The Importance of Immunonutrition

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Foreword

Nutrients have a tremendous potential to modulate the actions of the immune system, a fact which has a significant impact on public health and clinical practice.

The concept of pharmaconutrition – a central element of intensive care management – implies a bridge between drugs and nutrition. During the last decade, the role of nutrition, beyond providing the calories and the macro- and micronutrients for survival, has been well established and clinically proven. At the 77th Nestlé Nutrition Institute Workshop held from October 28th to November 1st 2012, world experts gathered in Panama City to present their latest findings on how nutrient status can modulate immunity and improve health conditions in pediatric patients. The 3 sessions of this workshop covered major aspects of the interplay between nutrients and the regulation of immunity and inflammatory processes.

The first session explored the pharmaceutical value of specific amino acids (arginine and glutamine) and hormones for addressing immune disorders and infant development. It is now understood that some amino acids have the ability to speed up the recovery of children admitted to intensive care. We took a closer look at the relationship between arginine metabolism and asthma, the role of this amino acid in T-lymphocyte function, and investigated the rationale for glutamine supplementation to improve outcomes in premature infants.

Many immune disorders and diseases are associated with dysregulation of the gut microbial homeostasis. The second session revolved around gut function and immunity, and the right balance of probiotics. The right microbiome can modulate the immune system and help protect from infectious disease, obesity and allergy. Getting the right mix of probiotics is key to unlocking their full benefits. The overview of the MetaHIT project presented during this session showed that individuals can be clustered based on their microbial metagenome profile, thus laying the framework for profiling health and disease.

The third session explored the role of lipid mediators and how their types and proportions can tip the balance in favor of health or disease. Given in the right time and conditions, lipids can prevent allergy, modulate the inflammatory process in the gut and play a protective role when cell homeostasis is threatened by neurodegeneration. It was discussed that
early LC-PUFA supplementation not only supports cognitive function but also may program brain development in later life stages.

We wish to thank the three chairpersons – Prof. M. Makrides, Prof. J. Ochoa and Prof. H. Szajewska for establishing an excellent scientific workshop program. We are also indebted to the renowned speakers who have further debated and increased our understanding of this important topic through their presentations and participation. We thank the many experts who came from across the globe to review and discuss the importance of immunonutrition.

Finally, we wish to thank and congratulate Luis Carlos Delgado and his team from Nestlé Nutrition LATAM for their excellent logistical support and hospitality that allowed us to not only enjoy the scientific program but also experience the historical spirit of Panama City.

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Arginine and Asthma

Claudia R. Morris

Recent studies suggest that alterations of the arginine metabolome and a dysregulation of nitric oxide (NO) homeostasis play a role in the pathogenesis of asthma. L-Arginine, a semi-essential amino acid is a common substrate for both the arginase and NO synthase (NOS) enzyme families. NO is an important vasodilator of the bronchial circulation, with both bronchodilatory and anti-inflammatory properties, and is synthesized from oxidation of its obligate substrate L-arginine, which is catalyzed by a family of NOS enzymes. Arginase is an essential enzyme in the urea cycle, responsible for the conversion of arginine to ornithine and urea. The NOS and arginase enzymes can be expressed simultaneously under a wide variety of inflammatory conditions, resulting in competition for their common substrate (fig. 1) [1].

Accumulating data show that low L-arginine bioavailability contributes to inflammation, airway hyperresponsiveness and remodeling of the asthmatic airway. Arginase plays an important role in this paradigm. Through substrate competition, arginase decreases bioavailability of L-arginine for NOS, thereby limiting NO production with subsequent effects on airway tone and inflammation. Arginine depletion may also contribute to NOS dysfunction by inducing the uncoupling of NOS and the formation of the proinflammatory oxidant peroxynitrite in the airways, further adding to the asthmatic milieu of oxidative stress. Finally, arginase can contribute to chronic airway remodeling through formation of L-ornithine with downstream production of polyamines and L-proline, which are involved in processes of cellular proliferation and collagen deposition. Ornithine and arginine also share the same intracellular cationic amino acid transporter. By shifting arginine metabolism away from NO towards ornithine, arginase can also impact intracellular transport.

Asymmetric dimethylarginine (ADMA) is an endogenous NOS inhibitor that competes with L-arginine for binding to NOS. It may also contribute to inflammation, collagen deposition, nitrosative stress and abnormal lung function in asthma. High levels of ADMA were recently demonstrated in both mouse and asthmatic human samples. Endogenous administration of nebulized inhaled ADMA to naive control mice, at
Fig. 1. Altered arginine metabolism in hemolysis: a path to pulmonary dysfunction. Dietary glutamine serves as a precursor for the de novo production of arginine through the citrulline-arginine pathway. Arginine is synthesized endogenously from citrulline primarily via the intestinal-renal axis. Arginase and NOS compete for arginine, their common substrate. In sickle cell disease (SCD) and thalassemia, bioavailability of arginine and NO are decreased by several mechanisms linked to hemolysis. The release of erythrocyte arginase during hemolysis increases plasma arginase levels and shifts arginine metabolism towards ornithine production, limiting the amount of substrate available for NO production. The bioavailability of arginine is further diminished by increased ornithine levels because ornithine and arginine compete for the same transporter system for cellular uptake. Despite an increase in NOS, NO bioavailability is low due to low substrate availability, NO scavenging by cell-free hemoglobin released during hemolysis, and through reactions with free radicals such as superoxide and other reactive NO species. Superoxide is elevated in SCD due to low superoxide dismutase activity, high xanthine oxidase activity and potentially as a result of uncoupled NOS in an environment of low arginine and/or tetrahydrobiopterin concentration or insufficient NADPH. Endothelial dysfunction resulting from NO depletion and increased levels of the downstream products of ornithine metabolism (polyamines and proline) likely contribute to the pathogenesis of lung injury, pulmonary hypertension and asthma in SCD. This model has implications for all hemolytic processes as well as pulmonary diseases associated with excess arginase production. This novel disease paradigm is now recognized as an important mechanism in the pathophysiology of SCD and thalassemia. Abnormal arginase activity emerges as a recurrent theme in the pathogenesis of a growing number of diverse pulmonary disorders. Regardless of the initiating trigger, excess arginase activity represents a common pathway in the pathogenesis of asthma and pulmonary hypertension. Reproduced with permission from the American Society of Hematology [1].
doses consistent with levels observed in the allergic inflamed lungs of the mouse model resulted in augmentation of the airway hyperreactivity in response to metacholine [2].

