NUTRICIA
LIFE-TRANSFORMING NUTRITION

MEDICAL **EVIDENCE**

The science behind Nutricia's solutions for preterm infants

CONTENTS

ABBREVIATIONS

AAP	American Academy of Pediatrics
AOS	Acidic oligosaccharides
AGA	Appropriate for gestational age
ALA	Alpha-linolenic acid
ARA	Arachidonic acid
BM	Breast milk
CA	Corrected age
DHA	Docosahexaenoic acid
ELBW	Extremely low birth weight
EPA	Eicosapentaenoic acid
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology, and Nutrition
EFA	Essential fatty acids
FA	Fatty acid
GLA	Gamma-linolenic acid
HCP	Healthcare professional
HMF	Human Milk Fortifier
HMO	Human milk oligosaccharides
IUGR	Intrauterine growth restriction
LBW	Low birth weight
LcFOS	Long-chain fructo-oligosaccharides
LCPUFA	Long-chain polyunsaturated fatty acids
LGA	Large for gestational age
LA	Linoleic acid
MCFA	Medium-chain fatty acids
NCD	Non-communicable diseases
NEC	Necrotising enterocolitis
PER	Protein/energy ratio
PL	Phospholipid
PDF	Post-Discharge Formula
ScGOS	Short-chain galacto-oligosaccharides
SGA	Small for gestational age
TG	Triglycerides
VLBW	Very low birth weight
WHO	World Health Organization

THE FIRST **1000 DAYS**

The first 1000 days is the period from conception to two years of age and is a time of extreme growth and organ development. It is also the time when the fetus, infant or young child is most susceptible to environmental influences that could have both short- term and life-long health and physical consequences.

(Thousand, 2017). Nutrition during the first thousand days is a modifiable environmental factor that can have a profound effect on these consequences (Wrottesley et al., 2016). Maternal over- and under- nutrition influences embryonic development and fetal growth, and deviations from optimal infant growth patterns are a risk factor for health in the short-term, e.g. gestational diabetes, and long-term risk of non-communicable diseases for the offspring, e.g. cardiovascular disease, obesity and diabetes (Adair, 2008, de Jong et al., 2012, Gluckman et al., 2005, Godfrey et al., 2007, Hanson et al., 2015). Provision of optimal nutrition during the first 1000 days will positively impact on the development of organs and biological systems and short-and long-term health risks. Provision of optimal nutrition will ensure a desirable growth trajectory, which is also known to be a positive factor in reducing later health risk.

IMPORTANCE OF THE FIRST 1000 DAYS IN THE PRETERM INFANT

The third trimester of pregnancy is characterised by rapid fetal body growth, and functional development of especially the brain, lungs, gastrointestinal, immune systems along with a marked increase in lean mass and deposition of body fat (Kugelman et al., 2013, Lapillonne et al., 2013, Ramel et al., 2014). Although the date of birth is estimated to occur 40 weeks after the first day of the last menstruation, an infant is born at term when the 36th week of pregnancy has been completed. Infants born earlier than 37 weeks face challenges because, firstly they need to survive, and secondly, complete these maturation steps outside their mother's womb. Even late preterm infants (born between 34 -< 37 weeks gestation) having missed three weeks of intrauterine growth may be vulnerable to short- and long-term implications (Kugelman and Colin, 2013, Santos et al., 2009).

Once survival has been secured, provision of optimal nutrition during the first weeks and months in the life of preterm infants is fundamental to their health, well-being and future prognosis.

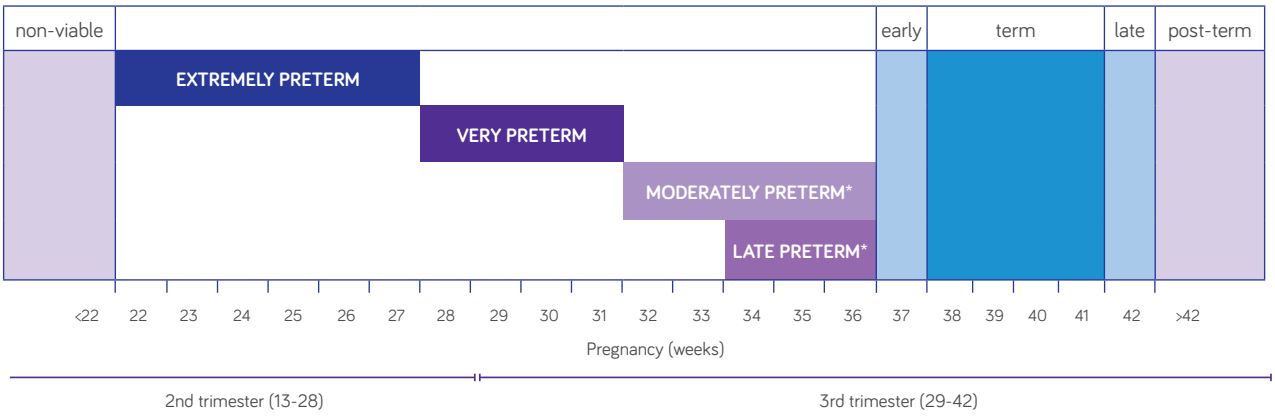
PREMATURITY

DEFINITIONS USED IN PREMATURITY

Preterm infants are those born before 36 weeks + 6 days of gestation were completed, corresponding to less than 259 days. The preterm population can be further classified either by degree of prematurity such as extremely, very, moderately, or late preterm or by birth weight (Figure 1).

