Early Nutrition Influence – Preventive and Therapeutic Aspects

24th–25th September, 2019
Nestlé Competence Center | Frankfurt | Germany
Early Nutrition Influence – Preventive and therapeutic aspects

The importance of early nutrition for long-term health is becoming increasingly impressive. The situation of premature babies in particular reveals this connection like a magnifying glass. Here nutrition is a decisive factor for growth and development, it influences the risk of life-threatening diseases and possible late damage. In this sensitive area, however, the latest research findings give us hope – and provide indications of what measures could also be useful for mature babies.

Following the discussions about protein, human milk oligosaccharides (or HMOs for short) have moved into the focus of science. After all, they appear to play a significant role in the positive effects of breast milk. The possibilities of supplementation with selected HMOs are still in their infancy, but the first results open up far-reaching perspectives for the future, while at the same time raising new questions.

There is no doubt that adequate early nutrition is an exciting field – as the topics and speakers at this NNI European Meeting will show.

Mike Poßner
Medical Director Europe, Nestlé Nutrition Institute
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AA</td>
<td>Amino acids</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse effects</td>
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<tr>
<td>ARA</td>
<td>Arachidonic acid</td>
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<tr>
<td>ARI</td>
<td>Acute respiratory infection</td>
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<td>BPD</td>
<td>Bronchopulmonary dysplasia</td>
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<tr>
<td>BF</td>
<td>Breast-Fed / Breast-Feeding</td>
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<td>BUN</td>
<td>Blood urea nitrogen</td>
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<tr>
<td>CMF</td>
<td>Cow's Milk Formula</td>
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<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
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<tr>
<td>DHM</td>
<td>Donor human milk</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>eHF</td>
<td>Extensive Hydrolysate Formula</td>
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<td>EN</td>
<td>Enteral Nutrition</td>
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<tr>
<td>FAP</td>
<td>Functional abdominal pain</td>
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<tr>
<td>FGID</td>
<td>Functional gastrointestinal disorders</td>
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<tr>
<td>GA</td>
<td>Gestational age</td>
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<tr>
<td>HC</td>
<td>Head Circumference</td>
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<tr>
<td>HMO</td>
<td>Human Milk Oligosaccharides</td>
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<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
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<tr>
<td>ID</td>
<td>Iron Deficiency</td>
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<tr>
<td>IGF1</td>
<td>Insulin-like Growth Factor 1</td>
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<tr>
<td>IGSQ</td>
<td>infant gastrointestinal symptoms</td>
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<tr>
<td>LBW</td>
<td>Low Birth Weight</td>
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<tr>
<td>LRTI</td>
<td>Lower respiratory tract infection</td>
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<tr>
<td>MFGM</td>
<td>Milk fat globule membrane</td>
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<tr>
<td>OMM</td>
<td>Mother's own milk</td>
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<tr>
<td>NCD</td>
<td>Non-Communicable Diseases</td>
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<tr>
<td>NEC</td>
<td>Necrotising enterocolitis</td>
</tr>
<tr>
<td>FGID</td>
<td>Functional gastrointestinal disorders</td>
</tr>
<tr>
<td>NEC</td>
<td>Necrotising enterocolitis</td>
</tr>
<tr>
<td>NICU</td>
<td>Neonatal Intensive Care Unit</td>
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<tr>
<td>pHF</td>
<td>Partial Hydrolysate Formula</td>
</tr>
<tr>
<td>PBMC</td>
<td>Human peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
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<tr>
<td>PICU</td>
<td>Paediatric Intensive Care Unit</td>
</tr>
<tr>
<td>PN</td>
<td>Parenteral nutrition</td>
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<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>PVL</td>
<td>Periventricular leukomalacia</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
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<tr>
<td>RDI</td>
<td>Recommended dietary intakes</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SGA</td>
<td>Small for Gestational Age</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptors</td>
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<tr>
<td>VOC</td>
<td>Volatile Organic Compounds</td>
</tr>
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</table>
## Early Nutrition Influence – Preventive and Therapeutic Aspects

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**Podium discussion**

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**Speakers**

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<th>Speakers</th>
<th>Pages</th>
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<td>NNI Portrait</td>
<td>25</td>
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“Today we have wonderful guidelines, however, are we able to apply these guidelines to our patients?”

Virgilio Carnielli, during the meeting
Vitamin D requirements in preterm infants

The role of the nutritional environment throughout early development in modulating later health outcomes is in the spotlight. As the evidence base guides both public policy development and clinical practice guidance, it is timely to consider the current state of the art on vitamin D requirements in infancy and in particular, in preterm infants.

Pregnancy

It is accepted that an infant’s vitamin D status at birth is completely dependent on maternal status. However, the definition of healthy vitamin D status during pregnancy, typically indicated by circulating concentrations of 25-hydroxyvitamin D (25(OH)D), has been challenged by a lack of data from well-powered, well-controlled trials in pregnant women. Despite the publication of many systematic reviews of the role of vitamin D supplementation during pregnancy, there is insufficient evidence that physiological requirements for 25(OH)D are higher during pregnancy than in non-pregnant adults.

At the same time, there is evidence for a high prevalence of low vitamin D status during pregnancy, typically indicated by circulating concentrations of 25(OH)D < 30 nmol/L at minimum) is required to prevent metabolic bone diseases such as nutritional rickets and osteomalacia, which have severe and lasting consequences for bone growth and skeletal integrity throughout life. The prevalence of low vitamin D status among newborn infants is common; in Ireland where there is no maternal vitamin D supplementation policy, 35% of >1,000 umbilical cord sera had 25(OH)D < 25 nmol/L (Kiely et al., J Steroid Biochem Mol Biol. 2017). (Fig. 1)

Thus, prevention of infantile 25(OH)D (reflected in umbilical cord sera) <25 nmol/L has been proposed as a potential target for protection of the fetal/infant skeleton (Kiely et al., Ther Adv Musculoskelet Dis. 2017). O’Callaghan et al. (Am J Clin Nutr. 2018) reported the first dose-response study in pregnant women to address the question of how much vitamin D would prevent cord 25(OH)D < 25 nmol/L. When maternal 25(OH)D concentrations were > 50 nmol/L during the 3rd trimester, cord sera were > 25 nmol/L at delivery. The vitamin D3 intake required to maintain maternal 25(OH)D > 50 nmol/L among almost all mothers (97.5%) was 30 µg/day.

Infancy

In an elegant dose-response randomised controlled trial (RCT) among infants from <1 month to 12 months, Gallo et al. (JAMA 2013) (Fig. 2) reported that 10 µg/day of vitamin D3 maintained 97% of infant 25(OH)D concentrations >25 nmol/L at delivery. The vitamin D3 intake required to maintain maternal 25(OH)D > 50 nmol/L among almost all mothers (97.5%) was 30 µg/day.

1: Distribution of 25(OH)D in umbilical cord samples

2: Effect of Different Dosages of Oral Vitamin D Supplementation

**Fig. 1**

- Distribution of 25(OH)D in umbilical cord samples

- Mean = 34.92
- Std. Dev. = 18.13
- N = 1,052

- Cord 25(OH)D (nmol/L)
  - < 10: 3
  - < 20: 24
  - < 25: 35
  - < 30: 46
  - < 50: 80

**Fig. 2**

- Achieving ≥ 75 nmol/L of plasma 25(OH)D
- Achieving ≥ 50 nmol/L of plasma 25(OH)D

- Error bars indicate 95% Cls.
- 25(OH)D indicates 25-hydroxyvitamin D
- *P < .05 vs 400 IU group

**Table:**

<table>
<thead>
<tr>
<th>No. of infants</th>
<th>0 months</th>
<th>6 months</th>
<th>12 months</th>
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<tr>
<td>25(OH)D &lt; 50 nmol/L</td>
<td>29</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>25(OH)D &lt; 30 nmol/L</td>
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<td>28</td>
<td>12</td>
</tr>
<tr>
<td>25(OH)D &lt; 20 nmol/L</td>
<td>10</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>25(OH)D &lt; 10 nmol/L</td>
<td>29</td>
<td>30</td>
<td>13</td>
</tr>
</tbody>
</table>

**Figure captions:**

25(OH)D > 30 nmol/L and is consistent with rickets prevention (Munns et al., J Clin Endocrinol Metab 2016). Recommendations for children >1 year and adults vary and are typically ~10–25 µg/day (400–1,000 IU).