Numerous studies of allergic asthma in various animal models have demonstrated an increase in arginase activity in the inflamed airways. Studies in human asthma confirm the importance of arginase in the pathogenesis of experimental asthma. Increased arginase I activity, mRNA and protein expression have been demonstrated in inflammatory cells and airway epithelium from bronchial biopsies and bronchoalveolar lavage samples from asthmatic patients. Single nucleotide polymorphisms in both arginase 1 and arginase 2 have been associated with atopy and increased risk of childhood asthma. Increased arginase in the plasma of patients experiencing an acute asthma exacerbation has been demonstrated, while plasma L-arginine levels and the arginine/ornithine ratio (a biomarker that inversely correlates to arginase activity) were simultaneously reduced [3]. Clinical improvement in asthma symptoms corresponded temporally with reduction of arginase activity and increase in plasma L-arginine levels and the arginine/ornithine ratio [3]. The lung function of severe asthmatics correlates directly with L-arginine bioavailability, and inversely with serum arginase activity [4]. Furthermore, animal models of specific arginase inhibition have demonstrated prevention or reversal of airway hyperresponsiveness associated with allergen challenge [5]. The development and study of inhaled arginase inhibitors represents a promising area of research. L-arginine supplementation is another potential therapeutic method, although the results of limited human trials are less promising than animal studies.

Aberrant arginine catabolism represents a novel asthma paradigm that involves excess arginase activity, elevated levels of ADMA, altered intracellular arginine transport, and NOS dysfunction. Addressing the alterations in arginine metabolism may result in new strategies for treatment of asthma.

References

Sepsis is a significant health problem in children and the most common cause of death worldwide, but it is different from adult sepsis in epidemiology and pathophysiology. In adults, the amino acid arginine has received much attention in the context of critical illness and sepsis, but is less known in children.

Arginine Metabolism

Besides being needed for protein synthesis, arginine has other important functions [1]. It is catabolized by arginase to ornithine and urea, and by nitric oxide synthase (NOS) isoenzymes to nitric oxide (NO) and citrulline. Furthermore, arginine is metabolized into agmatine and creatine. Hence, it has a role in ureagenesis, immune function, wound healing, cell growth and differentiation and vasodilatation. Arginine is derived from dietary intake, body protein breakdown and de novo arginine synthesis from citrulline. During inflammation, NOS isoenzyme 2 is induced to a great extent, enhancing cytotoxic effects in macrophages on the one hand, but leading to (excessive) vasodilatation on the other hand. Meanwhile, microcirculation is compromised due to reduced NOS3 activity [2].

Arginine Deficiency during Critical Illness and Sepsis

In critically ill adults and children, plasma arginine and citrulline concentrations are severely decreased, especially during sepsis. This points to an arginine-deficient state. From stable isotope studies, it became apparent that arginine availability is reduced because of increased arginine disposal (in part by increased arginase activity and increased protein synthesis) on the one hand, which is not met by endogenous arginine production on the other hand. Important in this respect is the reduced de
novo arginine synthesis [3]. The latter most likely results from reduced citrulline availability, which is either caused by reduced precursor availability (glutamine) or impaired intestinal function. As a result of arginine deficiency, NO synthesis may be reduced. These metabolic changes seem to be dependent on the severity of inflammation [4]. See figure 1 for a proposed hypothesis.

**Factors Affecting NO Synthesis**

Reduced arginine availability, as a substrate for NOS, may limit NO synthesis. Other factors are competition between NOS and arginase for arginine, competition between arginine and lysine and ornithine for transport into the cell and inhibition of NO synthesis by asymmetrical dimethylarginine (ADMA) [2]. The latter is increased in critically ill adults and related to increased mortality. Especially the ratio between arginine and ADMA seems of importance, which is reduced in septic adults, primarily by reduced arginine concentrations, and associated with increased hospital mortality. Restoration of the arginine-ADMA balance by increasing arginine availability could therefore be a therapeutic target [5].

**Arginine Supplementation**

Hence, arginine or citrulline supplementation in severe inflammation seems to be a logical next step. Reviews on immunonutrition containing arginine in adults have been not been uniform. In critically ill children, one group has studied immunonutrition, but no effects on clinical outcome were found. Arginine and citrulline supplementation in critically ill children has not been studied. Arginine supplementation in critically ill adults improved arginine concentrations, without adverse effects on hemodynamics. Oral and intravenous citrulline supplementation improved arginine and citrulline concentrations in children undergoing cardiac surgery, which may be valuable in critically ill children as well. Another approach to improve arginine availability and NO synthesis could be to use a protein-energy-enriched formula, as we have recently shown.

**Conclusion**

Critical illness and sepsis in children are arginine-deficient states, the extent of which depends on the severity of inflammation. There may be a role for arginine or citrulline supplementation, although the use of protein-energy-enriched formulas may be an initial step to improve arginine
Fig. 1. Hypothesis of changes in arginine (Arg) metabolism in moderate inflammation and severe inflammation (or sepsis). Plasma arginine is slightly reduced during moderate inflammation. This is due to a slight reduction in arginine synthesis, because of moderately reduced citrulline availability, and a moderate increase in arginine catabolism. These changes are augmented during severe inflammation, leading to a severe reduction in de novo arginine synthesis and extensively enhanced arginine utilization for protein synthesis and by arginase. As a result, plasma arginine concentrations are further decreased, leading to reduced availability for NO synthesis, possibly compromising microcirculation via NOS isoform NOS3. Reproduced with permission from American Society for Nutrition [6].
availability. Because pediatric sepsis is a significant health problem differing from adult sepsis, pathophysiological mechanisms and possible interventions in arginine metabolism in critically ill and septic children should be investigated.

References

Arginine Deficiency Caused by Myeloid Cells: Importance, Identification and Treatment

Juan B. Ochoa

Suppression of T lymphocyte function is characteristically observed after trauma or surgery and is also described in various illnesses such as certain forms of cancer, and in chronic infections. In all of these, T lymphocyte suppression contributes to poor outcomes by increasing the susceptibility to infection and increasing the risk of wound breakdown, and worsens the prognosis of patients with cancer.

Arginine supplementation in the diet has been used to overcome T lymphocyte suppression. Arginine is an essential nutrient for normal T lymphocyte function. In surgery, arginine supplementation has been demonstrated to significantly decrease the risk of infection postoperatively. Yet, its use in septic patients is highly contentious with contrasting reports of benefit or harm. Arginine supplementation in cancer is also controversial with evidence that it can alternately induce or suppress tumor growth. As a result, arginine supplementation remains controversial and poorly utilized.

Understanding arginine metabolism by the immune system is central to unraveling the apparently contradictory results of clinical studies. A breakthrough came from the discovery of a heterogeneous group of immature myeloid cells, which are induced during illnesses. Arginine is metabolized alternately through inducible nitric oxide synthase or by arginase 1 (ASE), which is also inducible. Regulation of T lymphocyte function can occur through the production of nitric oxide by iNOS or through arginine depletion by myeloid cells expressing ASE; hence, these cells are identified by the name myeloid-derived suppressor cells (MDSC).