Classifications of degree of prematurity are not universally agreed upon, for example, the (World Health Organization) WHO defines a late preterm infant as being born between 32 weeks + 0 days and 36 weeks + 6 days, whereas other institutions use a range between 34 weeks + 0 days to 36 weeks + 6 days (Fewtrell et al., 2016).

DEFINITION BY GESTATIONAL AGE



DEFINITION BY BIRTH WEIGHT

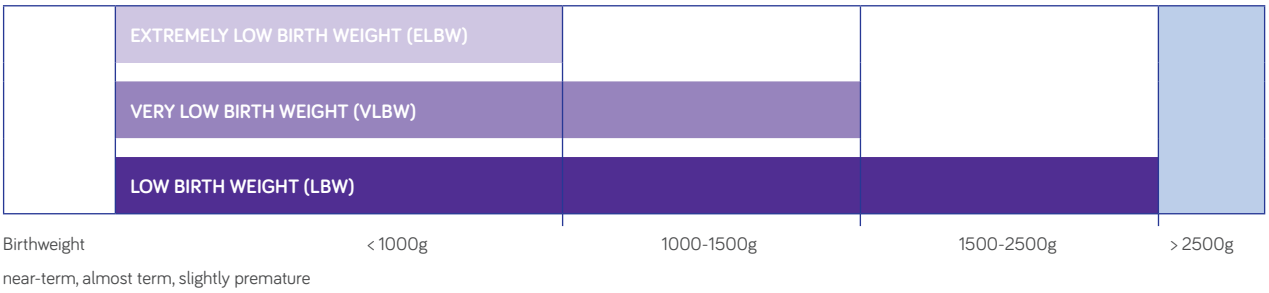


Figure 1 Classification of preterm infant populations, either by gestational age and degree of prematurity (top) or birth weight (bottom)

Classifications based on birth weight include low, very, and extremely low birth weight groups (Figure 1). Here it is noteworthy to consider that the low birth weight group (LBW) (<2,500 g) comprises a mixture of term and preterm infants, whereas it is rare (although possible) for a term infant to be born with less than 1,500 g very low birth weight (VLBW) (Fewtrell et al., 2016).

A third set of definitions are used in the description of (pre)mature infants; these are small for gestational age (SGA), appropriate for gestational age (AGA) and large for gestational age (LGA). Intrauterine growth restriction (IUGR) which is usually diagnosed based on at least two fetal ultrasounds may result in the birth of a SGA infant (Fewtrell et al., 2016).

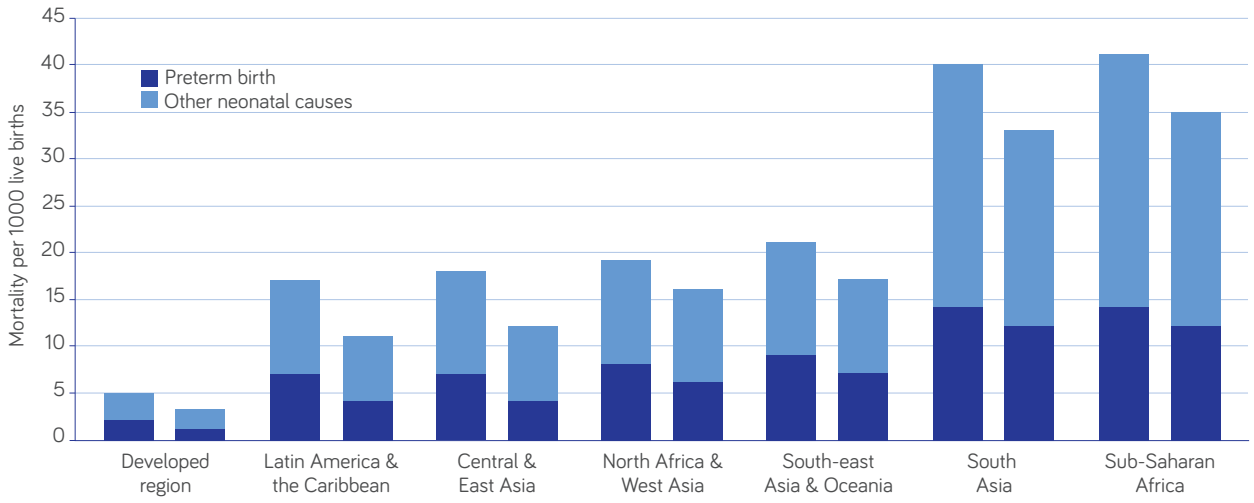
Several terms are used to describe the age of premature infants, often depending on their developmental stage. Shortly after birth, gestational age will be used to describe the progress of pregnancy or the degree of prematurity (Table 1). Definitions used to describe the age of a preterm infant are shown in Table 1; corrected age is used most frequently in the clinical setting.