### Preterm infants

Preterm infants have a higher vulnerability to very low vitamin D status than term babies. Burris et al. (Paediatr Res 2014) compared 25(OH)D between term infants and preterms born < 32 weeks and between 32 and <37 weeks of gestation and found that the distribution of 25(OH)D was lower and the prevalence of 25(OH)D < 50 nmol/L was higher among babies born <32 weeks. The maternal transfer of 25(OH)D across the placenta appears to be lower earlier in gestation (Kassai et al., BMC Pregnancy and Childbirth 2018), who reported a lower correlation between maternal and cord 25(OH)D among preterm vs term dyads. Berry et al. (PLoS ONE 2017) compared international vitamin D recommendations among healthy term and preterm infants and identified variability in recommendations between 5 g (200 IU) and 25 g (1,000 IU) per day to reach target 25(OH)D concentrations of 50–80 nmol/L for bone and non-skeletal effects. Several dose response studies of vitamin D among preterm infants have been described.

Natarajan et al. (Pediatrics 2014) randomised 96 infants at 28 to 34 weeks of gestation to two different doses of vitamin D and reported a prevalence of 25(OH)D < 50 nmol/L of 67% in the 400 IU group vs 38% in those receiving 800 IU/day. Similarly, Fort et al. (J Pediatr 2016) conducted a dose-response study among 100 infants of 23 to 26 (+ 6 days) gestation equivalent to a daily dose of 200, 400 or 1,000 IU/day when routine feeding was accounted for. At birth, 67% of infants had 25(OH)D < 50 nmol/L; by postnatal day 28, 41% of those in the 200 IU group, 16% receiving 400 IU and 0% in the 800 IU group were < 50 nmol/L. No adverse outcomes in these studies were attributable to the supplementation regime (Fig. 3).

In Nebraska, 32 infants born at 24–32 weeks’ gestation were randomised to receive 400 or 800 IU/day vitamin D3 in addition to their usual dietary supply (Berry et al., PLoS ONE 2017). Briefly, infants in the 800 IU group achieved higher 25(OH)D; only 4 babies between the two groups did not reach 50 nmol/L and there was no evidence of adverse effects, even among infants <1,200 g who responded similarly to their large counterparts.

While these studies were not powered to compare health outcomes, there is evidence between that dosages up to 1,000 IU/day are tolerated well. In Ankara, Bozkurt et al. (Early Human Development 2017) randomised 121 infants, gestational age of 24–32 weeks, to receive 400, 800 or 1,000 IU/day vitamin D. 25(OH)D increases were dose-related in the 3 groups, and by 36 weeks post-mensual age, 22.5, 10 and 2.5% were < 50 nmol/L of 25(OH)D in the 3 groups, respectively. Further, there was a significant decrease in PTH concentrations in the 1,000 IU group, with no additional health benefits. (Fig. 4)

### Take home messages

- **For the prevention of very low vitamin D status at birth** (25(OH)D <25 nmol/L or 10 ng/mL), provide 1,200 IU/day (30 g/d) of vitamin D3 for pregnant women.
- **Vitamin D status is lower among preterm infants than term infants and the association between maternal and cord 25(OH)D is weaker in preterm infants.**
- **Recommendations for vitamin D in preterm infants vary between 400IU & 1,000IU/day around the world but dose-response studies conducted since recommendations were published show that 400 IU is effective at raising 25(OH)D > 50 nmol/L (20 ng/mL) in most infants but not all.**
- **Some studies show that 800-1,000IU delivers very high 25(OH)D without evidence of toxicity. Recommendations are to conduct larger, well-controlled RCTs among preterm infants, with care to the study design, sample size and controls.**
- **The use of heterogenous populations including stratification for GA as well as body size is encouraged. It is important to measure and include an estimate of the background vitamin D, calcium and phosphate availability and to use gold standard LC-MS/MS analysis where possible, accounting for vitamin D metabolites, which are variable in neonates and confound much of the analytical data available.**

### 3: Comparision of 3 Vitamin D regimens

![Graph showing comparison of 3 Vitamin D regimens](image)

**Fort P et al; J Pediatr 2016;174:132**

### 4: Effect of vitamin D supplementation

![Graph showing effect of vitamin D supplementation](image)

**Bozkurt O et al Early Human Development 112 (2017) 54–59**
Gastrointestinal Tolerance in very preterm babies

Feeding tolerance in preterm babies < 32 weeks is getting more and more a topic because the survival rates are getting better. However, many of these babies have growth failure and end up being too small to be 36 weeks old, the time they are usually discharged. Even perhaps more importantly, over the last 20 years these babies did not get any neurological advantage. In addition these babies develop a nutrition deficit. Nick Embleton et al. concluded that preterm babies accumulate a significant nutrient deficit in the first few weeks of life that will not be replaced when current recommended dietary intakes are fed (Peds, 2001). This deficit can be directly related to subsequent postnatal growth retardation. Poor growth velocity and neurodevelopmental outcomes had been brought forward by Richard Ehrenkranz et al. (Pediatrics, 2006). They studied a cohort of 600 babies (500 g–1,000 g) from the US and showed marked correlation between slow in-hospital growth and poor neurological outcome. Same findings were also reported in the UK. These are cohort studies, but only few randomized clinical trials are available. You have to be really careful when interpreting this type of data. So we became interested in seeing what happened in our unit, what happened in different places.

Today we have wonderful guidelines, however are we able to apply these guidelines to our patients? Some of these babies are less critically ill, some are more critically ill. We developed a statistically valid software and we are able to collect what is being the target, the prescription and the administration of nutrients for each parental and enteral nutrition. With that nutrition dashboard you get all the calculations automatically on the bedside. You can see what is the target value, how far are you from target, you can even see the metabolic complications and the association between medication and illnesses. Next to it we have the growth data. Every patient’s growth rate is plotted and we can monitor weight gain, head circumference gains and so on.

One of our major interests is, if the outcome of the baby is nutrition-dependent or disease-dependent, and whether there is an interaction between these two issues. In our Ancona cohort there are nearly 1,500 babies from January 2004 up to March 2019. 1,129 babies < 1,250 g are available for evaluation. They get routine parental nutrition and slower enteral nutrition progression. If they are > 1,250 g they get no routine parenteral nutrition and a faster progression enteral nutrition. That is our feeding scheme (Fig. 1) according to the day of life and according to birth weight segments.

Feeding intolerance and low enteral nutrition

There are quite different ways to define feeding intolerance. According to our definition of low enteral nutrition (EN): EN volume < 85 % of the target scheme over a given period time.

We did a concordance study and for instance we investigated if those being less than 85 % of the target value had a good concordance with the days reaching 120 mL/kg/d. We compared five different methods to measure intolerance on the very same population and there is no difference no matter what method you use. The next step was to define critical illness. Sometimes you have a simple score especially given in the first days of life, but we came up with something a little more complex, where we took in account medications, signs, symptoms, and diagnoses. Every one of our patients gets this type of scheme with a score number for each individual day from day 1 to day 28. The sum of the score numbers tells you an approximation for severity and you can come up with the grade of sickness during the first 28 days of life or daily sickness of a given time period, or you can even do a cumulative calculation of points. We arbitrarily choose 15 points to separate the less critically infants and the more critically ones (>15 points). Comparing infants tolerating less than 15% of the target EN with control infants who tolerate the prescribed target value, profound difference in fluids and energy intakes was found. What happened to low enteral nutrition tolerance <85% of the target? The more critically ill infants occurred in 70% of the cases, less critically ill infants in 30% and vice versa in control babies (28% and 72%, respectively). If you are sick, tolerance is less of course.
There are some risk factors during pregnancy and the intolerant babies, in spite of the contribution of parenteral nutrition, at 36 weeks are smaller, grow less.

