

# Nutritional Factors Influencing Infections in Preterm Infants<sup>1,2</sup>

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## Abstract

In contrast with clinical studies in term infants or older children, it is very difficult to investigate possible immunoregulatory effects of a novel infant formula composition in preterm infants. This is mainly because of the multicausal origin of infections in this high-risk population that is usually admitted to the neonatal intensive care unit. Possible effects of nutrition composition on onset and incidence of nosocomial infections in these very small infants have to be compared with infections that may have originated in utero. The development of the gastrointestinal tract may be inhibited after severe intrauterine growth retardation, leading to functional impairment of the gut shortly after birth. This may be related to the onset of necrotizing enterocolitis of the newborn. However, this disease in very small preterm infants is possibly also related to the initiation of oral feeding and/or the amount of feeding. Specific infection risks of neonatal intensive care as a result of invasive techniques such as artificial ventilation or total parenteral nutrition using indwelling umbilical and/or Silastic lines and so-called "all-in-one" mixtures may influence the incidence of infections. Widespread use of intravenous antibiotics in the neonatal intensive care unit may create an even larger infection risk. Investigation of possible immunomodulatory effects of factors such as prebiotics and probiotics added to the nutrition of preterm infants should always be considered along with other nutritional factors known to influence the immature immune system. J. Nutr. 138: 1813S–1817S, 2008.

## Introduction

Recently, many clinical studies have been performed to investigate the possible beneficial effects of adding immunoregulatory substances to the nutrition of infants. In particular, the addition of nucleotides, prebiotics, or probiotics to the infant formula may create a so-called bifidogenic effect on the flora of the gut, which may reduce the number of latent invasive bacteria (1–3). It has been suggested that this may reduce the risk of infections and may also have a long-term beneficial effect on immune function (4). It has even been postulated that this type of "immunonutrition" will modulate the onset of infections and allergy in later life (4,5). However, as this article demonstrates, it is considerably more complex to investigate the effects of immunonutrition in preterm infants. Investigations into the effects of specific nutritional components on the intestinal microflora of very small preterm infants are complex because effects on

the gut caused by intrauterine growth retardation (IUGR)<sup>3</sup>, hypoxia after birth, the timing of the onset of enteral feeding of the immature gut, and the effects of infections caused by the invasive neonatal intensive care unit (NICU) treatment of these infants create a wide scatter of insults. Before one can study the possible beneficial effects of a certain gut microflora, all other factors influencing the gut bacterial flora over a prolonged time period should be considered and investigated thoroughly (Table 1).

## Origin of infections before birth

Infections in preterm infants may originate in utero (6). In particular, intraamniotic bacterial infections in relation to premature rupture of membranes may cause preterm birth followed by a severe threat of immediate postnatal sepsis and/or meningitis. Another cause of infections of which the origin can be found in severe IUGR caused by poor placental function during the second and last trimester of fetal development may be traced to intrauterine infections. In the Western world, this is often caused by severe preeclampsia, leading to a poor perfusion via the placenta and resulting in a relative sparing of the brain but a very poor development of the splanchnic organs including the gut. The underdeveloped gut, often suffering from relative hypoxia, is very sensitive to poor motility, leading to stasis in the lumen and rapid overgrowth of (potentially) pathogenic bacteria (7). This may lead to the typical disease seen in high-risk preterm infants:

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<sup>3</sup> Abbreviations used: GI, gastrointestinal; IUGR, intrauterine growth retardation; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit.

**TABLE 1** Multicausal origins of infections in preterm infants

## Factors influencing the onset of infections in preterm infants

|                                                                      |
|----------------------------------------------------------------------|
| Origin of infections before birth                                    |
| Intrauterine (prenatal) onset of infections                          |
| Poor development of splanchnic organs of the newborn because of IUGR |
| Immature GI tract                                                    |
| Origin of infections because of NICU environment                     |
| Invasive NICU care including mechanical ventilation                  |
| Misuse of intravenous antibiotics at the NICU                        |
| Total parenteral nutrition using indwelling lines                    |
| Origin of infections related to the GI tract                         |
| Early/late onset of enteral feeding?                                 |
| Amount of nutrition (minimal enteral nutrition)                      |
| Relation to the origin of NEC                                        |
| Postnatal malnutrition particularly in infants <26 wk                |
| Effects on infections by specific food components                    |
| Effects of amino acids, nucleotides, pre- and probiotics             |

necrotizing enterocolitis (NEC) (7). That these risk factors causing infections in preterm infants often originate before birth should always be considered in investigating the effects of pre- and/or probiotics added to the nutrition of these infants. The hostile environment in the lumen of the gut of preterm infants may be caused by pathogenic factors before birth, and the growth of pathogenic bacteria during the early postnatal period may be enhanced by relative hypoxia from poor ventilation.