Gestational age	Time elapsed between the first day of the last menstrual period and the day of delivery. Example: the child has a gestational age of 34 weeks.
Chronological age	Time elapsed since birth in days or weeks. Example: The child is born prematurely at 34 weeks but is 21 days old
Corrected age	Chronological age in weeks or months minus gestational age in weeks; especially used after term age (40 weeks) was reached. The child was born at 34 weeks, has a PMA of 52 weeks and is now 3 months term corrected (or term equivalent) age(CA).

Table 1 Definitions used to describe the age of a preterm infant (RCPCH, 2011)

PREVALENCE OF PREMATURE BIRTH

The WHO estimates that 15 million infants are born prematurely every year. Preterm birth is the number one cause of mortality and morbidity in children under 5 years of age around the world (March of DIMES et al., 2012). Figure 2 shows mortality as a result of preterm birth by region in 2000 and 2010. According to WHO over 60% of preterm births occur in Africa and South Asia. The ten countries with the highest numbers include Brazil, the United States, India and Nigeria, demonstrating that preterm birth is truly a global problem. Of the 11 countries with preterm birth rates of over 15%, all but two are in sub-Saharan Africa. In the poorest countries, on average, 12% of babies are born too soon compared with 9% in higher-income countries. Within countries, poorer families are at higher risk (March of DIMES et al., 2012).



Source: Child Health Epidemiology Reference Group and World Health Organization estimates of neonatal causes of death (Liu et al. 2012)

CAUSES OF PREMATURE BIRTH

Preterm birth occurs for a variety of reasons (March of DIMES et al., 2012):

- Elective (birth mode and time);
- Related to maternal factors;
- Related to fetal factors.

Some preterm births result from early induction of labour or caesarean birth whether for medical or non-medical reasons. Most preterm births happen spontaneously. Common causes include: multiple pregnancies, infections and chronic conditions, such as diabetes and high blood pressure and lifestyle factors, such as smoking and obesity; however, often no cause is identified. There is also a genetic influence (March of DIMES et al., 2012).

KEY POINTS

- The first 1000 days, the period between conception and two years is a time of extreme growth and development.
- In the third trimester of pregnancy, growth velocity is high for the fetus and there is rapid development of the brain, organs, immune and metabolic system.
- The right nutrition in the first 1000 days has the power to influence preterm infants' health for life.
- Prematurity is the leading cause of death in children under 5 years old and the single most important cause of death in the critical first month after birth.

MAJOR HEALTH CHALLENGES OF **PREMATURE BIRTH**

Infants born prematurely face substantial challenges that are more severe the earlier the child is born. The first challenge that they, their parents, and the hospital staff face is survival. The quality of medical care a preterm infant receives at this time is critical to their survival. Premature infants are especially vulnerable to temperature instability, feeding difficulties, low blood sugar, infections and breathing difficulties (March of DIMES et al., 2012).

THERMOREGULATION

Premature infants have a large body surface in relation to their body weight. Fat tissue is deposited during the third trimester of pregnancy, so most preterm infants have only a small amount of subcutaneous fatty tissue and low energy stores for generating heat. This means that premature infants have difficulties in keeping a stable body temperature

(Agostoni et al., 2010). Extremely low birth weight (ELBW) infants are particularly sensitive to heat loss immediately after birth. Low body temperature and cold stress may have negative effects on metabolism; cold stress increases oxygen consumption and ultimately compromises growth (Degorre et al., 2015).

It is therefore very important that premature infants are kept warm to reduce stress and improve growth. This is why most preterm infants are kept in an incubator which keeps the infant warm, reduces water loss and decreases the risk of infection. Kangaroo Mother Care was a solution to incubator shortages that was developed in the 1970s (March of DIMES et al., 2012). In Kangaroo Mother Care, the premature baby is put in early, prolonged and continuous direct skin-to-skin contact with its mother or family member to provide warmth; this also encourages breastfeeding (March of DIMES et al., 2012).

RESPIRATION

Immaturity of the lungs is a major cause of mortality and morbidity. In the womb, the fetus is supplied with the oxygen required from the mother's blood via the placenta. After birth, the infant acquires oxygen by breathing, but this may be problematic in preterm infants: firstly, the lungs are not yet fully developed and, secondly, the infant may be too weak or ill. The breathing of extremely immature infants is often supported with a ventilator – sometimes for weeks or months.

Furthermore, the premature infant poses a health challenge for four key physiological processes and nutrition plays a central role in minimising the deleterious effect of these challenges. Avoidance of growth failure which affects later life health and compromises brain development (Ehrenkranz et al., 2006, Stephens et al., 2009) is a central challenge to preterm nutrition (Ehrenkranz et al., 2006, Stephens et al., 2009, Ziegler, 2015).

GROWTH AND METABOLISM

The aim for preterm infants is to grow at a similar rate outside the womb as they would in the womb (Agostoni et al., 2010, Klein, 2002a) . The desired growth rate in preterm infants may be three to five times higher than in term infants (Clark et al., 2014, Klein, 2002a), therefore their nutritional requirements are high.

Rapid weight gain has been associated with disease risk in later life. Associations between prematurity and risks of non-communicable diseases (NCDs) in later life have been demonstrated in numerous studies as outlined below (Aggett et al., 2006, Hay, 2008, March of DIMES et al., 2012).