Our work has paid particular attention to the systemic development of arginine deficiency in illnesses through the preferential induction of MDSC expressing ASE. We explored the hypothesis that arginine deficiency caused by systemic activation of MDSC expressing ASE explains
the state of T cell suppression in illnesses such as trauma. Furthermore, we reviewed the hypothesis that diets containing supraphysiologic levels of arginine may overcome arginine deficiency and improve outcome in disease processes where MDSC expressing ASE may cause pathologic T cell suppression.
Glutamine Supplementation in Neonates: Is There a Future?

Josef Neu

Over the past couple of decades, glutamine (GLN) has emerged as an important metabolic intermediate signaling molecule and nutrient that becomes rapidly depleted and therefore critically important during stress. In very low-birthweight infants, a population of patients in whom supplementation of GLN should provide major benefits, GLN is provided in subnutritional quantities because standard parenteral nutrition solutions do not contain GLN, and most of these babies do not receive full enteral feedings until several weeks after birth. Results of efficacy on a limited number of outcomes have been mixed, but there is controversy regarding the validity and interpretation of these trials [1]. Some authors suggest that further study in this area is no longer warranted [2]. The use of GLN supplementation in very low-birthweight infants has therefore not become routine.

However, in premature infants there is a strong theoretical rationale for supplementation with GLN. Premature birth leads to a sudden cessation of a special combination of nutrients, including GLN, specifically suited for the rapidly developing fetus [3]. In the first weeks of life, these infants frequently derive most of their nutrition from the parenteral route, which contains no GLN, and are deprived of luminal nutrients, which contain GLN, because of the reluctance of neonatologists to use the enteral route. Furthermore, these infants are highly stressed and have an increased utilization of GLN during their first several weeks of life [4].

There are several inherent dilemmas common to trials of GLN supplementation (and perhaps many other types of nutritional supplementation studies):

1. Should isonitrogenous controls be used?
2. How long should the GLN supplementation last, and what is the correct amount that should be supplemented?
3. Is it better to supply GLN via the enteral or the parenteral route?
4. What outcomes should be evaluated in future studies? The primary outcome in many of the studies was sepsis. Is hospital-acquired sepsis a good outcome to evaluate in multicenter trials?

In conclusion, there is a recent reluctance to continue research on GLN-mediated amelioration of morbidity in premature infants. Based on the large body of evidence available from studies in animals and adults, as well as several of the recent studies in preterm infants, the notion that additional GLN research in premature infants should not be a priority is probably injudicious. As with the studies in adults, trials encompassing a large variety of premature infants with an array of problems may dilute effects. A variety of dosages have never been evaluated. If one extrapolates the dosages used in studies of enteral GLN administration in adults normalized to total protein requirements, the dosages used in the infant studies were relatively low. The route of administration (enteral vs. parenteral) may be critical. Downregulation of intestine-derived inflammation, apoptosis and stabilization of heat shock responses would theoretically occur to a greater degree with direct enteral application than if administered by the intravenous route, and this has not been investigated in preterms. The use of dipeptides of GLN also provides new avenues of research where the dosages can be increased and absorption improved. A thoughtful reevaluation of future applications and trials of GLN in premature infants is warranted.

References

Insulin in Human Milk and the Use of Hormones in Infant Formulas

Raanan Shamir and Naim Shehadeh

Introduction

Breastfeeding is the ‘natural and advisable way of supporting the healthy growth and development of young children’ [1]. Most advantages of breastfeeding can be attributed to human milk which contains cytokines, antibodies, enzymes, many hormones and growth factors (table 1). Studies in animal models show that some of these peptides (e.g. insulin, IGF-1, EGF) have an effect on the small intestine after orogastric administration.

We detail the rationale, evidence and plans for adding one of these hormones (insulin) to infant formulas.

Insulin in Human Milk

Insulin taken orally affects gut maturation and mucosal enzyme expression in newborn animal models [2]. Human milk contains significant amount of insulin, about 4-fold higher than amounts found in fresh pooled cow’s milk. Also, insulin is present in milk of mothers who gave birth to pre-mature infants as well as in milk of breastfeeding mothers for 15 months [3].

Insulin and Gut Maturation

Intestinal receptors for insulin were documented in mammalians in fetal life, during the suckling period, after weaning and in adults [3]. Insulin given orally to rats or piglets results in increased intestinal mRNA, protein, various intestinal enzymes' activities and enhanced maturation of the pancreas.

Insulin is a macromolecule that is usually not absorbed in the gut and its effect may be local and limited to the suckling period [3]. However, we were able to demonstrate, in a rat model, that the trophic effects of oral insulin on the intestine are observed 6 weeks after weaning.

In a rat model of short bowel syndrome (75% small intestinal resection), oral insulin significantly increased mucosal adaptation [4]. Furthermore,
oral insulin given to premature infants and children with short bowel syndrome or chronic intestinal failure provided clinical benefit in 3/6 premature infants and one child on home parenteral nutrition [4]. Also in humans, Shulman [5] demonstrated that oral insulin (8 preterm infants) increased lactase activity, reduced gastric residuals and shortened time to full feeds in treated infants compared to historic controls [5]. Oral insulin also influences intestinal permeability and has systemic indirect effects [3].

### Insulin in Infant Formulas

**Safety**

No adverse events including hypoglycemia and production of autoantibodies were reported in premature infants [4, 5]. In the DPT-1 trial, which aimed to test whether oral insulin can prevent type 1 diabetes in children at risk of developing type 1 diabetes within 5 years [6], rates of hypoglycemia were similar in insulin-treated children compared to controls.

**Adding Insulin to Infant Formulas**

In order to add insulin to infant formula, a product was designed containing a dry powder composed of bioactive insulin microencapsulated within a matrix of maltodextrin and vitamin C. The microencapsulation process enables insulin bioactivity protection until its immersion and consumption within infant formula.
In a preliminary study providing oral insulin (InsuMeal, Nutrinia, Israel) or placebo added to liquid infant formula (final insulin concentration of 400 μU/ml), 8 preterm infants were enrolled (table 2). The results suggest that preterm infants in the treatment group gained more weight during the first month (p < 0.02) with a trend of arriving earlier at full oral feeding (p < 0.09). However, the sample size is minimal and can only serve as guidance to a properly sized prospective study. Indeed, such a study is currently ongoing.

If the addition of insulin to preterm infant formulas results in better growth and accelerated intestinal maturation, future studies will need to address insulin supplementation in term infants and assess the efficacy of such supplementation in enhancing gut maturation and preventing non-communicable diseases such as allergy, autoimmune diseases and obesity.

**References**


**Table 2. Weight gain and time to full feeds**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight at day 1, g</th>
<th>Gained weight, g</th>
<th>Time to full feeds, days</th>
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<td>Control</td>
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Weight gain and time to full feeds in 8 preterm infants fed with control formula and a formula with added insulin during days 1–28 after birth.
Humans live in constant association with microbes. The number of microbes that are present on surfaces and in cavities of our body largely exceeds that of our own cells, and the number of genes they encode largely exceeds that of our own genes. This complex and dynamic microbiota has a profound influence on human health and disease. Defining this dynamic diversity represents the next frontier of understanding human biology, which will require not only knowledge of the human genome but also of the human microbial metagenome.

