NNI European Meeting

Nutrition and growth in the first 1,000 days of long-term impact

NRC, Vers-chez-les Blanc/Lausanne
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Various studies have shown that nutrition in the first 1,000 days of life, in other words, during pregnancy and the first two years of life, can have a long-term impact on the healthy growth of infants, thus putting this early childhood development into particular focus.

How can we work together to implement the newest scientific findings for nurturing a healthy generation? This NNI Meeting targeted the topic from various angles, with internationally renowned speakers presenting the latest insights and developments.

More than 100 European pediatricians and scientists accepted the invitation from Nestlé Nutrition Institute to share and discuss the latest insights about the long-term impact of nutrition and growth in the first 1,000 days. The focal points included new insights about the epigenetic influence on development and lifelong health, as well as new studies about the low-protein hypothesis and the importance of probiotics.

This acclaimed meeting also provided ample opportunities for discussions among the international participants, who engaged in a lively and highly successful exchange of views and ideas about new concepts for healthy growth.

We know that epigenetics plays a role. Nutrition in pregnancy influences both pregnancy outcomes and later health outcomes of the infant. The first 1,000 days are a precious opportunity for epigenetic and early nutritional programming, which influences lifelong health outcomes by lastingly modifying gene expression.

“Together we provide nutrition for a better future”: this sentence underscores the importance of the crucial period of the first 1,000 days. It is also a call for action: firstly, for action in research to better understand the mechanisms; secondly, for action by the industry to develop modern nutritional concepts. And finally, for action by healthcare professionals to provide advice to families during this period.

We need to better understand the importance of these first 1,000 days. And we need to share know-how with you.
A student to Albert Einstein:
“The questions of this year’s exam are the same as last year’s!”

Albert Einstein replied:
“True, but this year all answers are different …”
Impact of dietary interventions on pregnancy outcome

The westernised diet, which rich in carbohydrates, is thought to be one of the factors that contribute to overweight and obesity. Glucose, the primary energy substrate for the foetus, passes directly from the mother to the foetus; no transport mechanism is required. Maternal glucose concentrations below those diagnostic of diabetes are associated with increased birthweight and adverse outcomes (IHAP 2008).

Maternal diet, especially its carbohydrate type and content, influences maternal blood glucose concentrations, but not all carbohydrate foods are created equal. Chart 1 shows the incremental area under the blood glucose curve after ingestion of 50 grams of a test food. This could be important in pregnancy because if the mother ingests a high-GI meal, she under- goes a peak in glucose and transfers it to her fetus, who experiences fetal glucose intolerance.

Beginning in the early weeks of pregnancy, a maternal glucose level at the upper range of normal is associated with increased birthweight. We hypothesise that a low glycemic-index diet during pregnancy could prevent recurrence of foetal macrosomia.

Results
- There was no difference between the two groups in baseline maternal characteristics.
- There was no difference in GI at the beginning; but after introduction of the low GI diet, the intervention group had a lower GI index in the second (56.1 vs. 57.8) and third trimesters (58.0 vs. 57.7).
- There was no difference in gestational weight gain and maternal glucose level at the upper range of normal is associated with increased birthweight. The ROLO study (Walsh et al., 2012), which was conducted at the National Maternity Hospital in Dublin, we analysed the effect of a low glycemic-index diet intervention on the incidence of macrosomia (birth weight > 4kg) and gestational weight gain. The ROLO inclusion criteria were healthy women, second-trimester pregnancy, with a birthweight > 4000 g; the exclusive criterion was previous gestational diabetes. We recruited and randomized the participants at the first antenatal consultation. The primary outcome was birthweight, the secondary outcome gestational weight gain.

Conclusions
- A low glycemic-index diet in pregnancy does not reduce the incidence of large for gestational age infants in a group at risk of fetal macrosomia.
- It does, however, have a significant positive impact on gestational weight gain and maternal glucose intolerance.
Mechanistic insights into programming from experimental models

The author
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Recent studies in humans and animal models have suggested that diet during fetal and early postnatal life may be particularly important as it can have long-term effects on the risk of an individual developing metabolic conditions such as type-2 diabetes and obesity.

Although initial focus was directed towards the detrimental effects of under-nutrition and low birthweight, in light of the growing epidemic of obesity, increased attention is now being directed towards understanding the consequences of maternal over-nutrition during pregnancy and lactation on the long-term health of her offspring. The importance of the early postnatal environment is also being increasingly recognised, with rapid postnatal growth being associated with later obesity – independent of the growth in utero. Following on from observational studies showing associations between patterns of early growth and long-term metabolic health, the field is now making rapid progress in understanding the molecular mechanisms underlying such programming.

Accelerated cellular ageing

One of the best indicators of cellular ageing is telomere length. Telomeres at the end of our chromosomes shorten with every cell division. When they become critically short, they signal to the cell to stop dividing. It was recently shown that telomeres shorten in response to oxidative stress. We have shown how maternal diets can impact on telomere length in a number of tissues including pancreatic islets that not only play an important role in regulating glucose homeostasis, but also because the pancreas is very vulnerable to oxidative stress.

If you look at recuperated rat offspring who were exposed to a low-protein diet that restricted their growth in utero and then grew very rapidly in early postnatal life, you find that more short telomeres and less long telomeres compared to young aged-matched controls. This is functionally significant as it is accompanied by premature induction in mediators of cellular senescence including p21 and p16. The data on p16 is particularly noteworthy because p16 is regarded as one of the best markers of ageing among many species, including humans.

HNF4α as a predictor

HNF4α is a developmental transcription factor that plays an important role in the development of the endocrine pancreas. HNF4α also plays an important role in regulation of adult beta cell function because it regulates transcription of a number of genes involved in the glucose sensing mechanisms. Pancreatic islets of type-2 diabetics have much less HNF4α compared to the islets in a glucose-tolerant individual.

Using the maternal low-protein model, we demonstrated that there was a reduction in both mRNA and protein levels of this important gene. Two main epigenetic marks regulating gene transcription in mammals are DNA methylation and histone modifications (Chart 2).

Epigenetic programming of gene expression

The final potential mechanistic area is the role played by epigenetic programming of gene expression. Changing methylation and histone modification patterns could form the basis for the cellular memory. In simple terms, what we mean by epigenetics is that cells containing the same DNA can have very different phenotypes. For example, DNA in our brain cells is exactly the same DNA sequence as the DNA in our fat cells. But brain cells are very different from fat cells. This is because the cell expresses different genes with the DNA in different cell types and at different levels. So-called “epigenetic mechanisms” regulate these processes.