Preterm birth has been associated with a number of increased disease risks:

- Type 2 diabetes (Hovi et al., 2007, Kajantie et al., 2010, Mercuro et al., 2013)
- Metabolic abnormalities (Abitbol et al., 2012)
- Cardiovascular disease (Kajantie et al., 2014, Kerkhof et al., 2012, Skilton et al., 2011)
- Atherogenic lipid profile (Hovi et al., 2013)
- Advanced pubertal growth (Wehkalampi et al., 2011)
- Renal disease (Abitbol and Rodriguez, 2012)
- Bone mineral density (Hovi et al., 2009)

Immature kidneys and increased water loss through the skin means that the water and electrolyte balances of premature infants must be carefully monitored (Agostoni et al., 2010).

Small body stores and increased stress mean that low birth weight infants also have problems in maintaining stable blood sugar levels.

A recent study in young adults born VLBW showed that additional protein intake at relatively low neonatal protein levels was associated with a healthier body composition and higher metabolic rate (Matinolli et al., 2015).

Young adults (aged 19–27 years) who were born preterm were shown to have a less healthy diet in one study; they consumed less vegetables, fruits, berries and milk products with calcium and vitamin D (Kaseva et al., 2013). This study also found that they were less physically active, perhaps as a consequence of complications e.g. cerebral palsy, motoric control, visual impairment and hearing impairment (Kaseva et al., 2012).

GROWTH MONITORING AND GROWTH CHARTS

Some preterm babies are born SGA, which means that they have already experienced growth restriction in utero. Preterm infants are often born with VLBW, and despite displaying catch up growth when nutritional provision is adequate, are at an increased risk of early stunting (Santos et al., 2009). Growth faltering often begins at birth and in some premature babies continues during their hospital stay, this is particularly apparent in the extremely premature infants with significant complications. There is strong evidence that preterm infants have a higher risk of growth faltering in the first two years of life (Santos et al., 2009). On the one hand, rapid catch up growth confers potential short-term advantages in terms of survival and neurodevelopment, whilst on the other hand, it is also associated with a possible increased risk of metabolic disorders in later life, including hypertension, insulin resistance and cardiovascular disease (Fewtrell et al., 2016).

Appropriate weight gain is a desirable outcome nonetheless to ensure survival, optimal growth and neurodevelopment and preterm infants should undergo regular growth assessments. The growth goal is to mimic the estimates of fetal growth, although this will not always be possible in the extra-uterine environment. The WHO international growth charts are not suitable for preterm growth assessment prior to term corrected age (World Health Organization, 2017). Several preterm charts are available internationally to monitor growth trajectories in preterm infants after birth; the Fenton charts are the most well-established of these (Fenton et al., 2013a, Fenton et al., 2013b). These charts are based on an international meta-analysis of six large population surveys comprising nearly four million preterm infants. The charts depict expected body weight, length and head circumference in infants born from 23 weeks' gestation; they are designed to link with the WHO infants growth charts around 50 weeks' gestational age (Fenton and Kim, 2013a). The charts are available to download from the University of Calgary's website (accessed 19/02/2017): http://ucalgary.ca/fenton/2013chart

The International Fetal and Newborn Growth Consortium for the 21st century, or INTERGROWTH-21st is a global, multidisciplinary network of more than 300 researchers and clinicians from 27 institutions and in 18 countries and is coordinated from the University of Oxford in the UK. In 2014, INTERGROWTH-21st published a set of sex-specific international standards for fetal growth to complement the WHO standards for post-natal growth. They can be downloaded from the INTERGROWTH-21st website (accessed 19/02/2017): https://intergrowth21.tghn.org/articles/intergrowth-21st-fetal-growth-standards/

A year later, INTERGROWTH-21st published postnatal preterm charts which are likely to be used more frequently despite the study numbers being smaller than in the Fenton study (growth book). These charts can also be downloaded from the INTERGROWTH-21st website (accessed 19/02/2017): https://intergrowth21.tghn.org/articles/new-intergrowth-21st-international-postnatal-growth-standards-charts-available/

BRAIN DEVELOPMENT

Ensuring optimal brain development is a priority for the premature infant. The last trimester of pregnancy to two years of age is the sensitive period of brain development. Any growth deficits will compromise brain development, which may have long-term effects on multiple organ systems.

Preterm infants are also at a substantial increased risk for neurodevelopmental impairments and psychiatric difficulties (Aggett et al., 2006, Hay, 2008, March of DIMES et al., 2012). Prematurity has been associated with an increased risk of: impaired motor control, cerebral palsy, visual impairment (blindness) and dyslexia, hearing impairment (deafness) and subsequent reduced academic achievement (Pyhala et al., 2011, Strang-Karlsson et al., 2010). Prematurity has also been associated with behavioural difficulties, such as attention deficit hyperactivity disorder, autism, increased anxiety and depression (March of DIMES et al., 2012).