To rise to this challenge, MetaHIT has developed an approach that we term quantitative metagenomics, schematically represented in figure 1. Its central element is the reference catalog of the human intestinal bacterial genes. We have established a catalog of 3.3 million such genes, which represents rather well genes found in studies conducted in Europe, US and Japan and was dubbed ‘our other genome’ [1]. High throughput sequencing of total DNA from the stool samples lets us determine presence and abundance of the catalog genes in any individual – the gene abundance profiles – by matching the large number of short sequencing to the genes and thus counting the genes. The profiles let us cluster the individuals and reveal similarities and differences related to health, thus laying the ground for diagnostics.

Quantitative metagenomics focusing on sequenced genomes rather than genes has revealed the existence of particular microbial communities in different individuals that we termed enterotypes [2]. We reported 3 enterotypes characterized by a dominant bacterial genus as Bacteroides (green), Prevotella (red) and Ruminococcus (blue); their number, contours and composition are currently being debated as some studies have been able to reproduce the observation [3, 4] while others reported only 2 enterotypes [5].
Fig. 1. Schematic representation of quantitative metagenomics.

References

The human gut contains a vast number of microorganisms that have been collectively characterized as the ‘gut microbiota’ through the use of high-throughput DNA sequencing technologies. All three kingdoms of life, Archaea, Bacteria, and Eukarya, are represented in the gut microbial community. An estimated $10^{11}$ individual bacteria belonging to over 1,000 species reside in the mammalian gut with a collective genome predicted to be 150-fold greater than that of its host. Humans have coevolved to exist with our gut microbiota in a mutualistic relationship, where we provide a uniquely suited environment in return for physiological benefits provided to us by our gut microbiota. Despite its importance in maintaining the health of the host, growing evidence suggests the gut microbiota may also be an important factor in the pathogenesis of various diseases, a number of which have shown a rapid increase in incidence over the past few decades such as diabetes, inflammatory bowel diseases, colon cancer, atherosclerosis, and asthma. The composition of the gut microbiota has been shown to be ‘dysbiotic’, with an alteration in community structure, in a number of diseases, although studies are still required to demonstrate that dysbiosis plays a role in the pathogenesis of human disease rather than simply being a result of the disease process. Nevertheless, evidence in animal models suggests that the former may be true, at least under certain circumstances.

Factors including age, genetics, and diet may influence microbiota composition. Of these, diet is easiest to modify, and presents the simplest route for therapeutic intervention. In studies to be presented, we used diet inventories and 16S rDNA sequencing to characterize fecal samples from 98 individuals [1]. Fecal communities clustered into previously described enterotypes [2] were distinguished primarily by levels of *Bacteroides* and *Prevotella*. Enterotypes were associated with long-term diets, particularly protein and animal fat (*Bacteroides*) versus simple carbohydrates (*Prevotella*). Although the distinction of enterotypes as either discrete clusters or a continuum will require additional investigation, numerous studies
have demonstrated the coexclusion of the closely related \textit{Prevotella} and \textit{Bacteroides} genera in the gut microbiota in healthy human subjects [3], where \textit{Prevotella} appears to be a discriminatory taxon for residence in agrarian societies [4, 5]. A controlled feeding study of 10 subjects showed that microbiota composition changed detectably within 24 h of initiating a high-fat/low-fiber or low-fat/high-fiber diet, but that enterotype identity remained stable during the 10-day study. Thus, alternative enterotype states are associated with long-term diet. Interestingly, we have also shown that diet is also associated with alteration of the human gut virome that is primarily composed of bacteriophage [6].

Having demonstrated the impact of diet on human gut microbiota composition, we are now focusing on the impact of a dietary intervention known to be effective in the treatment of Crohn’s disease, namely a defined formula diet, on the composition of the human gut microbiota. The results of these studies may help to identify bacterial taxa that play a role in the pathogenesis of Crohn’s disease and/or serve as biomarkers that may help to predict response to therapeutic interventions. Ultimately, it is hoped these, and other studies, will help to define the ‘healthy diet’ for individuals genetically predisposed to the development of chronic inflammatory diseases such as inflammatory bowel disease.

References

Understanding Immunomodulatory Effects of Probiotics

Bruno Pot, Benoît Foligné, Catherine Daniel and Corinne Grangette

The intestinal microbiota is known to be the driving force in the development and maintenance of the immune system. While substantial shifts in the microbiota composition may influence the immune functionality in the longer term, short occasional changes might also be sensed. The latter opens considerable perspectives for the use of nutritional interventions intended to modulate immune functionality in a desired direction. Probiotics are one of the most promising ways currently used in a multitude of attempts to redirect immune parameters into a 'safer' direction. While it is already clear that probiotics will not rescue ‘heavily damaged’ immune systems, they may play a critical role in preventing disease or redirecting immune parameters that are subclinical but will increase the risk for disease in the longer term. Moreover, the understanding of their effects on immune tolerance parameters, their metabolic and physiological benefits, or their microbiological effects on pathogens, will yield fundamental knowledge leading to the development of new drugs and therapies. Although hopes are high that these approaches will be as effective as existing pharmaceutical homologs, even less effective compounds or strains will be welcomed as adjunct therapies because of the lack of side effects, too often seen with the current treatments.

Screening pipelines that use simple in vitro models allow to select strains that seem to perform well in e.g. animal models of inflammation. Further investigation using modern immunological techniques, metagenomic approaches, expression analysis, knockout animals, etc., has pointed out clear mechanisms of microbiological, metabolic or immunological activity, in some cases even allocated to active molecules at the bacterial surface or to metabolites produced under certain conditions. Knowledge of these mechanisms allows to understand and possibly circumvent differences in environmental conditions (skin, mouth, gut, vagina), lifestyle (drug, diets, stress, hygiene) or host status (genetic background, newborn, adult, elderly; healthy or diseased), which make clinical trials with probiotics extremely difficult.
We focused on only a few of these mechanisms, involving regulatory cells, cytokines, chemokines, defensins, which that manage immune responses, fight infections and toxins or cause immune-mediated anomalies. When these ‘natural’ control mechanisms fail, external intervention is necessary. Probiotics clearly have the potential to assist in this process. As an example, we described a pipeline that starts with a simple screening for ‘anti-inflammatory’ strains on peripheral blood mononuclear cells followed by a confirmation in a simple mice model for colitis, allowing the selection of two strains with opposite properties. Further testing using bone marrow-derived cells, pulsed or not with the respective bacteria, pointed out that dendritic cells were the host effector cells involved. Since Nod2−/− knockout mice revealed that the protection against colitis was Nod2 dependent, the hypothesis was raised that peptidoglycan (PGN) at the bacterial surface might be involved. Purified PGN injected intraperitoneally yielded again comparable results as obtained with the intact strains and moreover was dose dependent. A final comparison by high-performance liquid chromatography of the chemical composition of PGN of both strains yielded a significantly different peak, identified as the monomer GlcN-MurNAc-l-Ala-γ-D-isoGln-l-Lys, released only by the anti-inflammatory strain. Using the chemically synthesized pure molecule, it was possible to mimic the probiotic effect of the live strain in mice. Further mechanistic research revealed that regulatory CD103+ DCs are involved, preferentially CD4+Foxp3+ T regulatory cells, involved in intestinal homeostasis.