The functional gene product, which has the effect, is the protein of a particular gene. To get the functional protein, a number of processes must occur. Firstly, the appropriate gene must be transcribed into messenger RNA (mRNA). The mRNA is then translated into the functional protein product. So both transcription and translation are tightly regulated processes. You can see how the early environment could impact on the level of particular proteins, by either regulating the rate of DNA transcription, or the regulating translation, or both. If the process operates at the transcription level, you see a difference in the mRNA and in the protein. If it operates at the translation level, you won’t necessarily see a difference in the mRNA, but you will see a difference in the protein (Chart 1).

Animal models and human studies

It is always important to integrate our findings from animal models to human studies and vice versa. And it is always very significant when we find that similar things occur in humans.

A study by the University of Copenhagen tested a cohort of 19-year-old Danish men who were born with low birthweights. These young men had an impaired fasting glycemia, but were not diabetic. Impaired fasting glycemia is a good predictor of future development of diabetes. We studied adipose tissue biopsies from these individuals and observed reductions in expression of a very specific panel of insulin-signaling proteins, including P110β and as we predicted from the animal model (Chart 4).

How can we intervene?

We cannot assure that every woman begins pregnancy with a healthy BMI, so our first option is to intervene with the mother during pregnancy. But this is complex because fetal nutrition is not the same as maternal nutrition, therefore postnatal interventions may be more realistic. However we still need to identify the time period when our intervention has the maximum effect as well as identifying which individuals will benefit most from such an intervention.

Chart 1

Programmed Changes in Gene Expression

- HNF4α and Histone Modifications

Chart 2

Epigenetics and Gene Transcription

- Nucleosome
- Active histone modification
- Repressive histone modification
- DNA methylation
- Transcription
- No transcription

Chart 3

Adipose Tissue Signalling Proteins

Animal models and human studies

How can we intervene?

Conclusions

- Early nutrition and growth influence long-term health.
- Multiple mechanisms are involved.
- Further understanding of mechanisms could yield potential for targeted intervention.

| Year | Birthweight | Mortality | Growth
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<tr>
<td>2000</td>
<td>3.4 kg</td>
<td>0.5%</td>
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<tr>
<td>2005</td>
<td>3.5 kg</td>
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<td>2010</td>
<td>3.6 kg</td>
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Birthweight is a very crude index for in utero experiences. We need better markers of disease risk. If something is a good marker, it has got to be something that is expressed in a clinically accessible tissue. Placental tissue is of particular interest because it is available very early in life and is usually very clinically accessible. Using samples from the Southamption Women’s Survey, we observed a strong correlation between birthweight and placental P110β expression. How can we intervene?

Conclusions

- Early nutrition and growth influence long-term health.
- Multiple mechanisms are involved.
- Further understanding of mechanisms could yield potential for targeted intervention.
Breastfeeding may also produce long-term benefits for the health of children and their mothers (Chart 1). However, there are still discrepancies among the various studies and no definitive conclusion could be drawn regarding, for example, the relationship between later cardiovascular mortality and a history of breastfeeding. Smaller studies often report a greater protective effect for breastfeeding; adjustment for confounding is a source of heterogeneity among study results. The problem is that most of these studies are observational research with differing definitions and classifications. Various factors can blur associations between breastfeeding and subsequent outcomes. Misclassifications of individuals related to the definition of exposure to breast milk or breast milk substitutes are a problem too: Are we dealing exclusively with breastfeeding or with mixed feeding of breast and formula milk? Differences in familial background (education, socio-economic status) are likely to impact on other aspects of lifestyle such as diet and patterns of physical activity. Country- and culture-dependent differences exist in low- versus high-income countries as well as with regard to influences on infant feeding practices (Broin 2011, Fall 2011). Significant changes have occurred in the composition of milk substitutes during the past 40 years. Diverse weaning practices could also play a role.

### Allergy prevention

In full-term infants, a significant 42% reduction in the risk of atopic dermatitis (95% CI 8% to 59%) was reported in children with a family history of atopy who were exclusively breastfed for at least three months compared with those who were breastfed for fewer than three months. The relationship between breastfeeding and the risk of asthma in older children and adolescents remains unclear. A moderate protective effect of breastfeeding for at least three months was reported in subjects without a family history of asthma, with a higher effect (Gidalevich 2001, Van Oijik 2003).

“The problem is that most of the studies use different definitions and classifications.”

### Effects on inflammations and infections

Bioactive factors in human milk provide protection, promote immune development, and facilitate the development of immune tolerance and appropriate inflammatory response. Protection against gastrointestinal and respiratory infections is evident in developing countries. A review of 21 studies (Duijts 2009) conducted in industrialized countries found that all studies reported a protective dose/duration effect. Country- and culture-dependent variations exist in low- versus high-income countries as well as with regard to influences on infant feeding practices (Broin 2011, Fall 2011). Significant changes have occurred in the composition of milk substitutes during the past 40 years. Diverse weaning practices could also play a role.

### Cognitive development

Some studies reported better scholastic performance in late adolescence or young adulthood by breastfed subjects. When full-term infants in developed countries were studied with specific adjustment for maternal intelligence, little or no evidence could be found for a correlation between breastfeeding in infancy and cognitive performance in childhood. Beneficial effects may be more pronounced in preterm and growth-restricted infants. However, a recent systematic review confirmed the positive correlation between breastfeeding and IQ later in life (Horta 2013, Chart 2).

Brain development could also be affected by other factors, e.g. the quality of the weaning diet. Higher full-scale and verbal IQ, and better memory performance, were found in children who had been nourished at 6 and 12 months according to an “infant guidelines” dietary pattern with high consumption of fruit, vegetables and foods prepared at home (Gale 2011, Fisk 2011).

### Overweight and obesity

The association between breastfeeding and obesity remains debatable. The magnitude of the correlation decreases when all confounders are factored into the analyses. A 4% reduction in the risk of becoming overweight in adult life has been reported for each additional month of breastfeeding during infancy. However, cautious interpretation is required because of the possibility of residual confounding factors. Most studies were performed in high-income countries, where duration of breastfeeding was lengthier in families with parents who were more highly educated and earned higher income (Chart 3).

Consideration of only 16 studies (Horta 2013) that included a large number of subjects and that were controlled for confounding by socioeconomic, birthweight or gestational age and parental anthropometry yielded a pooled OR = 0.88 [95% CI: 0.83;0.93]. Higher-quality studies suggest a small reduction in children, which is significant at a population level.