A meta-analysis of 2390 preterm children and 1549 controls showed that preterm children have substantial difficulties in reading, spelling, and arithmetic. Children born preterm scored 0.71 SD below full-term peers on arithmetic ($p<0.001$), 0.44 and 0.52 SD lower on reading and spelling ($p<0.001$) and were 2.85 times more likely to receive special educational assistance (95%CI 2.12 to 3.84, $p<0.001$) compared to term born peers (Twilhaar et al., 2017). A further longitudinal study also demonstrated that very preterm infants score lower on arithmetic, reading comprehension and spelling that persist throughout primary school, with increased educational assistance and grade repetition, and lower secondary education levels (Twilhaar et al., 2018). Very preterm infants showed a similar progression to full-term children suggesting that that deficits apparent in the first grade of primary school do not improve or worsen with progression through later grades. This also suggests intact learning abilities, providing potential opportunities for intervention (Twilhaar et al., 2018).

GASTROINTESTINAL FUNCTION

FEEDING CHALLENGES

The tiny stomachs of premature infants have a limited volume tolerance, which in turn limits the amount of feed that can be given at any one time (Klein, 2002a, March of DIMES et al., 2012). The preterm also has an immature gut and metabolic system and the digestion and absorption of nutrients may be less efficient, because not all enzymes have reached full activity. This is compounded by their limited nutrient reserves and low body fat stores. Premature infants born earlier than 34 weeks often struggle with coordinate latching, suckling, swallowing and breathing (Agostoni et al., 2010). In addition, intestinal muscle movement (peristalsis) is still weak and uncoordinated resulting in gastric residues and/ or constipation. These feeding challenges need to be overcome to prevent growth failure and its subsequent long-term health implications.

NECROTISING ENTEROCOLITIS

Necrotising enterocolitis (NEC) is a potentially fatal inflammation of the bowel and the most intestinal emergency in premature infants, particularly ELBW and VLBW infants. Severe NEC leads to death, sometimes within hours. Despite intensive research over the past 30 years, the causes of this multi-factorial event have still not been fully understood but microbiota overgrowth is suspected to be one of the factors in its aetiology. It has been shown that maternal milk protects against NEC which is one of the reasons that maternal milk is promoted so avidly amongst neonatologists (King et al., 2015).

IMMUNE HEALTH

Many premature infants die due to infections (March of DIMES et al., 2012) caused by their immature immune system. Furthermore, medical interventions (e.g. intravenous feeding) and the hospital environment which contains potentially harmful pathogens increase the risk of infection. The intestinal microbiota plays an important role in immune defence. Preterm infants have less 'gut friendly' bacteria than term infants due to varying factors including: Caesarian section or rapid vaginal deliveries, prolonged hospitalisation and antibiotics and invasive procedures. As a result they are immunocompromised and at increased risk of infection (Groer et al., 2014). Even if not leading to death, infection impacts negatively on the growth and development of the preterm infant.

KEY POINTS

- In utero development is interrupted with premature birth; thus, preterm infants present with significant health challenges.
- Prematurity is associated with physiological, psychological and behavioural health risks which have long-term health implications.
- Prevention of growth failure and retardation through nutrition is crucial for metabolic, brain, immune and gut development.
- Vigilant growth monitoring in hospital is required to ensure growth is proportional and appropriate to achieve optimal development and recovery growth and avoid excessive weight gain.
- An immature GI tract and underdeveloped metabolic processes make feeding the preterm infant particularly challenging.

NUTRITIONAL NEEDS OF **PRETERM INFANTS**

Nutrition during early life is now recognised not only as a key determinant for immediate neonatal survival, growth and mental development during infancy (Clark et al., 2003, Painter et al., 2005, Zhang et al., 2011), but also as a major conditioning factor for long-term health (Koletzko et al., 2014). The recognition of the long-term impact of nutrition and growth has been a significant development in scientific thinking in recent years.

The fetus grows very rapidly in the last trimester. From the 23rd to the 36th week of gestation, intrauterine weight gain is 10-18g/kg/day. Indeed the fetus doubles its body weight between weeks 30-36 of gestation. At the same time tissues develop and differentiate, and organ and biological systems continue to develop and mature. The high nutritional needs for growth combined with the immature gut and other organs has been described as a 'nutritional emergency' (Corpeleijn et al., 2011).

Preterm infants have small endogenous nutrient reserves (glycogen and fat), immature physiological systems, a higher metabolic rate, an unstable medical condition and an increased growth rate. It is recognised that any infant born <2,000g and <34 weeks gestation will benefit from higher nutrient intakes (Agostoni et al., 2010, Tsang, 2005). Theoretically, the endogenous reserves in a 1000g infant are only sufficient for four days if unfed (Henderson et al., 2007).

The principle of preterm nutrition is to provide the energy and nutrients required to achieve a similar rate of growth and development as a fetus born term in utero, despite the immature physiology of the preterm infant. Intrauterine growth is often used as assessment for nutritional requirements of preterm infants (Agostoni et al., 2010, Klein, 2002a, Tsang, 2005). The intrauterine growth rate is about three times higher than that of term infants (Clark et al., 2003, Klein, 2002a).