However, when these babies reached 2 years of age body size was identical. But not only body size is identical, the Bayley scores are 99 versus 97 (cognitive), 103 versus 103 (motor) in the Ancona cohort.

We did not find that postnatal growth retardation over milder degree was associated with long term deficit at 2 years, neither in terms of anthropometry nor in terms of neurodevelopment. Case control group

In a well-matched case-control study we could select 88 cases of low enteral intake compared to standard enteral intake. Those infants on low EN were much smaller at 36 weeks, showing lower body size, lower weight gain, lower growth velocity, significantly lower enteral intakes, somewhat higher parenteral intakes and lower total energy and protein (parenteral plus enteral intakes). What happened to these case-control babies? At 2 years identical body size and neurodevelopment were eventually found.

Some papers showed that human milk is better tolerated, so we compared human milk versus formula and the difference was trivial. In our unit, to our surprise, the tolerance of the human milk fed babies was similar to formula fed babies.

More critically ill infants

Is there any difference in terms of tolerance and of nutrients if the baby is much sicker? We have 276 of these infants and we could match 138 babies who got lower tolerance, <85% target EN, identical clinical characteristics, and quite identical severity. Again you get less enteral energy, you grow less and at 36 weeks all the data are in terms of SD score. They are all smaller, weight gain during hospital stay was slower and the energy was significantly less in the low EN, as expected. But even in this group of more critically infant, tolerance of human milk and infant formula was identical. We could not find a difference in intolerance. (Fig. 2)

At 2 years even in this more critically infants, 57 per group – because of drop out and our neurodevelopment assessment started later than 2004 – no difference was found.

Even in critically ill infant receiving < 85% of the EN target we could not find any difference in the cognitive and motor Bayley scores. That is different from what was published before.

We divided babies who tolerated < 85% EN intakes in less critically and more critically ill and the less critically ill did better, as you would expect. So the effect of illnesses was found to be the same in the infants who were intolerant and in the control EN. Also the less critically ill babies had higher neurodevelopmental scores than the more critically ill.

Multiple regression analysis

In a cohort of 1129 babies we tested all possible variables, with all possible tests focused mainly on the prediction of low tolerance for the babies who got < 85% of the EN target volume during their first 28 days of life. The risk factors were not related to the type of milk or the clinical severity. We also looked at the cognitive development at two years, testing all possible variables and what came out was that cognition at 2 years was associated with PVL, BPD, SGA and maternal schooling. Using our severity score instead of the selected condition, the severity score was highly significant.

Although the Ancona Cohort has nearly the same population and we used exactly the same criteria, we did not find the inverse correlation between in-hospital growth and indices of poor development reported in the US.

Conclusion

- Very important: HAVING “OPTIMIZED” NUTRITION of the low birth weight infants!
- Feeding intolerance in preterm infants of <32 weeks gestation is chiefly associated with sickness.
- Infants who received less than 15% of the ANCONA target enteral nutrition volume were smaller at 36 weeks PMA (discharge) however they caught up at 2 years (body size and neurodevelopment comparable to control infants).
- The use of Parenteral Nutrition was larger in Low-EN infants.
- Enteral tolerance of infant milk formula was similar to own mother’s milk.
- Neurodevelopment in Ancona cohort was mainly affected by disease severity and parental education.
Protein requirements – are the recommendations too high?

The fetus receives high amounts of amino acids and some carbohydrates, whereas the term newborn infant gets a low protein diet, lots of carbohydrates and, lots of fat. In preterm infants this transition from fetal nutrition to term newborn nutrition happens in the neonatal ICU and actually, we do not know the best nutrition for the preterm infant.

Especially growth in the NICU has been linked to neurodevelopmental outcome in preterm infants. In ELBW infants (< 1,000 g) postnatal growth failure has been documented repeatedly. E.g. Ehrenkranz (1999) has shown that at term equivalent age up to 90% of ELBW infants developed extrauterine growth retardation defined by a weight below the 10th percentile. More recently this data has been reconfirmed (Cole et al., ADC Fet Neo 2014). An important point to take into consideration is, that in term SGA infants it has not been proven that better nutrition improves neurodevelopmental outcome. Therefore, it has been hypothesized that in preterm infants’ outcome improvement by better nutrition or better growth may only be possible early during early time in hospital.

In a retrospective study in ELBW infants more amino acids (AA) given in the first week of life significantly increased mental developmental index at 18 months and the same was true for energy. (Fig. 1).

Is the recommended protein intake too high?

In 2010, the ESPGHAN committee on nutrition recommended a protein intake of 4.0–4.5 g for infants up to 1,000 g and 3.5–4.0 g for infants from 1,000–1,800 g. Protein supply should compensate for a cumulative protein deficit. Some excess of protein had not been shown to be detrimental. Similar recommendations have been published by others as well. The US Life Science Research Office recommended 3.0–4.3 g/kg/d of protein in 2002, and Koletzko B et al. (World Re Nutr Diet, 2014) 3.5–4.5 g/kg/day protein.

Are these recommendations too high? Human milk is our preferred feeding; it is associated with less necrotizing enterocolitis (NEC). There are a very few trials challenging the recent increased enteral protein recommendations. In a systematic review seven RCTs have been found. (Fig. 2) All studies used standard protein fortification for the control group (on average 1 g of protein / 100 ml of human milk).

The majority of studies did not find an effect on weight or on length gain. In a carefully conducted straightforward trial however, Jacques Rigo (JPGN 2017) used a high protein human milk fortifier in comparison to a standard human milk fortifier and found a significant effect on weight gain. Therefore, in certain settings it is definitely possible to achieve better weight gain with higher protein supplementation. The study by Maas et al. (JAMA Paediatr. 2017) was quite remarkable. 1st The low protein group achieved normal weight gain on day 28. 2nd There was a trend towards better weight gain with lower protein fortification.

1: First week protein and energy intake and neurodevelopmental outcome at 18 months

<table>
<thead>
<tr>
<th>First week protein (g/kg d)</th>
<th>First week energy (kcal/kg d)</th>
<th>MDI at 18 months</th>
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<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
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<td>3</td>
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<td>70</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>60</td>
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2: High protein fortifier in VLBW infants

<table>
<thead>
<tr>
<th>Study</th>
<th>High protein g/100 ml</th>
<th>Low protein g/100 ml</th>
<th>Intake high g/kg/d</th>
<th>Intake low g/kg/d</th>
<th>Effect on weight</th>
<th>Effect on length</th>
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<tr>
<td>Miller 2012</td>
<td>1.4</td>
<td>1.0</td>
<td>4.2*</td>
<td>3.6*</td>
<td>no</td>
<td>(no)</td>
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<tr>
<td>Maas 2017</td>
<td>1.8</td>
<td>1.0</td>
<td>4.1 (0.39)*</td>
<td>3.5 (0.35)*</td>
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<td>Reid 2018</td>
<td>1.8</td>
<td>1.0</td>
<td>4.2 (1.31)*</td>
<td>3.5 (0.33)*</td>
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<td>Kim 2015</td>
<td>1.6</td>
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<td>3.9</td>
<td>3.3</td>
<td>no (yes?)</td>
<td>no</td>
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<tr>
<td>Moya 2012</td>
<td>1.54*</td>
<td>0.94*</td>
<td>4.48</td>
<td>3.54*</td>
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<td>Rigo 2017</td>
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<td>4.48 (0.38)</td>
<td>3.81 (0.43)</td>
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<td>no</td>
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<td>Rochow 2017</td>
<td>1.0</td>
<td>4.5</td>
<td>3.4*</td>
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<td>?</td>
<td>?</td>
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More recent data by Christian Fusch may provide an explanation for these observed effects. In a RCT he studied target fortification of human milk and exactly measured the intake of fat, amino acids / protein and carbohydrates and optimized the supplementation by adding as much protein and as much energy as required. The effect was remarkable. At 36 weeks of corrected age growth was adequate with target fortification. However, in the standard fortification group growth was adequate in infants with high protein human milk and there was growth failure in low protein human milk infants (donor milk or low protein mother’s milk). Therefore, the actual human milk protein concentrations may have significantly confounded the results of the previous studies. However, the exact protein concentration has not been measured in most of the trials.