Using results of ongoing metagenomic work, it is very likely that other bacteria than the traditional lactobacilli and bifidobacteria will come up as possible therapeutic alternatives. The term pharmabiotics has been mentioned before and will probably be further elaborated to include heat-killed or irradiated strains as well as metabolically or genetically engineered bacteria. These will make optimal use of mechanistic studies providing insights in the molecular basis of the intended effects. The future of the *-biotics without any doubt is brilliant.
The intestinal mucosa is exposed to an enormous antigenic load of alimentary and microbial origin. To protect its surface from potentially harmful aggressions while guaranteeing tolerance towards harmless antigens (i.e. of alimentary origin or the commensal microbiota), this intestinal mucosa evolved several strategies, resulting in an efficacious epithelial barrier and a highly organized mucosal immune system. There is good experimental evidence that the intestinal microbiota is a main driver of the development of the mucosal immune system, with a critical phase during initial colonization after birth [1, 2]. Therefore, it is easily understandable that perturbations/changes of the colonization process may cause health problems with short- and eventually long-term consequences. Examples of diseases resulting from a disturbed microbial-host interaction are numerous, such as allergic diseases or dysimmune disorders, particularly those involving the intestinal tract, such as inflammatory bowel diseases [3, 4]. Research over the last years allowed gaining profound insight into the regulation of the immune system, the dialogue with the microbial environment, as well as the potential impact of food-derived antigens on pathological inflammation. These advances were largely stimulated by the introduction of new experimental disease models, such as experimental colitis models, as well as by the discovery of distinct human diseases, highlighting defects in key steps of immune regulation. However, many open questions remain, i.e. the initial triggers causing chronic intestinal inflammation, such as inflammatory bowel disease, the cause of food intolerance or the reason for loss of tolerance or inability to acquire tolerance. Given the important task to protect the intestinal mucosa, while enabling the uptake of large amounts of nutritional products, it is not surprising that the intestine harbors over 70% of immune-competent cells and produces a high amount of immunoglobulins. For the recognition of antigenic
or foreign structures (of alimentary of microbial origin), macrophages and dendritic cells (DC) play an extremely important role. These antigen-presenting cells sense microbial and other antigenic structures in the intestinal lumen and initiate immune responses. It is surprising to note that an immune response elicited by intestinal DC differs markedly from those initiated by spleen-derived DC: intestinal DC induce anti-inflammatory and tolerogenic responses to harmless antigens such as those derived from the resident microflora or harmless food allergens, while systemic immune activation results in a strong inflammatory TH1/TH17 reaction. Recent research has clearly confirmed that the functions of DC are regulated and imprinted by the microenvironment [5]: high concentrations of retinoic acid or vitamin D metabolites, as well as thymic stromal lymphopoietin and transforming growth factor (TGF)-β activate signaling programs in DC that result in the priming of regulatory and anti-inflammatory T cell responses (fig. 1). This interaction between DC and the microenvironment is highly dynamic, and the process is called DC priming or conditioning. TGF-β is one of the key factors implicated in intestinal immune regulation; it is produced by a large variety of cells in the intestinal mucosa, including intestinal epithelial cells, lymphocytes and monocytes/macrophages/DC. Three distinct isoforms of TGF-β exist exhibiting similar pleiotropic properties.

**Fig. 1.** DC conditioning. Effect of the environment on DC function and subsequent T cell priming.
An important anti-inflammatory effect of TGF-β on the immune system is the promotion and generation of FOXP3-positive regulatory T cells in the intestinal compartment. There are first and encouraging data from studies in Crohn’s disease, an inflammatory GI condition, that used enteral therapy with optimized concentrations of immunoregulatory peptides, such as TGF-β.

References

Microbiota Modulation: Can Probiotics Prevent/Treat Disease in Pediatrics?

Hania Szajewska

Microbiota manipulation, such as through the administration of probiotics, may potentially contribute to improved health outcomes. Here, some examples of current research related to this topic and published in the last 3 years are described.

Treatment of Acute Gastroenteritis

Select probiotics with proven clinical efficacy [e.g. Lactobacillus GG (LGG), Saccharomyces boulardii] may be used as an adjunct to rehydration therapy for the management of acute gastroenteritis [1]. A number of studies have been conducted to evaluate the effects of administering other probiotics (single or in combinations) to patients with acute gastroenteritis. Many, albeit not all, reported a shortened duration of diarrhea in the probiotic-treated group [2].

Prevention of Antibiotic-Associated Diarrhea

There is some evidence to support the use of probiotics (e.g. high dose of Lactobacillus rhamnosus or S. boulardii) for preventing antibiotic-associated diarrhea, but there is no evidence that use of probiotics is beneficial for treatment [2].

Nosocomial Diarrhea

The administration of LGG compared with placebo has the potential to reduce the overall incidence of healthcare-associated diarrhea, including rotavirus gastroenteritis [3] (fig. 1).

Infantile Colic

Exclusively or predominantly breastfed infants with infantile colic benefit from the administration of L. reuteri DSM 17938 compared with placebo (fig. 2) [4].
Prevention of Necrotizing Enterocolitis

Certain probiotics prevent NEC [2]. Before the routine use of probiotics in preterm infants, data regarding which products should be administered, at what dosages, and for how long are needed. In settings in which the incidence of NEC is high, one may consider the use of probiotics. However, care should be taken in choosing those that are the best studied, with the highest effect size and the best safety profile.

Prevention of Respiratory Tract Infections

The role of probiotics in preventing respiratory tract infections in children remains to be defined; however, there are substantial grounds to consider LGG as a good candidate.

Fig. 1. Effect of Lactobacillus GG on healthcare-associated diarrhea. Reproduced with permission from Szajewska et al. [3].
The composition of the gut microbiota differs between lean and obese individuals. Differences are apparent within the first week of life. It has been documented that a shortage of bifidobacteria in early gut microbiota is followed by the later development of overweight and obesity in children [5]. In one study, LGG had a transient preventive effect on weight gain during the first years of life [6].

**Fig. 2.** *L. reuteri* DSM 17938 for the management of infantile colic. Primary outcome – treatment success (reduction in the daily average crying time ≥50%). CI = Confidence interval; RR = relative risk; NNT = number needed to treat. Reproduced with permission from Szajewska et al. [4].