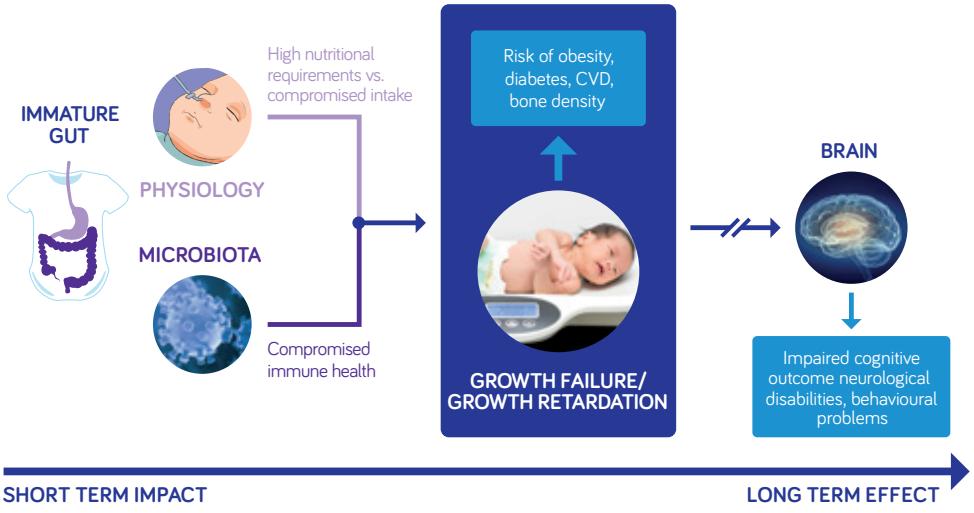
The degree of prematurity and birth weight will affect individual nutritional needs and as such a 'one size fits all' approach is not appropriate when feeding the preterm infant.

ROLE OF NUTRITION

Preterm nutrition supports optimal growth and development and specific nutrients are particularly implicated in the development of specific physiological processes and organs.

- Growth and metabolism – this requires high energy intakes, optimal protein intakes and protein/energy ratios (PER) (Ehrenkranz et al., 2006, Stephens et al., 2009), and high quality lipids.
- Brain development – iron and long-chain polyunsaturated fatty acids (LCPUFAs), specifically docosahexaenoic acid (DHA) and arachidonic acid (ARA)(Koletzko et al., 2014).
- Gut function and immune health – prebiotic oligosaccharides e.g. long-chain fructo-oligosaccharides (lcFOS) and/or short-chain galacto-oligosaccharides (scGOS) (Boehm et al., 2002, Knol et al., 2005).

These benefits are interlinked. Meeting the high nutritional requirements (especially protein) for growth and supporting the colonisation of healthy intestinal microbiome are essential to the improvement of gut and immune health. Suboptimal intestinal microbiota and an immature immune system may play a role in the risk of infection and NEC which would further compromise growth. Growth failure will also have an adverse effect on brain development.



Adequate growth is a major challenge for preterm infants. It is affected by an immature gut and metabolic physiology and as a consequence inadequate intake, digestion, uptake and usage of the nutrients. Moreover suboptimal intestinal microbiota and an immature immune system may play a role in the risk of infection and necrotising enterocolitis, further compromising growth. Together the factors/processes leading to growth failure also impact/compromise brain development.

Figure 3 The critical role of nutrition in the preterm infant for the short and long-term

ESTABLISHING FEEDING

Most infants born < 32 weeks will require parenteral nutrition. Parenteral nutrition provides energy, nutrients, fluid and electrolytes the premature infant needs to survive (Ziegler et al., 2002). Enteral nutrition is commenced via a feeding tube passed through the nose or mouth into the stomach (nasogastric tube).

As enteral feeds are tolerated at an increased volume, parenteral nutrition is concurrently reduced; the aim is to keep the parenteral feeding period as short as possible because of the associated risks. The time for this transition varies between preterm infants depending on their comorbidities. There has been reluctance to advance feeds too quickly because of the fear of increasing the risk of NEC. A randomised trial is currently underway which compares the effects of two speeds of increasing milk feeds in very preterm, <32 weeks or VLBW infants (Abbott et al., 2017). Outcome data will be collected throughout the hospital stay and at 24 months corrected age. This novel study will provide strong evidence on how to advance enteral nutrition in these vulnerable infants.

BREAST MILK IMPORTANCE IN PRETERM NUTRITION

Breast milk (BM) is acknowledged to be the best feeding for preterm infants (Gartner et al., 2005). The American Academy of Pediatrics (AAP) strongly recommends the use of BM because of its unique advantages with respect to host protection, its positive role in colonisation of the gut microbiota, its role in priming the gut for receiving enteral feeds and its positive effect on gut maturation (Gartner et al., 2005). This position is endorsed by the most recent recommendations which highlights the trophic properties of early feeding after birth (Koletzko et al., 2014). BM might in the short-term protect against infections, sepsis and NEC (Gartner et al., 2005). In the long-term, BM might also lead to improved neurocognitive development (Gartner et al., 2005). Recent work has shown that human colostrum (i.e. first milk) and later breast milk, which had been traditionally considered sterile, actually provides a continuous supply of commensal bacteria -harmless bacteria that are normally present in a given area of the body (Agostoni et al., 2010).

Besides the positive effect on infections, breast milk feeding protects infants from the development of atopic diseases, particularly if there is a family history (van Odijk et al., 2003) and other immune-mediated diseases (Brandtzaeg, 2003, Hanson et al., 2003).