### Hyperlipidaemia and type-2-Diabetes

High blood pressure, high total cholesterol and type-2 diabetes are related to an increased risk of cardiovascular diseases. Serum cholesterol is associated with the risk of ischemic heart disease. There is high cholesterol content in human milk. Higher intakes of cholesterol in infancy down-regulate hepatic hydroxymethyl glutaryl coenzyme A (HMG-CoA) and reduce cholesterol synthesis, but no significant difference in serum total cholesterol was found between breastfed infants and the others. Neither was there a long-term programming effect of breastfeeding on blood lipids. Type-2 diabetes could possibly be programmed early in life (Owen 2004). A small number (10) of high-quality studies (Horta 2013) show different effects depending on the age of the subjects. A protective effect is particularly evident among adolescents.

### Conclusions

- **Breastfeeding** has a global beneficial effect on health of mothers and infants.
- **Short-term positive effects** are well known, but there are also significant health benefits during adulthood.
- For some of these effects, further investigations are needed to determine whether the benefits differ for specific subpopulations.
Growth and Nutrition

Protein – a driver of human growth

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Proteins are crucially important for every living organism and serve as fundamental components of cell structure and function. Accordingly more publications in the field of infant nutrition have addressed proteins than any other nutrient. Research and clinical interest on protein supply in infants have traditionally focused on the risks of deficiency and their prevention and treatment. Since a protein supply below requirements has adverse effects on growth and development, traditionally generous intakes of protein have been promoted in infancy. But the concept of early infancy was considered might not always be true. We followed the hypothesis that an excessive protein intake during the first year of life is associated with rapid growth in infancy as well as an increased risk of obesity and associated disorders later in life, the “Early Protein Hypothesis”.

Numerous observational studies demonstrated that high rates of weight gain during infancy and the second year of life are associated with a significantly increased likelihood of obesity in childhood, adolescence and adulthood (Brandt, 2012). Based on observational studies in the USA, Stettrup proposed that rapid weight gain in infancy would explain 30% of the population’s obesity risk (Stettrup, 2003). These observations lead to the conclusion that modification of high early weight gain might be beneficial for prevention of later obesity.

Preventing rapid growth

A model for modulators of human growth was proposed by Karlberg in the 1980s, the Infancy-Childhood-Puberty (ICP) model of growth. He proposed that childhood growth occurs in three distinct phases with overlapping effects of three key regulating factors. During childhood, growth is regulated by human growth hormone whose action is mediated through the formation of insulin-like growth factor IGF-1. The puberal growth spurt is induced by the additional anabolic effects of sex hormones. In contrast, the rapid growth in infancy is stimulated by the supply of nutrients that stimulate the secretion of the growth factors insulin and IGF-1 (Chart 1).

Early protein hypothesis

Dietary carbohydrate intakes and blood glucose levels affect insulin secretion. Also protein exerts a strong effect on the regulation of the growth factors, which has been well documented in human and experimental studies. Amino acids stimulate the mTOR pathway. In experimental models, the maximal stimulating effect on mTORC1 can only be achieved by the simultaneous action of growth factors along with amino acids. Breastfeeding supplies less protein than conventionally used infant formulae. This is associated with a slightly different growth in breastfed and bottle-fed infants reported in many studies. At the end of the first year of life, infants who have been predominantly breastfed are significantly leaner than infants predominantly fed with conventional infant formula. Recent data indicate that breastfeeding in infancy is also associated with less body fat deposition at school age. In line with these data, numerous observational cohort studies and several meta-analyses found breastfeeding linked to a modest but consistent risk reduction for obesity, even after adjustment for relevant confounding factors. The protective effect size reported in meta-analyses is about 15–20%, which would represent a major public health benefit on a population-wide level. This would provide another strong argument for enhancing our efforts to support, promote and protect breastfeeding.

While infants cannot be ethically randomized to breast or formula feeding to establish firm evidence for a causal preventive effect of breastfeeding, it is possible to test the proposed underlying factor of reduced protein supply with breastmilk in a randomized study using infant formula with different protein content. An excess protein intake in formula-fed infants might increase the plasma and tissue concentrations of insulin-releasing amino acids, the levels of insulin and IGF-1, and thereby increase early weight gain and later obesity (Chart 2).

The Child Obesity Project trial

The Child Obesity Project (CHOP) trial is a double-blind, randomized, multicentre-intervention study that enrolled 1,678 healthy infants born at term in five European countries (Belgium, Germany, Italy, Poland and Spain). The intervention was provision of infant formula and subsequently follow-on formula with either higher or lower protein content for the duration of the first year of life. Of importance, the energy density of the paired products was the same, which was achieved by slight adjustments of the total fat content. A reference group included breastfed babies who were fully breastfed for at least three months (Chart 3).

Our hypothesis was confirmed with regard to effects on the growth factors: plasma IGF-1 and C-peptide excretion in urine were significantly lower in the low compared to the high protein groups, and some metabolites almost albeit not identical to levels found in breastfed infants. Weight for length at two years was significantly different between feeding groups. At two years, children fed higher protein formula during the first year of life were significantly heavier than previously breastfed children. However, children fed the lower protein formula in infancy reached the same weight at two years as the breastfed group and thus were protected from excessive weight gain. IGF-1 levels in infancy predicted body weight at six months and at two years. Follow-up of the children at early school age showed a persisting difference (unpublished data).

Reducing protein in infant feeding is relatively easy to accomplish, inexpensive, and requires no lifestyle changes, while it can have a sizeable effect on preventing subsequent obesity. Therefore, we believe it is prudent to avoid feeding an excessively high supply of protein during the first year of life by promoting breastfeeding, by avoiding cow’s milk as a drink during the first year of life because cow’s milk has a very high protein content, and by choosing infant and follow-on formula with limited amounts of protein but high protein quality (Chart 3). Many questions remain to be resolved. These include, for example, the extent of persistence of protective effect with increasing age, the causes and impact of observed gender differences in the IGF-response, the interaction of postnatal diet with parental and prenatal influences, and the identification of potential particularly susceptible subgroups defined by genotypes or other predictors, the regulatory role or further dietary components and metabolites, the definition of particularly sensitive age windows for preventive interventions, and further clarification of underlying mechanisms.

Conclusions

Breastfeeding: Actively promote, protect and support breastfeeding which reduces later obesity risk.

Avoid excessively high protein supply to normalize early weight gain and to reduce the later risk of obesity.