### Chronic Conditions

**Overweight and Obesity**

The composition of the gut microbiota differs between lean and obese individuals. Differences are apparent within the first week of life. It has been documented that a shortage of bifidobacteria in early gut microbiota is followed by the later development of overweight and obesity in children [5]. In one study, LGG had a transient preventive effect on weight gain during the first years of life [6].
Concluding Remarks

‘The microbes are coming’ [7]. This statement recently made by Gregor Reid speaks for itself.

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Membrane Composition and Cellular Responses to Fatty Acid Intakes and Factors Explaining the Variation in Response

Carlo Agostoni, Patrizia Risé and Franca Marangoni

The relative abundance of polyunsaturated fatty acids (PUFA) in the diet has a major influence on the composition of membrane bilayers. Moreover, the association between membrane composition and the intake of n-6 and n-3 PUFA with the diet and especially the dietary n-3/n-6 ratio may be attributed to the capacity of PUFA belonging to the two metabolic series to substitute for each other [1]. Both n-6 and n-3 PUFA are the biologically active fatty acids (FA). Within the n-6 series, linoleic acid, the first recognized essential FA, gives origin by desaturation and elongation processes to arachidonic acid, precursor of the eicosanoid families, and therefore one of the most essential components for life. The compound of the n-3 family with a shorter chain and lower unsaturation degree, α-linolenic acid, can be converted to the more biologically active very long-chain n-3 PUFA eicosapentaenoic acid, and docosahexaenoic acid (DHA). Also in this case the process occurs by a series of desaturation and elongation reactions.

Within this context, by increasing the contents of eicosapentaenoic acid and DHA in membranes, it is possible to modify the pattern of production of different lipid mediators [2]. These changes can affect membrane order, intracellular signaling processes, and, most important, gene expression, leading to changes in the production of both lipid and peptide mediators. Neurocognitive performance, visual development, immune function, inflammatory reactions and surrogates of the metabolic picture connected with the cardiovascular health (i.e. arterial blood pressure, cardiac rhythm, insulin sensitivity, overweight and obesity development) represent the main functional outcomes, at least in neonates and children. They share similar biological mechanisms and have been evaluated in relation to changes in FA intake to explain the association with dietary FA.
In the last years, the prominent role of the interindividual genetic variability in metabolism, incorporation, synthesis of biochemical intermediates and even effects on gene expression, has been shown to be closely connected with the individual asset of the aplotypes, including single-nucleotide polymorphisms (SNPs), associated with PUFA metabolism. Indeed, in addition to diet, common polymorphisms in the FA desaturase (FADS) gene cluster have very marked effects on human PUFA and LC-PUFA status. In intervention studies on the biological effects of linoleic acid, α-linolenic acid and LC-PUFA, and the effects of genetic variants in FADS1 and FADS2, 5-LO and cyclooxygenase-2 should be taken into consideration both in the determination of nutritional requirements and chronic disease risk [3]. New data have become available to show that FADS SNPs also modulate DHA status in pregnancy as well as LC-PUFA levels in children and in human milk. There are indications that FADS SNPs modulate the risk for allergic disorders and eczema, as well as the effect of breastfeeding on asthma symptoms and later cognitive development [4]. Based on these observations in human-based research, two take-home messages may be derived: (1) that the genetic variability may have a transgenerational effect via breastfeeding, and (2) that the genetic variation in human desaturase genes affects enzyme activity and, consequently, disease risk factors. Smoking and alcohol consumption may influence the absorption, biosynthesis, or metabolism of serum FA. The negative effects of smoking observed in the maternal-fetal dyad are summarized in table 1, and ethanol consumption may negatively affect the supply of FA from the maternal compartment to the fetus [5]. The differences observed in PUFA metabolism associated with variants in human genes and environmental factors suggest that different amounts of dietary PUFA may

<table>
<thead>
<tr>
<th>Table 1. Effects of smoking on PUFA metabolism observed in the maternal-fetal dyad</th>
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<tr>
<td>• In mothers: higher plasma lipid levels and lower milk total fat and DHA content in the first months of lactation</td>
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<td>• In mammary gland cells: exposure to cigarette smoke negatively affects the synthesis of n-3 LC-PUFA from the precursor</td>
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<tr>
<td>• In infants: reduction in LC-PUFA pools, particularly of the n-3 series, in infants born to smoking mothers in spite of lack of differences in maternal dietary intakes vs. nonsmokers in association with reduced fetal growth</td>
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<td>• Speculative associations: in breastfed infants, a lower total fat content of human milk is negatively associated with developmental indices at 12 months; in adults: negative relationship between maternal smoking during the third trimester and offspring adult intelligence</td>
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In the last years, the prominent role of the interindividual genetic variability in metabolism, incorporation, synthesis of biochemical intermediates and even effects on gene expression, has been shown to be closely connected with the individual asset of the aplotypes, including single-nucleotide polymorphisms (SNPs), associated with PUFA metabolism. Indeed, in addition to diet, common polymorphisms in the FA desaturase (FADS) gene cluster have very marked effects on human PUFA and LC-PUFA status. In intervention studies on the biological effects of linoleic acid, α-linolenic acid and LC-PUFA, and the effects of genetic variants in FADS1 and FADS2, 5-LO and cyclooxygenase-2 should be taken into consideration both in the determination of nutritional requirements and chronic disease risk [3]. New data have become available to show that FADS SNPs also modulate DHA status in pregnancy as well as LC-PUFA levels in children and in human milk. There are indications that FADS SNPs modulate the risk for allergic disorders and eczema, as well as the effect of breastfeeding on asthma symptoms and later cognitive development [4]. Based on these observations in human-based research, two take-home messages may be derived: (1) that the genetic variability may have a transgenerational effect via breastfeeding, and (2) that the genetic variation in human desaturase genes affects enzyme activity and, consequently, disease risk factors. Smoking and alcohol consumption may influence the absorption, biosynthesis, or metabolism of serum FA. The negative effects of smoking observed in the maternal-fetal dyad are summarized in table 1, and ethanol consumption may negatively affect the supply of FA from the maternal compartment to the fetus [5]. The differences observed in PUFA metabolism associated with variants in human genes and environmental factors suggest that different amounts of dietary PUFA may
be necessary in order to meet the requirements for these nutrients in
development and disease prevention on individual basis, but individual
phenotypic indicators are still lacking.