Although this protective effect has been attributed to the presence of secretory immunoglobulin and related components in breast milk, it is well established that the protective effect of breastfeeding is also related to the presence of oligosaccharides (Collins et al., 1999, McVeagh et al., 1997).

FORTIFICATION OF BREAST MILK

The high nutritional needs of the premature infant can be met by breast milk with careful fortification and supplementation. These requirements will vary between infants and must be continually monitored and adjustments made to the additions as clinically indicated (Agostoni et al., 2010). The negative outcomes of un-supplemented BM have been described in the literature (Arslanoglu et al., 2010). For instance, it has been reported that VLBW preterm infants fed exclusively un-supplemented breast milk grow more slowly, retain less nitrogen and have a higher occurrence of abnormalities of bone and mineral metabolism than those receiving a specially formulated preterm formula (Arslanoglu et al., 2010). For these reasons, it is recommended to supplement BM with a breast milk fortifier (HMF).

Multicomponent fortification, i.e. adding vitamins and minerals as a preparation to breast milk, has also been shown to be associated with good growth, i.e. short-term improved weight gain and head grow (Kuschel et al., 2004). Multicomponent fortification should start well before full enteral feeding volumes have been met (Koletzko et al., 2014). The composition of BM is influenced by the diet of the mother and even more by the phase of lactation. BM in the first week postpartum has, for instance, more proteins compared to later milk.

ALTERNATIVES TO MATERNAL MILK

Some mothers struggle to express their milk and are under intense stress which may affect their milk supply. An alternative to maternal milk may be donor milk and many large hospitals will have a milk bank for this purpose. Pasteurisation and storage conditions of breast milk influence the composition of the milk (King and Tavener, 2015).

In the absence of breast milk a nutritionally tailored formula meeting requirements of premature infants is an acceptable alternative (Agostoni et al., 2010, Tudehope, 2013). The order of preference of nutritional sources of enteral feeding is shown in Figure 4.

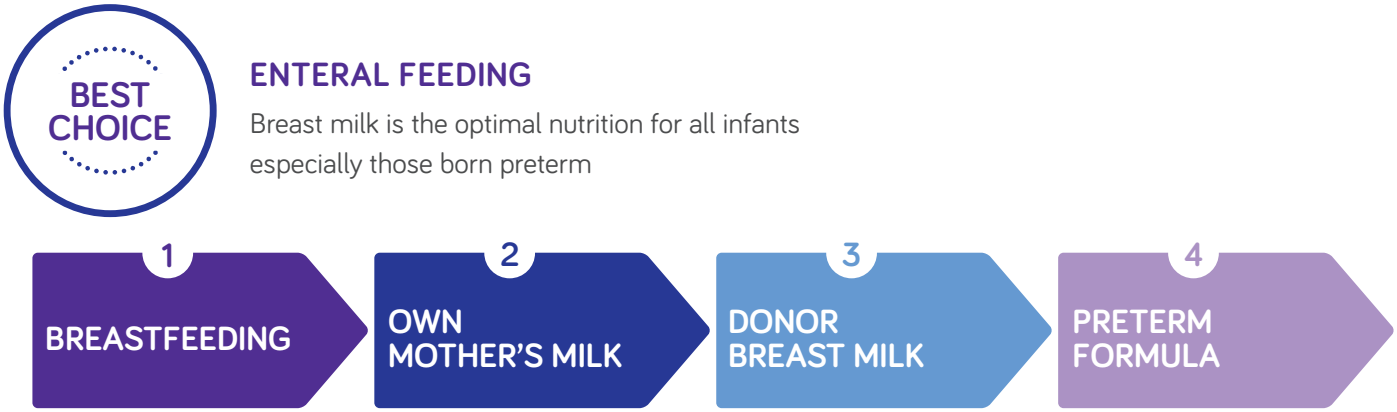


Figure 4 Feeding options for the preterm infant in order of preference

EXPERT RECOMMENDATIONS

Nutritional recommendations for preterm infants in hospital have evolved over the past three decades, as shown in Figure 5, due to an increased understanding of the physiological state and nutritional requirements of these vulnerable infants. The latest opinions of international experts were collected by Koletzko-Poindexter-Uauy (eds.) in 2014 which provides detailed guidance on nutritional requirements of preterm infants born at differing weights (Koletzko et al., 2014). The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) recommendations from 2010 had hitherto been the most up-to-date and definitive guidance (Agnostoni et al., 2010).