For infants that are not (fully) breastfed, choose an infant formula with a relatively low protein content but high protein quality.

Do not provide cow’s milk as a drink which provides far more protein that breast milk or modern infant formulae.

The infant formula with lower protein content was significantly lighter at two years of age than the conventional infant formula.

Weight gain and associated disorders

Enhanced secretion of insulin & IGF-1

High protein supply

Adipogenic activity

Anabolism and growth

Proteins

Blood glucose & amino acids uptake

Insulin receptor

Human growth hormone

Insulin like growth factor (IGF-1)

Insulin

Energy

Protein

Fat

Calcium
How can we reduce protein intake in infancy, especially in late infancy? A reduction would be desirable because we believe that by doing so, we may help infants avoid obesity later in life. Breastfeeding, and the longer the better, is a very easy answer. But for various reasons, not all of which are known, many women opt to feed their infants with formula. Rapid growth in infancy is associated with the risk of overweight and obesity in later life. It is plausible, but not proven, that slowing growth in infancy may reduce the risk of later obesity.

So my topic is how to reduce the amount of protein in formula to the lowest level that is still adequate. One important finding of two studies is that the protein content of a new low-protein formula is sufficient to support growth in infancy. The breastfed infant’s intake is on the low side. This makes evolutionary sense because the mother has to preserve as much protein as she can. It would be disadvantageous for her to feed her baby more protein than the infant really needs.

The graph for formula-fed infants is quite different. The protein content of the typical formula in the USA is 2.15. So formulae satisfy the highest needs during the first months of life. In the USA, we have only one type of formula that is used for the baby throughout the entire first year of life. As is clearly shown by the chart, from the second month and progressively thereafter, the baby ingests excess protein (Chart 2).

**High protein intake**

How much protein do infants actually receive? We have a number of studies, for example, the German DONALD study. Other studies show a similar range. The protein intake that babies receive after six months is always considerably higher than this upper limit, which already satisfies the needs of 97% of infants.

So we can say that in the later part of the first year of life, all infants receive more protein than they need. What could be the reason for this? Is it because the mothers think that their infants need protein? Or could it also be that babies crave more protein? Perhaps babies are just like most children and adults: we all eat more protein than we really need. Could it be that babies prefer protein and are somehow able to express this preference?

Where did the protein come from? Its sources are high-protein formula, cow’s milk, and (to some extent) meat.

**Low protein intake**

What about low protein? Nestlé conducted a study in Chile with children born to mothers with a BMI greater than 25. Beginning at three months, the babies were randomized to be fed formulæ lower or higher in protein content. Breastfed babies were in the reference group. Babies were fed formula until 12 months; a follow-up examination was undertaken at 24 months. Chart 3 shows the protein content of the two formulæ in relation to the protein requirements.

The weight gain between three and six months was significantly lower for babies who were fed the lower-protein formulæ. The difference in weight between the two formulæ groups became progressively greater and reached 517 g at age two years – and the intervention ended at 12 months! So the effect continued after the intervention, as it did in the European obesity study. Branched-chain amino acids and IGF-1 levels were significantly higher at six months in babies fed the higher-protein formulæ. Could it be that babies of the most obese mothers are affected differently? We found that if the mother’s BMI was greater than 30 (as it was for ca. 40% of the mothers), the difference in weight gain due to low protein compared to high protein was much larger. Nearly all the difference in weight gain for the group occurred in babies whose mothers had a high BMI. Babies who were above the 75th percentile for weight at three months were much more strongly affected by the low protein – they were, in fact, the only ones who showed an effect of formula protein content. It is thus clear that the low-protein formulæ almost exclusively decelerated weight gain only in babies who are born to overweight mothers and who are big themselves (Chart 4).

The low-protein formulæ was safe for all babies aged three months and older.

How is high protein intake linked to later adiposity? I speculate: High protein intake stimulates food (= energy) intake.

**Pickwick study Chile – summary**

1. Formula with low protein content (1.65 g/100 kcal) supports normal growth.
2. Low-protein formulæ slows growth compared to higher protein formulæ.
3. Low-protein formulæ slows growth especially in large infants and infants of overweight mothers, who are at increased risk of later obesity.

**Conclusions**

- High protein intake in infancy is linked to obesity in childhood.
- Formula with lower protein content changes serum markers toward those of breastfed infants.
- Formula with lower protein content decelerates weight gain among infants of overweight and obese mothers.
- Low-protein formulæ predominantly slows the growth of large and/or fast-growing infants.
Special proteins in infant nutrition

Protein supply for preterm babies and healthy growth

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Feeding preterm infants has always been described as challenging, and nutritional intakes are frequently below recommended levels. Concomitantly, extraterine growth restriction is also frequently observed and has been correlated with poor nutritional support. Several studies of preterm infants have shown that the quantity and quality of nutrition may significantly affect long-term growth, brain structure, neurocognitive function, and metabolic status. It is therefore crucially important to help premature babies develop and grow adequately taking into account that the growth rate during the third trimester is completely different from the rate during the first months of life (Chart 1).

Requirements for preterm infants

Intrauterine fetal growth represents the current model for postnatal growth in preterm infants. Nutritional requirements of preterm infants are derived from studies of nutrient accretion by fetuses and metabolic balance. Weight gain may not be solely used to evaluate postnatal growth because the gain of lean body mass is not the only factor to be considered; these practices were associated with insufficient growth velocity in the first month of life (Martin, Pediatrics 2009). Human milk must be fortified in order to meet the high nutritional requirements of preterm infants. Additionally, a survey in developed countries in different parts of the world recently demonstrated that many neonatologists wait a long time before fortifying human milk (Klingenberg, ADC-FNIN 2011). Moreover, classical human milk fortification does not take into account the high variation of human milk content, especially the variation in protein and fat content. Parenteral nutrition is considered critical for a period of about 3–4 days and during the perinatal period, the human milk is used as a bridge before starting parenteral nutrition. Parenteral nutrition is critical in the first days of life, corresponding to the physiological postnatal adaptation of body composition (i.e. extracellular water loss corresponding to 5–10% of birth weight). Protein and energy intakes should be increased during the first days of life, corresponding to the physiological postnatal adaptation of body composition (i.e. extracellular water loss corresponding to 5–10% of birth weight). Protein and energy intakes should be increased during the first days of life, corresponding to the physiological postnatal adaptation of body composition (i.e. extracellular water loss corresponding to 5–10% of birth weight).