References

Docosahexaenoic Acid and Its Derivative Neuroprotectin D1 Display Neuroprotective Properties in the Retina, Brain and Central Nervous System

Nicolas G. Bazan

The significance of the selective enrichment in omega-3 essential fatty acids (docosahexaenoyl, DHA, chains of membrane phospholipids, 22C and 6 double bonds) in the nervous system (e.g. synaptic membranes and dendrites) has remained, until recently, incompletely understood. While studying mechanisms of cell survival in neurodegenerations, we contributed to the discovery of a docosanoid synthesized from DHA by 15-lipoxygenase-1, which we dubbed neuroprotectin D1 (NPD1,10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid). This mediator is a docosanoid because it is derived from a 22C precursor (DHA), unlike eicosanoids, which are derived from the 20 C arachidonic acid family of essential fatty acids not enriched in the nervous system. We found that NPD1 is promptly made in response to oxidative stress, seizures and brain ischemia-reperfusion, and in the presence of neurotrophins. NPD1 is neuroprotective in experimental brain damage, oxidative-stressed retinal pigment epithelial cells, and in human brain cells exposed to amyloid-β peptide. Thus, we envision NPD1 as a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened by neurodegenerations. We provide here recent experimental examples that highlight the specificity and potency of NPD1, spanning beneficial bioactivity during initiation and early progression of neurodegenerations, epileptogenesis and stroke: (1) NPD1 increases during seizures in the hippocampus, and when we administered this docosanoid during pharmacologically induced epileptogenesis, it elicited a remarkable attenuation of pathological brain oscillations. This reflects attenuation of aberrant neuronal network activities that lead to spontaneous recurrent seizure. We used multi-microelectrode arrays in freely moving mice to record these
data. Thus, docosanoid-mediated signaling rescues neuronal network disruptions. (2) In brain ischemia-reperfusion, DHA administered (i.v.) 1 h after 2 h of middle cerebral artery occlusion leads to penumbra protection with an extended window of protection (up to 5 h) and with concomitant NPD1 synthesis. The availability of antiapoptotic BCL-2 proteins is positively modulated by NPD1, whereas proapoptotic BCL-2 proteins are negatively regulated, as is the arrival of leukocytes due to neurovascular unit breakdown. Recently, we identified a COX-2-mediated pathway in addition to the lipoxygenase-catalyzed route. The former gives rise to novel aspirin-triggered NPD1. This novel docosanoid is very potent in attenuating neuroinflammation and stroke-mediated brain damage. (3) NPD1 is drastically reduced in CA1 areas from Alzheimer’s patients. Therefore, we have explored the significance of NPD1 in cellular models that recapitulate part of the Alzheimer’s pathology. Human neurons and astrocytes challenged by amyloid-β or by overexpressing APPsw (double Swedish mutation) show that NPD1 downregulates amyloidogenic processing of amyloid-β precursor protein, switches off proinflammatory gene expression (TNF-α, COX-2 and B-94-TNF-α inducible pro-inflammatory element), and promotes neural cell survival. Moreover, anti-amyloidogenic processing by NPD1 targets α- and β-secretases and PPARγ receptor activation. The cell death cascade involves multiple checkpoints and signaling networks. NPD1 regulation targets upstream events of cell survival as well as neuroinflammatory signaling, in turn promoting homeostatic regulation of neural circuitry integrity.

Acknowledgements

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Branched-Chain Fatty Acids in the Neonatal Gut and Estimated Dietary Intake in Infancy and Adulthood

Rinat R. Ran-Ressler, Raymond P. Glahn, SangEun Bae and J. Thomas Brenna

Branched-chain fatty acids (BCFA) are primarily saturated fatty acids (FA) with a methyl branch, usually near the terminal methyl group. BCFA are abundant in bacteria, skin, and vernix caseosa but have seldom been studied with respect to human nutrition. They are generally at low concentration in the liver, brain, and other internal organs, but are major constituents of the lipids of the skin, being synthesized by sebocytes, and other skin glands, especially the meibomian and Moll’s glands of the eyelid where BCFA are found in membrane lipids.

In vernix, they appear with carbon chain lengths from 11 to 26 with a mean total concentration between 25 and 30% of total FA, the highest of any biological tissue or substance. In the last trimester of normal gestation, vernix sloughs off the fetal skin and becomes suspended as particulates that are swallowed by the fetus. BCFA are found in meconium with carbon lengths from 16 to 26, reflecting selective disappearance of the shorter chain BCFA in transit through the fetal alimentary canal. The few published reports of BCFA in human milk enable an estimate that breastfed infants consume 19 mg BCFA per 100 ml milk.

BCFA are major constituents of the membranes of 15–20% of bacteria, where they play biophysical roles similar to unsaturated FA but are not susceptible to damaging chemical oxidation. Moreover, BCFA availability in growing media modifies the function of some bacteria including the virulence of pathogens.

We recently investigated whether BCFA substituted for polyunsaturated FA in feeds protect against necrotizing enterocolitis (NEC) in the premature rat pup model. Dietary BCFA at levels similar to those found in human vernix reduced NEC incidence by more than 50% (fig. 1), increased the abundance of BCFA-containing bacteria in the nascent microbiota, and increased the expression of ileal anti-inflammatory IL-10.
These effects are all consistent with bioactive effects of BCFA enhancing the establishment of healthy microbial flora in the first days of life.

BCFA are prominent components of the adult diet. Published data and our own preliminary data indicate that BCFA enter the diet principally via consumption of milk fat, beef, mutton, and other ruminant products because rumen bacteria biosynthesize BCFA. A sampling of dairies supplying a major amount of the US fluid milk supply shows that more than 2% of FA are BCFA. Their carbon numbers are from 14 to 18 carbons and are dominated by two anteiso BCFA, anteiso-15:0 and anteiso-17:0. Because of the prominence of dairy and ruminant products, the diets of most adults are expected to include major quantities of BCFA.

Considering primarily milk fat and beef as the main sources of dietary BCFA, we calculate that US adults consume more than 400 mg BCFA per day. This estimate exceeds by severalfold the average dietary intake of bioactive FA that occupy much more research attention; for instance, eicosapentaenoic acid and docosahexaenoic acid intakes combined average about 100 mg/day.

We conclude that BCFA are bioactive, abundant but neglected components of the human food supply. BCFA are likely to influence establishment of microbiota in neonates, and alter microbiota throughout life, and early evidence suggests they influence gut inflammatory state.