**Are the high protein recommendations safe?**

To discuss this subject, I would like to highlight three studies:

- The working group of Carnielli V. (*Bellagamba, JPGN 2016*) studied the effect of an extra gram of protein in infants < 1,250g. No effect on weight gain or neurodevelopmental outcome has been found.

- The large randomized trial (*n=1440*) by Greet Van den Bergh (Fivez et al., *NEJM 2016*) studied parenteral nutrition in the PICU setting. In order to optimize nutrient intake, infants were randomized to receive parenteral nutrition in addition to enteral nutrition right from the first day of life or starting after day 7. With early parenteral nutrition there was a trend to more infections and more days in hospital.

- In a subgroup analysis in 209 term newborn PICU infants late parenteral nutrition (starting AA after day 7 – Verbruggen et al., *Lancet 2018*) increased the probability for live discharge. (Fig. 4) Therefore, we need to be careful with AA in sick PICU term newborns.

This leads back to target fortification which has been studied by Christoph. Fusch. However, target fortification is time consuming and may not be available for every neonatal department. Jacques Rigo was the first who suggested to measure urea or blood urea nitrogen regularly in infants on enteral nutrition and to adjust human milk fortification. If blood urea nitrogen and weight gain are low he suggested to increase protein and energy intake. If urea is high, you may reduce protein intake in infants who grow nicely or you may try to improve the energy intake in infants who do not grow sufficiently.

This approach has only been studied in one randomized controlled trial by Sertac Arsanoglu. The disadvantage is that it requires regular blood sampling.

**Conclusions**

- VLBWI are frequently undernourished
- Poor nutrition and poor growth are associated with poor neurodevelopment
- AA/protein is not always beneficial
- Be careful in sick neonates with early PN
- Be careful with routine protein recommendations above 4 g/kg/d
- High AA/protein intake may need to be individualized (target fortification or BUN/urea adjustment)

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**3: Higher protein human milk supplementation**

![Graph showing SDS Difference for Weight and Head Circumference](image)

**4: Which macronutrient may cause the harm in infants < 4 weeks**

![Graph showing Likelihood of live PICU discharge](image)
The Impact of Nutrition on Early Brain Development

The ability of human to perform complex mental activities including thinking, reasoning, remembering, problem solving, decision making, and learning new information are possible thanks to the ability of the brain to alter its functional and structural organization as a result of experience which is referred to brain plasticity. Moreover, development and full maturation of such a formidable organ is thought to take more than 3 decades.

During brain development, infancy and early childhood are particularly sensitive and characterized by rapid periods of brain growth that coincide with the emergence of nearly all cognitive, behavioral, and social-emotional functions. Moreover, throughout this period, brain’s networks are built and refined through processes that include axon growth, dendritic arborisation, synaptogenesis, and synaptic pruning and myelination. Therefore, providing the appropriate level of nutrition and the nutrients that support these physiological processes is key to guarantee optimal brain development.

Brain organization and plasticity

Ramón y Cajal (1852–1934) was a pioneer in brain anatomy and basically is the father of modern neuroscience. Using light microscopy and tissue silver staining approaches, Ramon y Cajal generated extremely accurate drawings disclosing the cytoarchitecture of the key cellular elements of the brain, including neurons and glia cells. Based on these observations he proposed 4 hypotheses that shaped modern neuroscience:

- the brain is composed of discrete individual signaling elements (neurons);
- Information passes from neuron to neuron across gaps (synapses);
- Information is polarized.
- Moreover, very importantly, he proposed that the brain has not a static structure but is dynamic and may adapt its structure depending on experience.

The ability of the brain to alter its functional and structural organization as a result of experience is referred to brain plasticity. The brain architecture is also incredibly complex: it is estimated that the human brain contains more than 200 billion neurons and non-neuron cells, 1 quadrillion of connections, 100 km of nerve fibers and 600 km of blood vessels. Such dynamic abilities and complex architecture require outstanding energy needs. Indeed, the adult brain accounting for a mere 2% of body weight is estimated to be responsible for 20% of oxygen (O₂) consumption and 20–25% of glucose utilization:

As noted earlier, brain development is characterized by massive growth that is due to several physiological processes that happen in overlapping waves. Neurogenesis and neural migrations happening first mainly before birth. And after birth, billions of neurons get connected by synaptogenesis, it is estimated that 700,000 synapses/second are formed. Myelination develops rapidly as well postnatal to enhance neuron communication throughout the entire brain. Moreover, early experience-dependent processes are triggering the plasticity and capacity for adaptation that is the hallmark of brain ability to adapt. We know that there are mainly 2 different factors that influence the brain development. Indeed, molecular processes can be either driven genetically but also influenced by environmental factors like social economic status, social interaction, urbanization, pollution, social mobility, stress and nutrition and food. Concerning the Z later, it has been shown that the total white and gray matter’s volume of malnourished and non-malnourished children are significantly different suggesting that appropriate level of nutrients is key for proper brain and cognitive development.

Executive functions

Brain has specialized areas and functional network to support cognitive tasks through information integration. Indeed, different parts of the brain need to structurally and functionally connect to each other in order to fully integrate different sensory information and make sense out of it. Therefore, proper connectivity is key for proper brain and cognitive functions. High brain connectivity allows complex cognitive processes such as executive functions (EFs). EFs are a set of key mental skills that act as a command center in the brain to get it organize, manage information and control behavior. Three core EFs are particularly important:

- Inhibition: the ability to suppress automatized or predominant responses.
- Cognitive flexibility: the ability to switch between cognitive sets or tasks.
- Working memory: the ability to organize and mobilize information that is stored in memory and is crucial for planning, for example.

EFs are necessary for life and learning and also for cognitive, social, and psychological development. Importantly EFs are not genetically encoded, and children are not born with EFs, but rather are develop them along time. Therefore, nutrition may play a key role in promoting brain development and being especially important for optimal development of EF. Interestingly EFs involve different part of the brain and relies heavily on brain connec-
The Impact of Nutrition on Early Brain Development

Myelination

Myelination is the process by which oligodendrocytes (OLs), specialized glial cells in the central nervous system (CNS), form a myelin sheath around axons and this is critical for proper brain connectivity. Myelin acts as an insulator by increasing axonal resistance and decreasing capacitance. Myelin sheath thickness, affects the conduction velocity of action potentials: compared to unmyelinated axon, the velocity of information in a myelinated axon is increased by 20 fold (Fig. 1).

In humans, myelination starts at mid-gestation, peaks during the first years of life and continues into adulthood. Interestingly, there is a strong overlap in the emergence of specific cognitive function and the myelination of brain regions and networks subserving these functions. (Dayanji/Uddin, Trends Neurosci 2015)

Recent studies suggest that the level and presence of specific nutrient may have significant impact on myelination and eventually on cognitive performance during brain development. Comparing infants that have been either exclusively breast fed or formula fed in an observational study showed first that breast fed infants have higher level of myelination in some brain areas and performed better in specific cognitive task compared to formula fed infants. Moreover, these observational data show positive associations between level of some nutrients present in breast milk and formula and myelination, like sphingomyelin DHA/ARA, choline, Vit B9, Vit B12 and iron (Schneider et al., eNeuro 2019). Furthermore, we showed that teh combination of these nutrients enhances de novo myelination in vitro (Hauser et al., Nutritional Neuroscience 2019).