Nutritional deficits

Current practice tries to improve nutritional support, but most studies still report intakes that remain below recommendations. A large recent European survey conducted in Germany, France, Italy, and the UK demonstrates that the intention to initiate parenteral nutrition practices is still frequently not in accordance with current European recommendations in the neonatal intensive care units that respond to the survey (74% of the units in the four countries). Indeed, it revealed that only 60% of the responding neonatologists begin feeding protein on the first day of life; only 40% start with ≥1.5 g/kg/d; and only 40% start with 1 g/kg/d (Lapillonne, BMJ open 2013). Intakes on enteral nutrition are also frequently below recommendations. In another large recent American survey, intakes were close to recommendations but did not quite meet them; these practices were associated with insufficient growth velocity in the first month of life (Martin, Pediatrics 2009). Human milk must be fortified in order to meet the high nutritional requirements of preterm infants. Additionally, a survey in developed countries in different parts of the world recently demonstrated that many neonatologists wait a long time before fortifying human milk (Klingenberg, ADC-FNIN 2011). Moreover, classical human milk fortification does not take into account the high variation of human milk content, especially the variation in protein and fat content. Parenteral nutrition is critical in the first days of life, corresponding to the physiological postnatal adaptation of body composition (i.e. extracellular water loss corresponding to 5–10% of birth weight). Protein and energy intakes should be increased during the first days of life, corresponding to the physiological postnatal adaptation of body composition (i.e. extracellular water loss corresponding to 5–10% of birth weight). Protein and energy intakes should be increased during the first days of life, corresponding to the physiological postnatal adaptation of body composition (i.e. extracellular water loss corresponding to 5–10% of birth weight). Protein and energy intakes should be increased during the first days of life, corresponding to the physiological postnatal adaptation of body composition (i.e. extracellular water loss corresponding to 5–10% of birth weight).

Neurodevelopmental outcome

Adolescents who were born very preterm are shorter and frequently have smaller head circumference and decreased whole brain volume, grey matter volume and hippocampal volume than term infants (Nosarti, Brain 2002). It has also been shown that neurodevelopmental outcomes are significantly influenced by the course of postnatal growth, with a major adverse impact of extraterine growth restriction (Lalit-Hajnal, J Pediatrics 2003). Brief nutritional intervention during a few weeks may have a significant impact on brain structure. In an interventional randomized study in preterm infants, caudate volume was influenced by early nutrition and related selectively to verbal IQ in males (Iolasco, Pediatrics 2008).

Two recent observational studies suggest that the first week of life is also crucial. This implies that any insufficiency or delay in initiating early nutritional support may be deleterious and may contribute to postnatal morbidity and to impaired mental and psychomotor development. In 2011, Shenkarzak et al. (Pediat Res) suggested that the severity of critical illnesses such as bronchopulmonary dysplasia and sepsis in the first weeks of life and later growth was associated with the amount of early nutritional support provided during the first week. In 2008, Stephens et al. (Pediatrics) estimated that increasing protein intake by 1 g/kg per day and boosting energy intakes by 10 kcal/kg per day during the first week of life might increase mental development index at 18 months by 8.6 and 4.2 points respectively. Most recent studies with increased nutritional intakes in VLBW infants have demonstrated positive nitrogen balances, increased protein synthesis, and improved growth without significant side effects. Current guidelines which include this approach should no longer be considered “aggressive” but are, in fact, optimal and recommended.

Conclusions

Despite current recommendations, insufficient nutritional supply and postnatal growth restriction are still commonplace today. Nowadays, such practices may be considered as iatrogenic malnutrition because they are clearly associated with adverse developmental outcomes. High protein supply should be promoted in preterm infants from the first day of life and throughout postnatal hospitalization to enhance anabolism and lean body mass accretion. Under-nutrition and postnatal growth restriction are evitable. Breastfeeding should be promoted. Further studies, including long-term studies, are needed to improve nutritional offers.

Protein intake and growth

We recently published an optimized nutritional policy for infants with birthweights below 1,250 g. This policy includes the use of a unique standard parenteral nutrition solution, which is ready to use and available 24/7. The regimen was simple and easy to understand and implement by the residents and physicians on the ward. The regimen began with 44 kcal/kg and energy and amino acids at 2.4 g/kg, then progressively increased to 120 kcal/kg/d and 4.1 g/kg of amino acids within the first week of life. Weight loss was observed in these infants during the first three to four days of life, corresponding to the physiological postnatal adaptation of body composition (i.e. extracellular water loss corresponding to 5–10% of birth weight). Postnatal weight gain occurred afterwards and birthweight was regained after 7–8 days on the average. This growth pattern might be considered optimal because it is similar to the one observed in term infants. Weight z-score evolution declined during the first 3–4 days with the physiological weight loss, but afterwards ceased to decrease, which means that weight gain was similar to fetal weight gain.

Metabolic tolerance

Very few biological anomalies were observed during this study. Blood urea increased during the first three to four days of life, decreased during the next three to four days, and stabilized during the second week of life. The variation of urea according to protein intake in different periods during the first two weeks of life found no positive correlation between protein intake and urea level. In fact, urea was mainly dependent on creatinine and body hydration. Blood urea elevation was quite similar to other studies with high protein intakes from the first day of life and reflects adequate protein oxidation, hydration and renal immaturity.

Although metabolic acidosis has been associated with high protein intakes in the past, the initial postnatal base deficit rapidly disappeared during the first week of life and no correlation between base deficit and protein intake was observed. Only 30% of infants develop metabolic acidosis (base deficit >5 mmol/l) after three days of life, and only 2% develop severe metabolic acidosis (base deficit >10 mmol/l). This observation is quite different from what was recently reported by Kermovant et al. (LIPIDN 2012), who noted frequent progressive and severe metabolic acidosis during the first week of life with important anomalies in electrolytes plasma concentrations.

Ninety percent of infants born before 34 postmenstrual weeks were found to have a base deficit >5 mmol/l during the first week of life (isocaloric 1.5 g/kg/d, Prot: 3.6 g/kg/d, 3.21 g/100 kcal (N=85). The physiological weight loss, but afterwards ceased to decrease, which means that weight gain was similar to fetal weight gain.

This observation is quite different from what was recently reported by Kermovant et al.
The role of hydrolysed protein in infant nutrition

The author
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The rationale for the use of cow’s milk protein hydrolysate infant formulas to prevent allergy is that familial atopy increases allergy risk for the offspring up to 80%. Early allergen exposure is a risk factor for later allergies. The oral antigen load in non-breastfed vs. breastfed infants is 10 times higher due to the intact cow’s milk protein in conventional cow’s milk-based infant formula. So the arms are reduced antigenicity of the formula protein and possibly induction of oral tolerance. But how much or how little reduction is best? Depending on the degree of hydrolyzation, we have partially hydrolyzed (pHF) and extensively hydrolyzed (eHF) formulas (Chart 1).