**Fig. 1.** Proportions of animals that were healthy (white) and ill with NEC (black) in each treatment group: DF (dam fed; no ill animals; a), control (formula fed, no BCFA; 17 of 31 animals were sick; b), BCFA (formula fed, 20%, w/w BCFA; 5 of 24 animals were ill; c). BCFA reduced NEC compared to the control group.
Clinical Overview of Effects of Dietary Long-Chain Polyunsaturated Fatty Acids during the Perinatal Period

Susan E. Carlson

Two long-chain polyunsaturated fatty acids (LC-PUFA) that have been studied in relation to infant and child development are considered: docosahexaenoic acid (DHA) and arachidonic acid (ARA), respectively, members of the n-3 or ω-3 and n-6 or ω-6 fatty acid families, and synthesized from linoleic and α-linolenic acids (see fig. 1). These LC-PUFA are found preformed in animal fats, including human milk. US infants fed human milk instead of infant formulas available in the late 1970s (which did not contain LC-PUFA) were observed to have a greater proportion of DHA and ARA in their red blood cell membrane phospholipids [1]. Research thereafter asked if DHA and ARA were ‘conditionally essential’ nutrients for infant development, i.e. if biochemical pathways for synthesis provided less than optimal DHA and ARA for function. Visual acuity and early cognitive development were outcome measures, because Martha Neuringer and her colleagues found reduced retinal and brain DHA, lower visual acuity and less mature attention in rhesus monkeys fed diets low in α-linolenic acid during development [2]. Their studies provided clear evidence that retinal and brain DHA were important for optimal visual acuity and a measure of early learning in n-3 deficiency. In the main, clinical studies did not limit α-linolenic acid and were designed to determine the need for LC-PUFA.

The first postnatal supplementation studies were done in the US in preterm infants, and supplementation was based on the amount of DHA in breast milk of US women. Preterm infants uniformly demonstrated higher (but probably not optimal) visual acuity with 0.2% DHA. Later, we learned that milk DHA content in the US is among the lowest in the world (~0.2% of total fatty acids). Both term and preterm infants have now been fed up to 1% of fatty acids as DHA. Preterm infants were fed approximately 0.3% compared to approximately 1% fatty acids as DHA until a corrected age of term birth had similar performance [3].
US term infants benefited from LC-PUFA with 0.32–0.96% DHA and 0.64% ARA compared to a formula without LC-PUFA, but infants fed the LC-PUFA-supplemented formulas had similar 12-month visual acuity [4] and cognitive performance to 6 years of age (fig. 2).

Challenges that I see for the future: (1) More finely grained measures of brain development are needed. These have shown benefits of LC-PUFA, whereas global measures designed to evaluate whether infants/toddlers are progressing normally generally have not [5]. (2) The effects of perinatal LC-PUFA supplementation may be easier to find later in childhood when children are able to be tested on more complex behaviors. For example, the effects of perinatal LC-PUFA supplementation emerged around 4 years of age in children subjected to tests of early cognition twice yearly between 18 months and 6 years of age (fig. 2). An exciting aspect suggested by some studies of brain development is that early LC-PUFA status may program development for later advantage. (3) Intrauterine exposure to LC-PUFA may be very important for fetal

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**Fig. 1.** Pathways for conversion of essential fatty acids to LC-PUFA.
brain development, but transfer from mother to fetus is highly variable and the reasons are not known. Randomized trials that use ITT compare groups with a great deal of overlap in newborn DHA status. We need to determine what variables influence this transfer. (4) Single-nucleotide polymorphisms in the fatty acid desaturase genes (FADS1/2) are now recognized to influence maternal and infant LC-PUFA status, and it might be hypothesized that they influence LC-PUFA requirement, but this has not been studied.

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**Fig. 2.** Emergence of differences in rule learning/inhibition in childhood after DHA supplementation in infancy.


Dietary n-3 LC-PUFA during the Perinatal Period as a Strategy to Minimize Childhood Allergic Disease

Maria Makrides

The prevalence of allergic diseases in many industrialized countries has increased over the last 30 years and is now estimated to be at least 20%. This increase in allergic disease has occurred too rapidly (within one to two generations) to be a result of population genetic changes, so is likely to be related to environmental changes. In this context, strategies to reduce the burden of disease through prevention are important.

The period of increase in allergic diseases has coincided with a substantial shift in dietary intake of fatty acids to favor n-6 fatty acids over n-3 fatty acids, leading to speculation that the change in dietary fatty acid balance may be linked to the increased prevalence of childhood allergic disease. Diets rich in n-6 fatty acids, via increased consumption of linoleic acid (18:2n-6)-rich vegetable oils as well as increased consumption of arachidonic acid (AA; 20:4n-6) through animal products, lead to a predominance of AA in tissues. AA gives rise to eicosanoids such as prostaglandin E₂ that can enhance the synthesis of T helper type 2 cytokines and immunoglobulin E antibodies – the hallmark of atopic responses to allergens. When diets are high in n-3 long-chain polyunsaturated fatty acids (LC-PUFA; e.g. fish) they are readily incorporated into cellular phospholipids, in the process displacing AA. This leads to a range of biochemical and immunological changes, including reduction of prostaglandin E₂ synthesis, alteration of receptor expression and activity and reduced pro-inflammatory cytokine responses. Thus, there are plausible mechanisms by which diets high in n-3 LC-PUFA may modulate the development of immunoglobulin E-mediated allergic disease and regulate immune responses.

With this rationale, several randomized controlled trials have been conducted to investigate whether n-3 LC-PUFA supplementation will lower the risk of developing childhood allergies. Most studies have included children with higher than normal risk of developing allergic disease because of their family history. These studies may be grouped...
according to whether supplementation with n-3 LC-PUFA occurred during the postnatal period or primarily during the prenatal period.

There are two major dietary intervention studies with n-3 LC-PUFA supplementation during the postnatal period. One trial randomly allocated newborn infants to receive a fish oil supplement (approximately 500 mg n-3 LC-PUFA/day) or an olive oil control until 6 months and assessed allergic outcomes including sensitization, eczema, and food allergy at 12 months of age [1]. There were no differences between the groups in the risk of allergic disease at 12 months [1]. The other trial was designed to increase n-3 LC-PUFA status through a combination of docosahexaenoic acid (22:6n-3)-rich tuna oil supplementation and a reduction in dietary linoleic acid intake from 6 to 18 months of age, and showed that dietary intervention lowered the prevalence of early asthma symptoms such as wheeze and cough at 18 months and 3 years, respectively [2, 3]. There was no effect of intervention at 5 years of age [4].

On the other hand, randomized trials that have commenced n-3 LC-PUFA intervention during pregnancy are producing interesting results. One of the earliest prenatal supplementation studies involved high-risk infants and showed changes in neonatal immune responses which were consistent with a less allergic phenotype in the fish oil group compared with the control group [5]. More recently, two studies have demonstrated that n-3 LC-PUFA supplementation with at least 1 g per day during the last half of pregnancy reduced the risk of atopic eczema during the first year of life and reduced the frequency of egg sensitization in infants who are at high hereditary risk of allergies [6, 7]. Of interest are the findings by Olsen et al. [8] who demonstrated that n-3 LC-PUFA supplementation during pregnancy reduced asthma in adolescence in a study including families at normal risk of allergies. However, the allergy outcomes from the trial of Olsen et al. [8] were obtained through linkage to a national registry of doctor visits. The expected event rates in their study are low, and it is not known whether diagnoses were made according to standard definitions.

In summary, supplementation with n-3 LC-PUFA during the perinatal period and before allergic response is established and may be a useful strategy to prevent early childhood allergic disease in children at high hereditary risk.

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