**Origin of infections related to the NICU environment**

Recently, we reviewed the literature on the development of the intestinal microflora of preterm infants and the factors influencing its environment (8). Most studies show that the intestinal bacterial colonization with beneficial bacteria is delayed in preterm infants (8).

The number of potentially pathogenic bacteria is high. The widespread use of antibiotics at the NICU has a negative effect on the development of the intestinal microflora. Many preterm infants receive prophylactic antibiotics at birth. Over the period 1998–2000, a surveillance study on nosocomial infections was performed at our NICU (9,10). During 50% of the admission time, at least 1 antibiotic was administered to the preterm infants (9). The risk of sepsis was negatively related to birth weight: <1000 g birth weight, 31% risk of sepsis; 1000–1500 g birth weight, 28% risk of sepsis; and 1550–2500 g birth weight, 4% risk of sepsis. Apart from low birth weight, another high-risk factor for sepsis in preterm infants is the use of total parenteral nutrition mixtures (9,10).

**Origin of infections related to the gastrointestinal tract**

Severe infections in preterm infants often originate from the gastrointestinal (GI) tract because of the developmental immaturity of the gut. Often the gut wall is not yet fully mature, and the ultimately tight junctions between the mucosal cell layers are still open, permitting bacteria from the intestinal lumen to penetrate into the interstitium or vascular system causing sepsis (11). The process of gut maturation is influenced not only by the gestational age but also by the effect of administering enteral feeding late or early by so-called gavage feeding using an indwelling plastic probe. Over the last 10–15 y, the concept of minimal enteral trophic feeding has been introduced into clinical practice: the early start of enteral feeding in small quantities (maximal  $12\text{--}24\text{ mL} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) in ventilated preterm infants during the period of

parenteral feeding to enhance the gut function and with the intention to enable full enteral feeding sooner after birth (12). Preferably breast milk is given by nasal drip into the stomach to enhance mucosal development because mucosal growth factors in human milk facilitate mucosal growth (13). Pulsed minimal enteral feeding with breast milk may stimulate gastric emptying and may cause less reflux than bolus feeding. There may also be several nonmucosal effects of minimal enteral feeding such as stimulation of the motor activity of the muscular layer of the gut and enhancement of the postprandial response: lowering of the vascular resistance of the gut wall and increased oxygen consumption (14). Breast milk consumption may also result in the release of endocrine and metabolic factors such as gastrin, enteroglucagon, motilin, neurotensin, gastroinhibiting peptide, and pancreatic polypeptide (15). Enhancement of *Lactobacillus* and *Bifidus* flora by breast milk, known to diminish the risk of overgrowth of (potentially) pathogenic bacteria has already been discussed above. All these factors may lower the risk of NEC, a clinical entity in preterm infants leading to extremely high morbidity and/or mortality (7,16,17).

It remains very difficult, however, to collect sufficient clinical evidence for the validity of the concept of minimal enteral feeding (18). Most relevant clinical studies have been reviewed in a Cochrane meta-analysis (12,16). We concluded that minimal enteral feeding diminishes the number of days to reach full enteral feeding without increasing the risk of NEC. A study by Mihatsch et al. (19) showed that, even in IUGR preterm infants, the first postnatal day that full enteral feeding can be administered is reached earlier by increasing the amount of nutrition by  $16\text{ mL} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  up to  $160\text{--}176\text{ mL} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  until enteral nutrition is reached. Following this protocol, no increase in the incidence of NEC was observed, and the infants left the NICU sooner. In contrast, Berseth et al. (20) in 2003 claimed that increasing the amount of enteral nutrition with  $20\text{ mL} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  in preterm infants could be responsible for the fact that the team observed more cases of NEC.

**Effects on infections by components of the nutrition**

**Quantitative aspects of important metabolic nutritional substrates: proteins.** Proteins/amino acids are important anabolic substrates for the developing fetus and newborn. From numerous kinetic metabolic studies in preterm infants using amino acids labeled with stable isotopes, we know that the daily protein gain in preterm infants may be as high as  $2\text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  of protein during preterm life (21). This can be reached only by feeding fortified human milk or special protein-enriched preterm formula.

As demonstrated by Embleton et al. (22), preterm infants, particularly those born below 30 wk gestational age, may suffer from an increasing cumulative energy/protein deficit despite all efforts to feed them properly. The catabolic situation that develops in such infants has a negative effect on their infection risk during their time of stay in the NICU.