A total of 2,252 high-risk children were recruited for the prospective, randomized GINI study (German Infant Nutritional Intervention Study), which was double-blind until three years of age. The intervention was with three different hydrolysed formulas vs. regular cow’s milk formula: pHF-whey (pHF-W – Nestlé Beba HA), eHF-whey (eHF-W – HIPP HA/ Nutricia Pepti) and eHF-casein (eHF-C – Mead-Johnson Nutra- migen). The protocol included feeding recommendations for at least four months of breastfeeding and/or only randomized study formula. Accordingly, the atopic history and the degree of hydrolyzation was almost parallel to the protein whey or casein. We also saw no effect on asthma. When we looked at sensitization of her’s egg, cow’s milk and aeroallergen at three years, the only effect was a significant reduction to cow’s milk allergen with the pHF-W. This could be interpreted as oral tolerance induction.

The children were followed by modified ISAAC questionnaires at one, two, three, four, six and ten years of age and by physical examinations at regular intervals. The outcomes were parent-reported physician-diagnosed atopic diseases (atopic dermatitis (AD) and allergic manifestations: AM: AD, urticaria, food allergy, asthma, hay fever/allergic rhinitis). Chart 3 maps the evolution of atopic eczema until ten years. It clearly shows that the increase in the study formulas was almost parallel after the age of one year. This means there was no rebound effect, which I regard as a very important finding.

We performed three different analyses for the ten-year analysis:

1. Intention-to-treat (ITT): All primarily randomized children (n=2,252)
2. Modified intention-to-treat (mITT): All children excluding those who did not receive any study formula during the first six months of life because they were exclusively breastfed (n=1,615)
3. Per protocol (PP): All infants fed (fully or partially) with study formula within the first four months, who were compliant with the study protocol (n=988)

The PP analysis for the cumulative incidence of physician’s diagnosis of atopic eczema shows a significant reduction at three and six years. The effect in the formula groups persists almost the same at ten years. The weakest effect is in the ITT analysis: this is because it includes all the dropouts, noncompliant and exclusively breastfed infants. When we excluded the breastfed children in the modified ITT analysis (mITT), the results came quite close to the PP analysis. This means that breastfeeding had obviously contributed to the lesser effect in the ITT population (Chart 4).

The GI宁 study

The results after three years showed that the effects develop in the first year and that the incidence becomes similar in the four study groups after the first year. Two formulas – the pHF-W and the eHF-C – developed a significant effect in the first year (Chart 2). These results were not actually new; they confirmed earlier studies. It was surprising, however, to find that the eHF-W formula had no effect. Because pHF-W and eHF-C were effective, but not the eHF-W, means that the effect depends neither on the degree of hydrolyzation nor on the protein whey or casein. We also saw no effect on asthma. When we looked at sensitization of her’s egg, cow’s milk and aeroallergen at three years, the only effect was a significant reduction to cow’s milk allergen with the pHF-W. This could be interpreted as oral tolerance induction.

Long-term effects
To answer questions regarding a long-term effect of the formulas, we followed the children up to six and ten years.

Are there long-term effects?

1. On eczema: cumulative incidence, prevalence, rebound phenomenon?
2. On respiratory allergies: asthma and allergic rhinitis?
3. On sensitization?
4. On growth?

The children were followed by modified ISAAC questionnaires at one, two, three, four, six and ten years of age and by physical examinations at regular intervals. The outcomes were parent-reported physician-diagnosed atopic diseases (atopic dermatitis (AD) and allergic manifestations: AM: AD, urticaria, food allergy, asthma, hay fever/allergic rhinitis). Chart 3 maps the evolution of atopic eczema until ten years. It clearly shows that the increase in the study formulas was almost parallel after the age of one year. This means there was no rebound effect, which I regard as a very important finding.

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Asthma and allergic rhinitis
At six years, there was no effect on asthma and on symptoms of asthma, i.e. wheezing. At ten years, there was a slight but insignificant increase in asthma in the ITT analysis. But we know that at school age, asthma may be higher in formerly breastfed children. In the mITT analysis – with the excluded breastfed children – we had very similar results to the PP analysis.

Cost-effectiveness
We analyzed the cost-effectiveness of infant hydrolysate formulas to prevent atopic dermatitis in children. Based on the data from the GI宁 study up to six years, we looked at the costs for the insurance and at the social costs. In the first year, the eHF-C and the pHF-W were cost-effective, at six years, both were even cost-saving, with a better effect for the pHF-W.

Conclusions

Cows’ milk protein hydrolysate infant formulas are effective in long-term prevention of eczema, but not of asthma or allergic rhinitis, nor on sensitization in high-risk children.

Differences in the magnitude of the effect seem not (solely) dependent on the degree of hydrolysation or on the protein source, but rather on the hydrolysing process itself.

For clinical use, only formulas with a documented effect should be used.

Which impact other components of the formulas (such as lipid composition or probiotics or changing of protein concentrations) have on the observed effect is not known.

Dietary prevention by the oral route with hydrolysated formulas is restricted to eczema in the GI宁 study. Other approaches are necessary for the prevention of respiratory allergies.
There is a marked increase in protein content in the diet during the complementary feeding period; from about 5 energy percentage (% E) in breastmilk to a content in the family diet of about 15 %. Typically 10–15 % in low-income countries and 12–18 % in high-income countries. There is increasing evidence that if this transition in protein intake deviates from recommended levels, it can have negative effects on growth, body composition and later risk of non-communicable diseases (NCD).

Effects on linear growth and IGF-I

When we examined linear growth and IGF-I, we found strong evidence that cow’s milk stimulates linear growth. We deduce that milk stimulates growth, even in situations where the nutrition intake is adequate. In one study, we gave healthy 8-year-old boys 1.5 lines of skim milk or 250 grams of meat per day, but only for one week. We wanted to see if we would find an acute effect in IGF-I and insulin. Milk significantly increased both IGF-I and insulin; meat had no effect (Chart 2).

Our SKOT cohort exhibits highly significant effects of breastfeeding. The more time per day you were breastfed, the lower your IGF-I level was (Chart 3).