Lipids

While the brain is, is mostly composed by water (77-78%), the brain is one of the richest in lipids (10-12%) among the body organs. Moreover, it has been estimated that there are around 100,000 different lipids in the brain, with different structures (Piomelli et al., Nat Rev. Neurosci. 2007). Lipids have three main functions: first they affect the cytoarchitecture of cells, organelles, and subcellular structures such as synapses, second they can act like signaling molecules, and finally lipids are used as a source of energy through beta-oxidation. Some of the lipids, called polar lipids, are especially important for brain development including synapse and myelin. Indeed, polar lipids such as sphingomyelin, and phospholipids are key to build brain connectivity and promote fast and efficient brain communication.

Conclusion

- The first 1000 days of life are a rapid and dynamic period of brain development.
- Brain connectivity is at the heart of high cognitive functions.
- Myelination is key to promote proper brain connectivity, it accelerates signal transmission between cells and thus supports brain communication.
- Myelin is mainly built during the first years of life and is modulated by specific nutrients.
- Polar lipids play an important role in brain, myelin and cognitive development, particularly phospholipids and sphingomyelin.
- Sphingomyelin is highly concentrated in the brain and naturally present in human milk
The idea that nutrition in early life may influence, or programme, later health first emerged in the 1960’s with the work of McCance (Singhal A, Lucas A., Lancet 2004). He showed that rats raised in small litters, and therefore overfed post-natally, were larger in adulthood. In humans, the strongest evidence for nutritional programming has emerged for the benefits of breast-feeding. Breast-feeding compared to formula feeding has been shown to reduce the propensity for obesity, dyslipidaemia, high blood pressure, and insulin resistance, the main risk factors for cardiovascular disease. Although the mechanisms for these effects are not known, we proposed that faster growth (upward centile crossing) as a result of relative over-nutrition in formula fed compared to breast-fed infants could adversely affect later cardiovascular health – the Growth Acceleration Hypothesis (Singhal A., Lucas A., Lancet 2004).

Consistent with the growth acceleration concept, many studies in healthy infants have confirmed an association between faster weight gain in infancy (upward centile crossing for weight) and later obesity, insulin resistance, and higher blood pressure. This association has been seen in >30 studies (summarized in 5 systematic reviews – [Singhal A., Proc Nutr Soc. 2016; /Patro-Got b B et al., Obes Rev 2016] including an individual-level meta-analysis in 47,661 participants from 10 cohorts (Druet C, Stettler N, Sharp S et al., Paediatr Perinat Epidem 2012). These effects are seen in both high- and low-income countries, for both weight gain and linear growth, in infants born preterm or at term, in infants with normal or low birth weight for gestation, and in both breast-fed and formula-fed infants (Savage JS, et al., JAMA Pediatr. 2016). In fact, the adverse long-term effect of faster early growth appears to be a fundamental biological phenomenon seen across animal species as diverse as insects, fish and mammals1. The magnitude of the effect is substantial. For example, over 20% of later obesity risk can be attributed to a high rate of infant weight gain; the relative risk of obesity associated with faster weight gain in infancy ranges from 1.2 to as high as 5.7; and a 3 mm Hg reduction in population diastolic blood pressure associated with slower infant growth would be expected to prevent >100,000 cardiovascular events/year in the USA alone. (Singhal A, Lucas A., Lancet 2004 /Patro-Got b B et al., Obes Rev 2016).

Central to the growth acceleration hypothesis is that fact that breast-fed infants grow more slowly than those fed formula probably because of the lower protein content of breast-milk compared to cow’s milk based formulas. (Fig 1) Formula-fed infants receive on average 0.5g/kg/d greater protein than breast-fed infants which could increase later adiposity possibly by mechanisms that involve programming of hormonal factors that affect appetite regulation and adipose tissue deposition. This difference in protein intake is most marked between 3 and 12 months of age when the protein concentration of breast-milk falls rapidly (Michaelson KF, Greer FR., Am J Clin Nutr 2014). Therefore, a lower protein intake in infancy that leads to slower weight gain might improve long-term health and particularly the risk of obesity – a hypothesis now confirmed in 5 randomised controlled trials (as reviewed – [Singhal A., Proc Nutr Soc. 2016]).

In the first RCT, infants born preterm and randomly assigned to a protein-enriched diet, that promoted faster weight gain in the first few weeks after birth, had higher blood pressure, fasting concentrations of insulin, cholesterol, and C-reactive protein, and greater risk of obesity in adolescence than controls. Similarly, infants born small for gestation at term and randomly assigned to nutrient-enriched formula that increased weight gain had higher diastolic blood pressure at age 6–8 years and, in 2 trials, 18–38% greater fat mass at age 5–8 years than controls (Singhal A., Proc Nutr Soc. 2016). (Fig 2)

In the largest study, the European Childhood Obesity Trial, infants randomised to a higher protein formula (2.05 g/100ml in first 6 months followed by 3.2 g/100 ml to 12 months of age) had 2.4x greater risk of obesity at age 6 years than those receiving standard infant formula (1.25 g/100 to age six months followed by 1.6 g/100 ml to 12 months) (Weber M, Grote V, Closa-Monasterolo R et al., Am J Clin Nutr 2014). Finally, in Chile, infants of mothers with a BMI >25 kg/m2, who were randomised to new low-protein formula between 3 and 12 months of age (protein: 1.04 g/100 ml, energy 62.8 kcal/100ml) had lower risk of obesity at age 2 years than those assigned to a standard protein formula (protein: 1.77g/100 ml, energy: 65.6 kcal/100 ml) (Inostroza J, Haschke F, Steenhout P et al., JPGN 2014).

Public Health Implications

The strength of the evidence supporting the growth acceleration hypothesis is challenging established public health practices. Professional bodies such as the Institute of Medicine in the US and the Royal College of Paediatrics and Child Health, and the Scientific Advisory Committee on Nutrition in the UK have recognized the role of faster infant weight gain in increasing the risk of long-term obesity. The WHO growth charts based on slower growing exclusively breast-fed infants are now widely available.
used and can help in the prevention of overfeeding in infancy. Furthermore, contrary to previous medical and public opinion, promoting catch-up growth by nutritional supplementation in healthy term infants born small for gestation may not be appropriate (Clayton PE, Cianfarani S, Czernichow P et al., J Clin Endocrinol Metab 2007). Finally, the benefits of a slower rate of infant weight gain as seen in breast-fed compared to formula fed infants has led to changes in infant formula to try to reduce the risk of over-feeding in formula-fed infants. These include reduction in the protein content of infant formulas and changes in recommendations for the composition of formula.

For example, the European Food Safety Authority recently recommended a reduction in the maximum permitted protein content in infant formula and suggested that “infant formula and infant follow-on formula should ensure that the growth and development of infants fed infant formula are similar to those of infants who are exclusively breast-fed during the first 6 months of life” (EFSA NDA Panel, EFSA Journal 2014). Therefore, using infant formulas with lower protein content could slow down the rate of weight gain in formula fed infants (closer to those given human milk) and have long-term benefits for health, and particularly for the risk of obesity. Finally, cow’s milk, a major source of excess protein for young children in richer countries, is not recommended below 12 months of age and even restricted to <500 ml/d in toddlers.

Clearly, the risk-benefit of faster early growth depends on the population involved. Faster weight gain may improve long-term cognitive function in infants born preterm and has short-term advantages for morbidity in infants with low birth weight from low-income countries (Singhal A., Proc Nutr Soc. 2016). However, in healthy infants born at term, the key message for health care professionals and parents is that ‘bigger is not necessarily better’.
The controversy around Vitamin D – Do we need to supplement beyond infancy?

Vitamin D is a key component for the growth and development of children and adolescents, influencing a multitude of functions. In Germany as well as in many other countries worldwide it has been recognized that there is a need for a higher vitamin D intake in all age groups, from infancy to older ages (Cashman et al. 2016, Kunz et al. 2018, Zittermann 2018).