**Qualitative aspects of important metabolic substrates: essential amino acids.** Apart from protein quantity, the protein quality of the enteral feeding may influence metabolic conditions in the luminal part of the developing gut. Essential amino acids such as glutamine and glutamate are important energy sources for the intestinal intraepithelial lymphocytes, which require these amino acids for their proliferation (23). Indirectly, these amino acids may contribute to important functions including production of secretory immunoglobulin preservation of the gut-associated lymphoid tissue system and intestinal integrity (maturation of

the crypt cells), prevention of bacterial translocation, and decrease of transcellular permeability (23–26). In a randomized controlled trial, Van den Berg et al. (27,28) investigated the effects of glutamine supplementation on the enteral feeding of 102 very low-birth-weight newborn infants. As clinical outcomes, feeding tolerance, serious infectious morbidity, and short-term outcome were investigated together with the postnatal adaptation of the gut and possible modulation of the immune response. No direct effects of enteral glutamine supplementation were seen studying the development of the intestinal microflora at postnatal day 30 (Table 2).

However, glutamine enrichment of the enteral feeding showed a positive effect on the number of serious infections in preterm infants during their stay at the NICU (Tables 3 and 4). The time to reach full enteral feeding was not reduced by supplementation of glutamine (Table 4). During the first 12 mo of life, preterm infants fed glutamine-enriched formula over the first 30 d of life showed significantly less atopic dermatitis but no difference in the incidence of bronchial hyperreactivity.

### Effects of breast milk

Breast milk contains numerous factors with direct trophic effects on the intestinal wall cell layer protecting the infant to infections, resulting in better absorption and motility (13). In this manner it may help to protect a preterm infant to the pathophysiological process of NEC (13). This protection may be caused by an inhibiting effect on the development of a potentially pathogenic intestinal microflora that results from stimulating the growth of Bifidobacteriae and *Lactobacillus* strains (29,30).

### Effects of nucleotides

Nucleotides are components of human milk that have been identified as affecting the immune function. In animal models, nucleotides have been shown to stimulate growth and maturation of the GI tract (31). Over the past 10 y, several clinical studies have shown effects of infant formula containing nucleotides on the immune status of infants (5,32). Most studies describe stimulated humoral responses after vaccination (5) and effects on the immune cell development (33). It is interesting to note that in both term and preterm infants, dietary nucleotides stimulate the intestinal circulation directly as observed by measuring the blood flow in the superior mesenteric artery (34,35). However, the clinical implications of these findings remain to be determined.

**TABLE 2** Adding glutamine to the nutritional formula of preterm infants does not affect the relative composition of fecal bacteria at postnatal d 30<sup>1</sup>

| Fecal bacteria <sup>2</sup>           | Glutamine group | Control group | P-value <sup>3</sup> |
|---------------------------------------|-----------------|---------------|----------------------|
| <i>Bifidobacteria</i> (%)             | 0 (0–54)        | 0 (0–93)      | 0.32                 |
| <i>Lactobacillus-Enterococcus</i> (%) | 6 (0–39)        | 6 (0–28)      | 0.57                 |
| <i>Streptococcus-Lactococcus</i> (%)  | 5 (0–25)        | 4 (0–22)      | 0.65                 |
| <i>Bacteroides</i> (%)                | 0 (0–11)        | 0 (0–33)      | 0.38                 |
| <i>Clostridium</i> (%)                | 0 (0–13)        | 0 (0–33)      | 0.64                 |
| <i>Escheria coli</i> (%)              | 21 (0–65)       | 33 (0–83)     | 0.12                 |

<sup>1</sup> Data adapted from Van den Berg (47).

<sup>2</sup> Relative percentage of bacterial species in the fecal microflora at postnatal day 30 in 37 preterm infants fed with a 0.3 g · kg<sup>-1</sup> · d<sup>-1</sup> glutamine-supplemented preterm formula (Glutamine group) and 37 preterm infants fed with a standard preterm formula (Control group).

<sup>3</sup> Mann-Whitney U-test.

**TABLE 3** Effects of glutamine supplementation to the nutrition of preterm infants on the number of postnatal days to reach full enteral feeding and on the number of serious infections during their stay at the NICU<sup>1</sup>

|                                                    | Glutamine, n = 52 | Control, n = 50 | Odds ratio (95% BI) | Hazard ratio (95% BI) | P-value (χ <sup>2</sup> ) |
|----------------------------------------------------|-------------------|-----------------|---------------------|-----------------------|---------------------------|
| Time to reach full enteral feeding, <sup>2</sup> d | 13 (7–31)         | 13 (6–35)       | 1.19                | (0.79, 1.79)          | 0.40                      |
| Serious infections                                 | 26/52 (50%)       | 38/50 (76%)     | 0.32                | (0.14, 0.74)          | 0.008                     |

<sup>1</sup> Adapted from Van den Berg et al. (27).

<sup>2</sup> Data are shown as mean (range): 50 preterm infants received 150 mL · kg<sup>-1</sup> · d<sup>-1</sup> of a preterm formula (control), and 52 preterm infants received the same amount of special formula supplemented with 0.3 g · kg<sup>-1</sup> · d<sup>-1</sup> glutamine until postnatal day 30.