We recently completed a study in Cambodia, where we randomized children to different complementary blended foods which contained milk, small dried powdered fish or dried powdered spiders. This is acceptable in Cambodia, where the nutritive value of spiders is appreciated. The results have not yet been published. Small fish are the world’s least expensive animal-based foods. The results have not yet been published. Small fish are the world’s least expensive animal-based foods.

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There is a dramatic change in fat intake from breastfeeding with 52 fat % to a family diet with 25–30 fat %. Agostini wrote for the same WHO meeting a review on the role of fats in the first two years of life as related to later development of NCDs. He concluded that there is no evidence of any convincing correlation between fat intake during the period of 6 to 24 months and later indices of adiposity.

Scientists have considered breastfeeding to be part of a healthy diet in early life. There are few studies that examine the effects of breastfeeding on growth and development. A recent systematic review by Gale et al. (2012) includes 15 studies on the difference in fat mass percentage between breastfed and formula-fed infants until 3 years. Breastfed babies gain more fat, and for the last six months they gain more lean mass. There is a pattern here: we assume that the pattern of the breastfed infants is the optimal one, but much more research remains to be done on the effect of diet and protein intake on body composition and the long-term effects of different patterns of body composition in early life.

Conclusions

All infants in high-income countries have sufficient protein intake during their first two years.

Protein intake during the complementary feeding period is very high among some children, mainly due to a high intake of cow’s milk.

A high protein intake (above 15 PE% or 5 g/kg/day) is likely to increase the risk of obesity and some risk markers for NCD during childhood and may also have an effect in adults.

Glutamine and glutamate

Agostini measured free amino acids in breastmilk and found that most FAA were at the same level, except for glutamine and glutamate. FAA increased dramatically during the three months when he examined them (Chart 4). These data suggest that the glutamate content in breastmilk could have an appetite-regulating effect. A recent systematic review by Gale et al. (2012) includes 15 studies on the difference in fat mass percentage between breastfed and formula-fed infants until 3 years. Breastfed babies gain more fat, and for the last six months they gain more lean mass. There is a pattern here: we assume that the pattern of the breastfed infants is the optimal one, but much more research remains to be done on the effect of diet and protein intake on body composition and the long-term effects of different patterns of body composition in early life.

Protein quality – free amino acids

I was recently asked at a meeting in Vietnam: “Is monosodium glutamate okay for babies?” Ventura, Beauchamp and Mennella have studied this question. Glutamate is a key signal for satiety in adults, it gives the umami taste, it is a neurotransmitter in the brain and is the most abundant FAA in breastmilk.

In a study, they found that infants eat less when they were fed hydrolysed protein formula with added glutamate, suggesting it has an appetite-regulating effect. A new study by Rolland-Cachera further strengthens this hypothesis. She examined the association between fat intake at age two and body composition at age 20, and found a negative correlation. She proposes a “low-fat programming” hypothesis: a restriction on dietary fat in early life could decrease serum leptin concentration, increase suscepti- bility to becoming overweight and reduce the risk of metabolic diseases later in life; it could also lead to leptin resistance later in life.

Protein intake – recommendations

At six months, the mean protein requirement is equal to ca. 5.6 %, based on the require- ments per kilo of body weight and the average energy requirements from the WHO’s 2007 publication, and it continues to increase up to around 3.8 (safe level 5.2) to 2.5 years. So the requirements are low.

The protein energy percentages are 56 % for breastmilk; 7–8% for infant formula, 15–20% for family food, 20% for whole cow’s milk, and 38% for skimmed milk. This is one reason why we shouldn’t recommend skimmed milk for young children. Meat has 30–60% protein, but infants don’t eat very much meat.

One study, compiled by Roland-Cachera (1999), examines protein intake in late infancy in differ- ent countries (Chart 1). I would like to point out the 90–95% percentile in Danish and Italian studies with 6–7 g/kg. So these infants ingest 6 to 7 times more protein than they need.

Protein intake in late infancy – Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>Protein intake: g/kg</th>
<th>Protein energy: % E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden (12 mo)</td>
<td>6.4</td>
<td>15.6</td>
</tr>
<tr>
<td>Belgium (12-24 mo)</td>
<td>4.6</td>
<td>15.6</td>
</tr>
<tr>
<td>Italy (12-18 mo)</td>
<td>4.1</td>
<td>15.6</td>
</tr>
<tr>
<td>France (10 mo)</td>
<td>5.6</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Protein intake during the complementary feeding period is very high among some children, mainly due to a high intake of cow’s milk.

A high protein intake (above 15 PE% or 5 g/kg/day) is likely to increase the risk of obesity and some risk markers for NCD during childhood and may also have an effect in adults.

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The role of intestinal flora in early infancy

Effects of probiotics in early infant nutrition

The author

Hanna Szażewska
Department of Paediatrics
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Infant formula with probiotics

In 2011, the Committee on Nutrition of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) evaluated the use of probiotics in pre- and infant formulas. It was considered that there is some evidence related to the addition of probiotic bacteria and prebiotics to infant formulas. The administration of probiotics often begins in early infancy, sometimes at birth, when the gut microbiota is not fully established. Factors that influence microbiota may persistently affect the development of the ecosystem. The second issue is the duration: the daily administration of such products is often prolonged for several weeks or even months. Delivery is in the form of very specific matrix (infant formula) that could be the only source of food for an infant. An initial issue is the persistence of the effect: beneficial effects, as well as unfavorable ones, can be seen years after the administration of probiotics.

Part of this ESPGHAN document comprises a systematic review of 20 RCTs evaluating the effects of the administration of infant formula supplemented with pro- and prebiotics to healthy infants. The Committee focused on probiotics and prebiotics supplementation during the manufacturing process. The Committee looked at outcomes such as growth, clinically relevant outcomes, and adverse events. The Committee considered two different types of administration: administration in the first month of life and administration beyond early infancy (Chart 1).

### Prevention of NEC

Between 35 and 50% of preterm infants with necrotizing enterocolitis (NEC) require surgery and up to 30% of them may die; the survivors have an increased risk of adverse neurodevelopmental sequelae. There are an increasing number of RCTs evaluating the effect of probiotics pre- and postnatal care. NEC is the most common gastrointestinal disease in newborns, and there is a need to prevent its occurrence. The prevention of NEC is a challenging task, and the use of probiotics is considered to be a promising strategy. The use of probiotics in the prevention of NEC has been studied in several RCTs. The results of these studies are conflicting, and more research is needed to determine the efficacy of probiotics in preventing NEC.

### Main conclusions from ESPGHAN

**Safety:** Administration of currently evaluated probiotic-supplemented formula to healthy infants does not raise safety concerns with regard to growth and adverse effects.