To reach a sufficient vitamin D status, pediatric societies such as DGKJ or ESPGHAN recommend a daily intake between about 400 IU and 800 IU and appropriate outdoor activities (Brägger et al. 2013, Reinehr et al. 2018). In Germany, vitamin D deficiency is rare in infancy due to the very effective vitamin D prophylaxis during the first 12–18 months of life with 400–500 IU, the costs which are covered by health insurances. After this time, no effort is made to guarantee a sufficient vitamin D status. This is surprising considering the high need for vitamin D in this critical time period beginning with puberty in which the assembly of healthy bones and an optimal peak bone mass is of paramount importance not only for growth and development but also for health in later life. Therefore, a particular emphasis should be put onto this critical time period in the first 20 years of life (Fig. 1).

The existing recommendations, however, cannot be followed in practice. To eat a variety of foods containing vitamin D ignores the accepted fact that food is a very poor source for vitamin D and contributes only about 5–10 % to the vitamin D status. The very detailed instructions of national and international societies to get enough sunshine (e.g. 20 min between 11 and 3 pm (Reinehr et al. 2018)) seem unrealistic to be followed by children and adolescents. More important, the recommendations are based upon studies by M. Holick’s group in Boston (USA) but has not been verified for countries in the middle of Europe. In this context, it is widely accepted that at a geographic latitude of >40° N there is usually no skin vitamin D production in the winter times, i.e. from October to March (Zittermann 2010).

As the daily vitamin D supply from food comprises only about 50–100 IU, some 500–700 IU need to be obtained from other sources (Kunz and Zittermann 2016).

As there is hardly any skin vitamin D production in winter, an improvement of the vitamin D status is difficult to achieve without the intake of vitamin D enriched food or supplements. However, vitamin D supplements are often not recommended by health authorities because of their potential negative effects, although there is no scientific evidence for a risk of hypervitaminosis D at these levels of intake, not even for high risk groups. It is important to emphasize that Vitamin D itself is inactive. Only if required, it will be converted into its active form 1,25(OH)2D (hormone) in the kidney (Holick 2017).

This activation is largely independent of the dietary vitamin D intake and from synthesis in the skin. No negative effects have been observed even after the intake of very high amounts. It is misleading for the general population that even in scientific publications there is often no distinction made between data obtained by the inactive native vitamin D and the hormonal form of vitamin D, i.e. the extremely potent 1,25(OH)2D; the latter is never recommended or given for preventive reasons!

The human body has several control mechanisms preventing a strong increase in 25 OH D, and hence potential negative effects:

- a local increase of vitamin D in the skin will finally lead to a self-regulated degradation within the skin,
- the transport binding proteins (DBPs) have a high capacity to bind large amounts of vitamin D and metabolites with the highest affinity for vitamin D and 25 OH D and, most importantly,
- there is a strong endogenous control of the renal production of the active vitamin D metabolite, 1,25(OH)2D, which will be synthesized only if the hormonal form is required.

As prevention of diseases is of paramount importance – and all authorities agree on this – it has to be underlined that the current cut-off for a sufficiency status is at least >50 nmol/L 25 OHD (IOM: Ross et al. 2011, ESPGHAN: Brägger et al. 2013, DGKJ: Reinehr et al. 2013) (Fig. 2). Other international societies recommend even much higher 25 OH D levels as only then can an increase of PTH with its potential negative effects be prevented. 25 OH D concentrations below 50 nmol/L are considered as “insufficiency” or even “deficiency” (Fig. 2). To be effective preventing a disease requires to start at an early point when vitamin D deficiency has not yet established. Therefore, if pediatric and nutrition societies consider a vitamin D status between 20 and 50 nmol/L as insufficient, then steps to improve this situation are strongly needed at this point in time and not waiting until a disease has developed.

In the following we summarize important issues regarding vitamin D, some of which are often misinterpreted in the public as well as in scientific discussions:

- Groups at high risk are, e.g., children and adolescents, older people, pregnant and lactating women;
- The recommended intake for children and adolescents is about 600–1000 IU/d, and even higher for children in risk groups (ESPGHAN: Brägger et al. 2013);
As the vitamin D intake (only 50–100 IU/day) via food is negligible, supplementation of the missing 500–700 IU is required; in children and adolescents vitamin D status, measured as serum 25 OH D, is often low which puts those groups at a high risk for negative effects, e.g. on bones; skin vitamin D production through UVB can be extremely effective; however, it is often prevented by the strongly recommended use of sunscreen for cancer protection; in addition, in Germany and in countries at the same latitude, it is not effective in 4–6 months (October to March); the assumption that storage of vitamin D in summer as a depot to be used in winter is not supported by the often insufficient vitamin D status in overweight and obese individuals; the assessment of the vitamin D status relies on serum 25 OH D measurements. The classification according to IOM, to which ESPGHAN and DGKJ adhere to is shown in (Fig. 2); it is often argued that increasing the daily vitamin D intake would easily lead to a high risk for potential negative effects of vitamin D. However, within the recommended intake, there is no evidence for any deleterious effect in the general population. The official statements regarding safety aspects of the IOM and EFSA to which DGKJ and ESPGHAN adhere, are given in (Fig. 3); according to the recommendations of the EFSA (European Food Safety Agency, 2012) and the Institute of Medicine (USA) 4,000 IU/day are physiologically safe, even during pregnancy and lactation (Fig. 3). Neonates should not receive more than 1,000 IU/day, young children up to 1 year of age not more than 2,000 IU/day. The upper limit for older children, adolescents and adults is 4,000 IU/day (Kunz/Zittermann 2015). not recommending supplementation or food enrichment because of potential adverse reaction ignores the high risk for vitamin D deficiency in large parts of a population (Fig. 4). vitamin D is one of the few nutrients for which the coverage of the recommended intake can only be met most of the year by taking supplements or by food enrichment strategies.

Finally, the often made argument that at 25 OH D level classified as insufficient “because rickets is not seen in practice” can be questioned as this would mean that initiatives to treat the disease would begin only at very low levels of 25 OH D corresponding to a deficiency status according to the classification of the pediatric societies. Preventive steps, however, need to start much earlier; indeed, at a point in time when 25 OH D concentrations are reached, which is considered as insufficient according to the classification show in (Fig. 2). For example, if 30–50 % of the German population are observed to have 25 OH D levels between 20–50 nmol/L, (ref) efforts are urgently needed to improve this situation and to guarantee at least the agreed minimum of about 400–1,000 IU/day for healthy bones of young children and adolescents; also, because of the long lasting effects of healthy bones later in life.

1: Changes in body mass throughout life

Environmental factors

- Hormonal status
- Nutrition
  - Age
  - Physical activities
  - Co-morbidities

Calcium
Vitamin D
Protein

2: Serum 25 OH D levels and Health according to IOM

<table>
<thead>
<tr>
<th>nmol/L</th>
<th>ng/ml</th>
<th>Health Status</th>
</tr>
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<tbody>
<tr>
<td>&lt; 30</td>
<td>&lt; 12</td>
<td>Associated with vitamin D deficiency, leading to rickets in infants and children and osteomalacia in adults</td>
</tr>
<tr>
<td>30 to &lt;50</td>
<td>12 to &lt;20</td>
<td>Generally considered inadequate for bone and overall health in healthy individuals</td>
</tr>
<tr>
<td>≥ 50</td>
<td>&gt; 20</td>
<td>Generally considered adequate for bone and overall health in healthy individuals</td>
</tr>
<tr>
<td>&gt; 125</td>
<td>&gt; 50</td>
<td>Emerging evidence links potential adverse effects to such high levels, particularly &gt;150 nmol/L (&gt;60 mg/mL)</td>
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</tbody>
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3: Tolerable upper intake levels