### Effects of probiotics

Feeding with breast milk leads to the development of a healthy intestinal microflora of *Lactobacillus* and Bifidobacteriae instead of the potentially pathogenic Enterobacteriae. In an attempt to create a similar effect, clinical studies have been conducted to investigate the addition of *Lactobacillus* and/or *Bifidus* bacteria directly to the formula of infants (2,36,37). In term infants, positive effects have been described on the development of *Lactobacillus* and *Bifidus* in the gut microflora (36,37). In particular, infants with diarrhea may restore their gut function more rapidly and dramatically after addition of probiotics to the formula (36). For preterm infants, it is feared that probiotics may contribute to the onset of bacterial resistance against antibiotics, whereas most

**TABLE 4** The effects of glutamine supplementation to the nutrition of preterm infants on the incidence of serious infections from sepsis or pneumonia during their clinical stay at a NICU<sup>1</sup>

| Infection                                | Glutamine formula       | Control formula |
|------------------------------------------|-------------------------|-----------------|
| ≥1 Serious infection, <sup>2</sup> n (%) | 26/52 (50) <sup>3</sup> | 38/50 (76)      |
| Episodes, n                              |                         |                 |
| 1                                        | 16                      | 28              |
| 2                                        | 8                       | 7               |
| 3                                        | 2                       | 3               |
| Microorganisms in culture                |                         |                 |
| Sepsis, n (%)                            |                         |                 |
| <i>Coagulase-negative staphylococcus</i> | 23/31 (74)              | 25/38 (66)      |
| <i>Staphylococcus aureus</i>             | 4/31 (13)               | 8/38 (21)       |
| <i>Escheria coli</i>                     | 1/31 (3)                | 4/38 (11)       |
| <i>Streptococcus</i> spp.                | 0/31 (0)                | 1/38 (3)        |
| <i>Klebsiella pneumoniae</i>             | 2/31 (6)                | 0/38 (0)        |
| <i>Pseudomonas aeruginosa</i>            | 1/31 (3)                | 0/38 (0)        |
| Pneumonia, n (%)                         |                         |                 |
| <i>Staphylococcus aureus</i>             | 0/7 (0)                 | 6/13 (46)       |
| <i>Klebsiella pneumoniae</i>             | 2/7 (29)                | 3/13 (23)       |
| <i>Pseudomonas aeruginosa</i>            | 1/7 (14)                | 0/13 (0)        |
| <i>Ureaplasma urealyticum</i>            | 3/7 (43)                | 2/13 (15)       |
| <i>Enterobacter</i> spp.                 | 1/7 (14)                | 1/13 (8)        |
| <i>Escheria coli</i>                     | 0/7 (0)                 | 1/13 (8)        |

<sup>1</sup> After glutamine supplementation, the number of serious infections in preterm infants at the NICU is reduced, but no effects are seen regarding the relative incidence of various pathogenic bacterial species. Data adapted from Van den Berg (47).

<sup>2</sup> Serious infections included sepsis, pneumonia, meningitis, arthritis, and pyelonephritis.

<sup>3</sup> P < 0.01 compared with control group.

*Bifidus* and *Lactobacillus* strains may be killed by the use of broad-spectrum intravenously administered antibiotics (38). Only Lin et al. (39) in Taiwan investigated the effects of the addition of probiotics in preterm infants. This study reported no side effects and showed beneficial effects on survival, infection rate, and incidence of NEC in preterm infants. However, it was surprising to see that the incidence of NEC was much higher in Taiwan than in either North America or Europe (39).

### Effects of prebiotics

The immunomodulatory effects of oligosaccharides in infant nutrition have been described frequently (40–43). Most studies describe a bifidogenic effect on the gut microflora and a positive effect on the incidence of infections at short term and possibly also at long term. The bifidogenic effect may also lead to less atopy (4). Characteristically, softer stools are also observed after introduction of galacto-oligosaccharides or mixtures of galacto- and fructo-oligosaccharides (1,2). Similar clinical beneficial effects have been described in a limited number of studies in preterm infants (3,44). One of the problems in preterm infants is the widespread use of broad-spectrum antibiotics, negatively influencing the bifidogenic development of the gut microflora (3). To study the long-term effects of adding prebiotics to infant formulas, more clinical studies are needed (45). At present, we are investigating the (possibly) beneficial effects of adding so-called acidic (46) and neutral oligosaccharides to the formula in preterm infants.

In summary, we conclude that studying the immunomodulatory effects of breast milk and dietary components such as pre- and probiotics and nucleotides added to the preterm formula in preterm infants is complex because of the multicausal risk of infections in these infants. A multitude of immunoregulatory factors, then, must be considered in clinical studies investigating the effects of immune components in the nutrition of preterm infants.

Other articles in this supplement include references (48–57).

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