**Routine use:** There is insufficient data to recommend the routine use of probiotic-supplemented formulae.

**Research:** The supplementation of formula with pro/prebiotics is an important field of research. There is a need for well-designed and carefully conducted RCTs.

### Alteration of gut microbiota

- **Infants with colic compared to controls:**
  - C. difficile
  - Escherichia coli species
  - Lactobacillus species
  - Certain Lactobacillus strains predominate in infants with colic
  - Certain Bifidobacterium and Lactobacillus species are protective against crying

**Infantile colic**

The etiology of infantile colic is unclear and it may not have an abdominal cause. Proposed etiology and risk factors are an extreme of the spectrum of normal crying, manifestation of underlying physiological or psychosocial factors, GER, food allergy and especially an alteration of gut microbiota.

The differences between colic and control microbiota in the first month of life show a reduced diversity in the colic group compared to a higher diversity in the control group. There were no significant differences at an age of about three to four months, when colic usually disappears.

The control infants showed a higher stability in the intestinal microbiota.

The authors’ suggestions were that the results offer opportunities for early diagnosis and for developing specific therapies.

Savino conducted two studies with Lactobacillus reuteri ATCC 65793 (Savino et al., 2007) and L. reuteri DSM 17 938 (Savino et al., 2010). The results of the latter study showed a reduction of crying time and a higher percentage of responders, defined by a 50% reduction in crying time from baseline.

The following conclusions were drawn:

- Not all probiotics are equal
- Quiet nights are more likely
- Difficult to withhold from

### The crying time was significantly reduced in the probiotic group compared with the placebo group throughout the study period (Chart 3).

There was also a significant reduction in the parental perception of colic severity for parents of infants in the probiotic group compared with the placebo group. In addition, VAS scores indicated improved parental/family quality of life throughout the study for parents and families of infants in the probiotic group.

Based on the results of our study, we concluded that exclusively or predominantly breastfed infants with infantile colic benefit from the administration of L. reuteri DSM 17 938 compared with placebo.

At the ESPGHAN meeting in 2013, Indrio et al. presented the results of a study on prevention of GI disorders in the first three months of life with probiotic supplementation. It was also a RCT, double-blind study of L. reuteri DSM 17 938, conducted at eight centers in Italy with more than 500 infants, half of them breastfed and the other half formula fed (Chart 4). The authors concluded that supplementation with L. reuteri DSM 17 938 could represent a new therapeutic strategy for preventing functional gastrointestinal disorders and crying in infants.

**Take-home messages**

- Infant formula with probiotics
  - No safety concerns
- Probiotics for preventing NEC
  - Difficult to withhold from parents and infants
- Infantile colic
  - Quiet nights are more likely
- Not all probiotics are created equal
  - Each product must be evaluated separately
The role of intestinal flora in early infancy

**Mechanism of action of probiotics**

**The author**

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Generally speaking, the desirable characteristics of probiotics are preferably (but not necessarily) human origin, safety (GMPs, QMS), absence of transferable resistance to antibiotics and pathogenicity or toxicity factors, and the ability to survive in intestinal conditions (acid pH enzymes, bile salts, etc.). In particular, probiotics should have functional characteristics such as: adhesion to intestinal epithelium, antagonism against pathogens, stimulation of the immunological system, and metabolic activity beneficial to the host. Finally, they are also technological characteristics, e.g. maintenance of the probiotics' activity, viability on technological treatments, and growth efficiency.

**Characterization of probiotics**

When we characterize a probiotic strain, we consider its mechanism of action; we need to isolate it and check its resistance to low pH and bile salts. We check its adhesion to human cells and, in addition, we identify its genotype and phenotype. We have recently isolated three bacteria, each of which was considered to be unique by the Institute Pasteur: Lactobacillus paracasei CNCM I-4034, Bifidobacterium breve CNCM I-4036 and lactobacillus rhamnosus CNCM I-4036.

**Reaction with pathogens**

To understand the mechanism of action of particular bacteria, we need to know in advance what is the completion, what are the genes and the phenotype, enzymatic activity, carbohydrate fermentation, etc. The three aforementioned bacteria were isolated from fecal material of exclusively breastfed infants. When we try to identify them, we check their adhesion to the mucosa. In the complete genome expression, we found significant increases in the expression of interferon lambda 3, which is active in the maturation of interleukin 1beta, could be one of the additional mechanisms by which bacteria act.

**Effects of new probiotics**

When we examine probiotics, we consider not only the living bacteria, but also the active substances that are secreted by the bacteria and that could potentially incorporate into the living bacteria and elsewhere. We published a study on the probiotic inhibition of the three recently isolated bacteria: not all bacteria have the same activity, but some are able to decrease the growth of rotaviruses (Chart 1).

We know that commensal pathogenic microorganisms can interact not only with TLR at the cell membrane. This is just one example of how, compared to the control Lactobacillus paracasei, the presence of various bacteria such as S. typhimurium and S. typhi can result in decreased secretion of interleukin 8 and significantly decreased production of TNFalpha (Chart 2).

**The health benefit attributed to one strain is not necessarily applicable to another strain, even within the same species.**

We recently developed a system using dendritic cells from human interstratum. This is quite new because most previous studies were done with peripheral dendritic cells, which have different receptors. Intestinal dendritic cells have a very specific pattern compared to other intestinal cells. We can cultivate these dendritic cells and expose them to probiotics. This structure is rather complex, but we can nevertheless evaluate how the activities of different bacteria lead to the activation of different profile genes.

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**Conclusions**

Some of the important mechanisms underlying the antigenic effects of probiotics on various microorganisms are: modification of gut microbiota, competitive adhesion to the mucosa and epithelium, strengthening of the gut epithelial barrier, and modulation of the immune system to convey an advantage to the host.

Recent characterization of host families of pattern-recognition molecules (e.g. TLR and NOD-like receptors), as well as modulating key signaling pathways (e.g. NF-kB and MAPK) with respect to their ability to enhance or suppress activation and influence downstream pathways, will shed light on the complex interplay of host-microbe interactions and, particularly, on the GALT and immune responses.

Stimulation of these receptors by commensal bacteria plays a crucial role in eliciting measured antimicrobial responses with minimal inflammatory tissue damage.

Probiotics seem to affect the expression of some genes at the intestinal mucosa level. In particular, a number of them are small nucleolar regulatory RNAs and others are directly related to adaptive immunity and regulators of G-protein signaling.
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