<table>
<thead>
<tr>
<th></th>
<th>EFSA (2012)¹</th>
<th>USA (2013)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>1000 IU</td>
<td></td>
</tr>
<tr>
<td>Children (1 to 11 years)</td>
<td>2000 IU</td>
<td></td>
</tr>
<tr>
<td>Children and adolescents (&gt;11 years)</td>
<td>4000 IU</td>
<td></td>
</tr>
<tr>
<td>Adults / Pregnant of breastfeeding women</td>
<td>4000 IU</td>
<td></td>
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</tbody>
</table>

¹ European Food Safety Authority; ² Institute of Medicine USA

4: Vitamin D Supplementation and/or Food Enrichment

YES
Risk of toxic effects low

NO
Risk of deficiency high

¹ within upper intake levels
The early-life gut microbiota establishes and matures sequentially during infancy and early childhood. An age-appropriate microbiota maturation is important for normal digestive, immune competence and metabolic development. The gut microbiome has to be seen as an ecosystem, it’s not an individual bacteria or an individual bacterial group, they are working together, also with the host. The early life microbiota trajectory is dynamic. At 3–6 months of age the infant gut harbours bifidobacteria dominated community types. These communities transit and change further until to about 2–3 years, when they resemble community types that are reported for adults. The first years of an infant’s life are characterized by a very dynamic microbiota establishment and obviously when you have different community types dominated by different microbiota taxa you have also different metabolic activity in the gut ecosystem.

The microbes in microbial activity in the mother can have an influence on the fetus. There are metabolites that can go in utero and probably modulate also the development of the fetus.

In early life you have different relevant events so microbiota seeding at birth – probably the most important event.

With the introduction of complimentary diet, you have a diversification of nutrition. If you do not diversify your nutrients you do not diversify your microbiota with a higher risk again for diseases later in life.

**Influencing factors**

Delivery mode and maternal intrapartum antibiotic use are major covariates related with variation in microbiota development seen in delayed bifidobacterium development in C-section born infants. (Shao et al., Nature 2019) There is a higher risk of atopic disease or food allergy, asthma related to C-section birth. But there is not always an association, this is a mystery still and it must be stratified better to understand the different risk factors for many of these diseases. Also, the early live microbiome could have a role for overweight obesity. Same for antibiotic use: A large study indicates that antibiotic use between 6–24 months had no effect, but < 6 months there was an effect seen, indicating the importance of the early life microbiota development.

The nutrition influences on microbiota in breastfeeding period is relatively easily done because we can compare breastfed infants and formula fed infants. The breastfed infants have a lower diversity and then it increases, while the cow milk formula or soy milk fed infants have already higher diversity into microbiota early on. There is a bifidobacteria dominance in the breastfed infants and a delay in the bifidobacteria appearance in the formula fed infants.

Possibly, the microbiota needs to grow slowly and have to have time to go through different phases.

**Human Milk Oligosaccharides (HMO)**

Among the major differences between breastmilk and formula milk are the human milk oligosaccharides (HMOs), structurally diverse elongations of the milk sugar lactose by enzymes that are also responsible for mucosal glycosylation. Breastmilk HMO composition is highly variable and understanding the underlying factors is key to have meaningful observational correlation studies with infant growth, development and health. HMOs shape the establishing early life gut microbiota and supposedly help the development of appropriate immune competence.
Factors influencing HMO composition in breastmilk include:

- Genetics (Secretor-, Lewis gene)
- Lactation stage
- Physiological status of the mother (e.g. BMI)
- Mode of delivery
- Infant gestational age
- Preterm and term delivery
- Diet

HMO 2’FL and LNFP II are proxies for the fucosyltransferases FUT2 and FUT3 genotypes and allow to stratify breastmilk into 4 milk groups in mothers. These most prominently affect HMO composition (Fig. 2):

- FUT2 / FUT3: pos/pos
- FUT2 / FUT3: neg/pos
- FUT2 / FUT3: pos/neg
- FUT2 / FUT3: neg/neg

There are different pathways how the HMO works: one major way is through the bifidobacteria that can have effects also to the host, then there are some reports on direct deflection or inhibition of pathogens, some reports on strengthening the gut barrier function and some reports on educating the developing immune system either through the bacteria or their metabolites. Or, directly through some HMOs that may go systemic. And then there is newer data that there might be modulation also of the nervous system and the brain function.

Clinical observations

Infants were observed to harbor microbiota community types with highest bifidobacteria abundance in presence of Bifidobacteria-containing the genes that can utilize fucosyl-HMOs. The gut ecosystem of those infants also have lowest pH and highest amounts of acetate, a typical short chain fatty acid produced by bifidobacteria. (Matsuki et al., Nat Commun. 2016)

In a Finnish cohort of mother-infant pairs, 2’fucosylated HMO in breastmilk were seen to alleviate the early life dysbiosis of C-section born infants compared to vaginal born infants (Korpela et al., Scientific Reports 2018). Among the microbiota changes the Bifidobacterium and specifically B. breve increased in abundance. Interestingly, B. breve strains were identified by Matsuki et al. 2018 to be able to utilise fucosylactose. In the same Finnish cohort associations of 2’Fucosyl-HMOs to allergies up to 5 years were studied. A faster onset of allergy was observed in the C-section born infants at 2 years of age if they get breastmilk deficient in 2’Fucosyl-HMOs.

Looking at the stratified data set of C-section and vaginal born infants, the effect of the 2 HMOs was strongest in C-section born infants, this both for the reduced risk for lower respiratory tract morbidity (LRTI) and for the microbiota changes.

Conclusion

- Clinical observation studies with breastfed infants suggests that specific 2’Fucosyl-HMOs may act through the microbiota, especially in situations of dysbiosis like upon C-section delivery, to help immune protection and development.
- RCT with formula fed infants suggests the 2 HMOs 2’FL and LNNt help protect from LRTI and antibiotic use, possibly through their effects on the early gut microbiome maturation and activity.
A real world study with an infant formula enriched with two HMO was done in Spain in 2018. The paper was presented at the last ESPGHAN meeting in June by Dr. Enriqueta Román Riechmann, who was the main researcher of the study. Here there are some of the results of that NEHMO study.

The idea why to perform a study on the formula that is already in the market was trying to follow the regulations of the legislation. The last regulations in infant formula said that when there are new ingredients in an infant formula first of all you have to review all the previous data and then you have to set new studies to answer the question regarding the benefits of the new ingredients. (Official Journal of the European Union, 2.2.2016)

The aim of performing this real world evidence study, when there is already done a RCT (Puccio et al., JPGN 2017) was two things:

- The overall purpose was to assess and document growth and feeding tolerance of healthy term infants consuming infant formula supplemented with 2’FL and LNnT for 8 weeks in a real-world setting.
- The second one was to see the behaviour of that formula in a group that was not yet present in the randomized controlled studies. The mixed-fed infant group that is, they are breastfed and receive some supplement infant formula several times a day.

Once the parents had decided the type of feeding they wanted for the kids, they were offered to enter in the study and some of them received the study formula (FF), some were exclusively breastfed (BF) and some were part of the mixed-fed group.

The outcomes were growth according to the WHO standards, the feeding tolerance and measured by means of the validated questionnaire infant gastrointestinal symptoms (IGSQ). As additional outcomes, parents were asked about satisfaction within the use of the study formula. As a safety measurement, total number of adverse events were also recorded. (Fig.1)

The study was done in six locations all over the country during the last four months of the last year. Once the informed consent was signed and the babies entered in the study they were first visited in the outpatient clinic, have a phone call after four weeks and were seen again in the outpatient clinic at the end of the study (8 weeks). At all the visits they have anthropometry and the questionnaire on the tolerance as well as they acceptance by the families evaluated. The study was approved by the Institutional Review Board in all centers and registered in ClinicalTrials.gov (NCT04055363). (Fig.2)

According to enrollment, 207 patients were included (114 receiving formula and 63 exclusively breastfed). From those receiving any amount of formula, 82 were exclusively bottle-fed.
Finally, in the per protocol analysis, 159 infants were considered as evaluable. Drop-outs were regularly distributed in both three groups, and mainly because of non-compliance.

The first of our goals was to see growth. The results were as normal as in the randomised control trial by Puccio et al. The infants were growing normally within 0 and -1 standard deviation from the WHO standard z-scores for weight and for height and also for the head circumference, without differences between the three groups (Fig. 3). Gastrointestinal tolerance was investigated by means of the infant gastrointestinal symptoms scale.

Infants of all the three groups in the study investigation – face to face and by phone – have no really gastrointestinal distress. They show low IGSQ composite scores at any time point. The comparable GI tolerance in formula, mixed and breast-fed infants was indicated by similar IGSQ composite scores at all time points. So the study formula was as good and well tolerated as breast feeding in all three periods.

The mean IGSQ scores of each domain were nearly the same for all of the infant groups in all three period domains, except for the stooling characteristics. For stooling, firmness was higher in formula fed group at the beginning of the study and similar to breast-fed group by the end of the 8 week period. (Fig. 4)

The incidence of AE in the study was low and comparable for all three groups, despite the high incidence rate of respiratory infections at the time of the study (fall and winter time).

In three patients receiving formula adverse events were considered in relation with the formula, although not confirmed. In the satisfaction questionnaire for parents at the end of the per-protocol study most of them were satisfied with the HMO-supplemented formula (90 %) and were willing to use the formula in the follow-up after finishing this study (> 90 %).

### Conclusion

In this first real-world-evidence study on HMO providing unique data on mixed-fed infants, infants exclusively or mixed-fed with formula supplemented with the two HMO 2’FL and LNnT had:

- **Age-appropriate growth in line with WHO standards and no significant difference observed between groups.**
- **Good digestive tolerance as indicated by low IGSQ scores and comparable to BF reference.**
- **Low incidence of adverse events (AEs) / illnesses and comparable to BF reference.**
- **High formula acceptance and parents were satisfied with the HMO-supplemented formula.**
The potential of HMOs in neonatology

Unlike the milk of many other mammals, human milk contains a high amount of diverse complex sugars called human milk oligosaccharides (HMOs). The composition varies between women and, to a certain extent, changes over the course of lactation. Current research focuses on the maternal factors that drive HMO variation and aims to understand how HMO composition impacts immediate and long-term infant health and development.

Maternal Drivers

The principal component plot (Fig. 1) includes HMO data from almost 10,000 milk samples collected from different sites around the world. Each dot in the three-dimensional space represents the HMO composition in one milk sample. The closer the dots are to each other, the more similar the HMO composition in those two samples. The further apart the dots, the more dissimilar or different the HMO composition. The plot highlights the milk samples from two different cohorts with the CHILD cohort samples in red and an India rotavirus cohort in green (ref). The samples cluster in slightly different locations, suggesting that HMO composition is different in different parts of the world. However, even more evident is the strong left-right separation that is independent of cohorts and geographic location, but can be explained by maternal genetics. In fact, single nucleotide polymorphisms (SNPs; the difference in one single base pair in a person’s DNA) is responsible for the two HMO “lactotypes”. The SNPs introduce a premature stop codon and inactivate an enzyme called fucosyltransferase 2 (FUT2), which participates in HMO synthesis. Secretor moms (right cluster) have an active FUT2 and are able to synthesize a specific HMO subset. Nonsecretor moms (left cluster) have an inactive FUT2 and are unable to synthesize these specific HMOs, but they can still synthesize many other HMOs. This one base pair change in mom’s DNA dramatically changes her entire HMO composition. The frequency of Secretors and Nonsecretors varies around the world. For example, in South America the abundance of Secretors is ~95%, in some parts of Africa it’s only ~65% (McGuire et al., Am J Clin Nutr 2017).

In addition to genetics, other maternal fixed and modifiable factors like diet and exercise, probiotics and other supplements, health status and drugs, may influence HMO composition and are currently under investigation.

Infant Health and Development

Understanding the maternal factors that drive the variation in HMO composition represents only one axis of the mother-milk-infant ‘triad’. The other axis links HMO composition with infant health and development, and the available data is a mixed bag of associations in observational cohort studies, mechanistic insights from tissue culture and animal models, and recently intervention studies with individual HMOs added to infant formula. To this extent, it is believed that HMOs serve as prebiotics, antimicrobials and antiadhesives, but also directly interact with epithelial and immune cells either locally in the gut or systemically after absorption in the circulation.

Prebiotic effects:

HMOs can be utilized by and provide a growth advantage to certain potentially beneficial bacteria in the infant gut. Other, potentially harmful bacteria, may not be able to utilize HMOs and are at a disadvantage. HMO utilization leads to the production of short chain fatty acids and other HMO biotransformation products that potentially benefit the infant, but also lower the pH in the gut lumen and help keep other bacteria in check. However, an overabundance of HMO-utilizers like specific Bifidobacteria may also create a disadvantage as HMOs, particular the higher molecular structures, are being used and are no longer available to exert other protective effects.

Antiadhesive effects:

Due to the constant flow and movement in the gut, many microbes need to be able to attach to the intestinal surface to avoid being washed out. Many microbes have specific anchor proteins that allow them to adhere to complex sugar molecules (glycocalyx) on the intestinal surface. HMOs, complex sugars themselves, resemble the structure of the intestinal surface sugars and serve as decoy receptors and block the adhesion of microbes, which are no longer able to attach, proliferate and in some cases invade and cause disease. For example, specific HMOs block the adhesion of enteropathogenic E. coli (EPEC) both in vitro and in suckling mice. EPEC is one cause of diarrheal
The potential of HMOs in neonatology is significant, given that more than 2,000 children under the age of 5 die daily from preventable diseases.

**Rotavirus infection and vaccination:**
Rotavirus is a major cause of infectious diarrhea and death, particularly in infants. A specific rotavirus strain, G10P[11], is prevalent in certain areas in India and affects neonates. Surprisingly, specific HMOs increase the infectivity of this strain in tissue culture and are associated with symptomatic rotavirus infections in a mother-infant cohort in India (Ramani et al., Nat Commun 2018).

Does this mean that pathogens are exploiting HMOs to their advantage? Or can we use this knowledge to develop new vaccination strategies that include HMOs?

**Growth:**
In addition to protecting from infections, HMOs may affect infant metabolism – either indirectly through shaping microbial communities or directly by affecting metabolically relevant host tissues like fat, liver, muscles – and influence body weight and composition in infancy and beyond. For example, an explorative study in the Danish SKOT III cohort with 30 mother-infant pairs showed that exclusively breastfed infants with extreme weight gain in the first six months of life received mother’s milk that contained more 2’FL and less LNnT than the normal weight group (Larsson et al., Frontiers Pediatrics 2018). A Finnish study within the STEPS cohort (N=802 mother-infant pairs) corroborated these results in healthy children: infants with higher concentrations of 2’FL and lower concentrations of LNnT in the mother’s milk were associated with higher length and weight during the breastfeeding period and up to 5 years of age. (Lagstrom/Rautava, manuscript in revision). While there is currently no data showing causalities or mechanistic understanding, these and other cohort studies suggest that HMOs are associated with infant body weight and composition, highlighting that it’s not only individual HMOs, but the relative abundance of different HMOs to each other that drive outcomes.

**Necrotizing Enterocolitis (NEC):**
5% of all VLBW preterm infants develop NEC. Remarkably, infants who receive human milk are at six-to-ten-fold lower risk to develop this devastating and often deadly disorder. HMOs improve survival and reduce NEC pathology in animal models, with disialyllacto-N-tetraose (DSLNT) being the most effective individual HMO. Human cohort studies with mothers and their VLBW infants corroborate these results, showing that infants who develop NEC receive less DSLNT with the mother’s milk than infants who do not develop the disorder. Data from animal studies and human cohorts together establish associations and causalities, strongly indicating that DSLNT is protective against NEC.
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