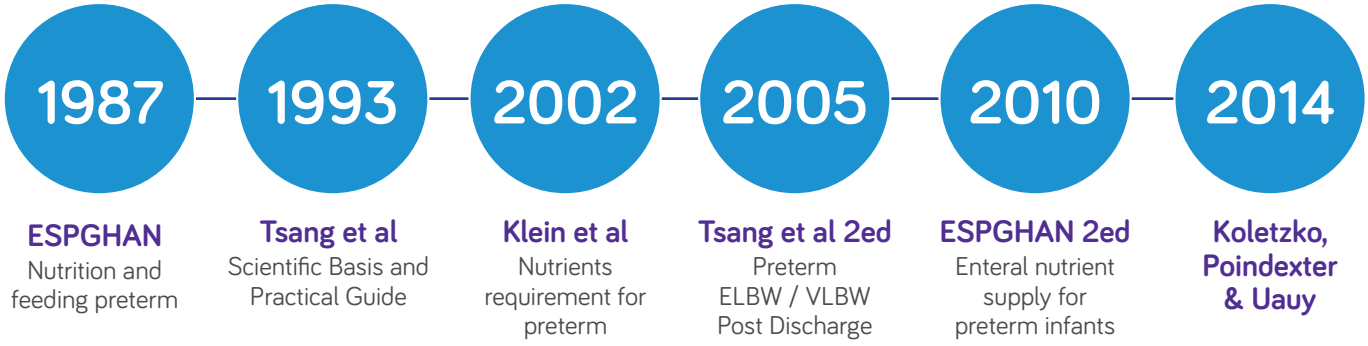


Figure 5 Timeline of nutritional guidelines for preterm infants

PROTEIN REQUIREMENTS

The supply of protein is of particular importance for the growth and health of a premature infant. The objective is to provide the correct quantity and quality of protein required to achieve adequate growth and the corresponding nitrogen retention (Koletzko et al., 2014). However, it is important to avoid the accumulation of potentially harmful end products of protein breakdown. Protein utilisation is influenced by the uptake of protein and non-protein nitrogen and by the biological value of the proteins taken up, the nutritional status, catch-up growth, the hormonal situation, clinical status and the PER.

Growth failure due to suboptimal protein intake can lead to impaired neurocognitive development, that preterm infants might show later in life. A large study on children born between 22 and 25 weeks, who were currently at school age found that 46% had severe or moderate disabilities, such as cerebral palsy, vision or hearing loss and learning problems (Lucas et al., 1998). For that reason, adequate nutrient provision including protein intake is relevant to improve growth and neurocognitive outcome (Ziegler et al., 2009).

In 2010 ESPGHAN revised their 1987 recommendations on nutrition and feeding of preterm infants (Agostoni et al., 2010). In these reccomendations, protein intake was specified for the first time by body weight. These recommendations are shown in Table 2.

Body weight	Daily protein requirements
<1000g (ELBW)	4.0-4.5g/kg 3.6-4.1g/100kcal
1000-1,800g (VLBW)	3.5-4.0g/kg 3.2-3.6g/100kcal

Table 2 Protein requirements in ELBW and VLBW infant

ESPGHAN based their recommended quantity of proteins on factorial data as well as on empirical data. In factorial research protein needs are based on both protein accreditation (growth) and protein losses (urinary nitrogen loss and dermal loss). Based on these data a protein level of at least 3.0 g /kg/day was set (Agostoni et al., 2010).

These figures also take account of the fact that the protein supply has to compensate for accumulated protein deficiencies that are seen in almost all premature infants and may vary in extent depending on the weight of the infant.

KEY POINTS

- An individualised approach should be taken to the provision of nutrition in the preterm infant to ensure that their high nutritional needs are met.
- The aim of preterm nutrition is to achieve the growth and development that would have occurred in utero in the third trimester and to reduce the risks of later health risks.
- Nutrition plays a central role in preventing growth failure and supporting the development of the metabolic system, brain, gastrointestinal function and immune system.
- Breast milk is the preferred choice of feeding for the preterm infant.
- Expert guidance exists to guide the feeding of the preterm infant and the composition of products.
- Adequate protein intake is critical to the growth and development of the preterm infant.

NUTRICIA'S **NUTRITIONAL EXPERTISE**

Nutricia are committed to supporting breastfeeding. For more than 40 years, our scientists have been dedicated to studying breast milk and its specific benefits.

BREAST MILK RESEARCH

Nutricia have and continue to actively study the composition of breast milk for its full spectrum of macro, micronutrients and beneficial bacteria. Nutricia have a broad understanding of pre-, pro- and synbiotics and study the absorption and digestion of these compounds in early life. In addition, Nutricia study the role of gut microbiota in stimulating the immune system and gastrointestinal system. The Nutricia Breastfeeding Research Team has published numerous papers on breast milk including analytical methods, human milk oligosaccharides (HMOs), proteins, lipids, beneficial bacteria and advancing breastfeeding knowledge. In 1977, Nutricia were the first company to improve the casein-whey ratio in their products according to findings in their breast milk research. In 1994, the team were the first to describe the complex spectrum of HMOs.

Nutricia Research strives to further investigate the benefits of breastfeeding to be able to optimally support mums and infants with innovative nutrition and services in The first 1000 days. It aims for a better understanding of how maternal, social and psychological factors may influence breast milk composition and breastfeeding behaviour and impact the health of the breastfed infant now and in later life. Nutricia will continue its journey to unravel the complexity of breast milk and the benefits of breast milk and breastfeeding.

PRETERM EXPERTISE

For over 60 years Nutricia has been developing expertise in preterm nutrition research. Nutricia and its subsidiaries have shared its knowledge in more than 305 peer-reviewed publications. In collaboration with external academic and/or clinical partners, Nutricia has published more than 150 papers advancing knowledge in preterm nutrition in four clinical benefit areas:

- Growth & metabolism
- Brain development
- Gastrointestinal function
- Immune health

PUBLICATIONS IN THE PRETERM FIELD

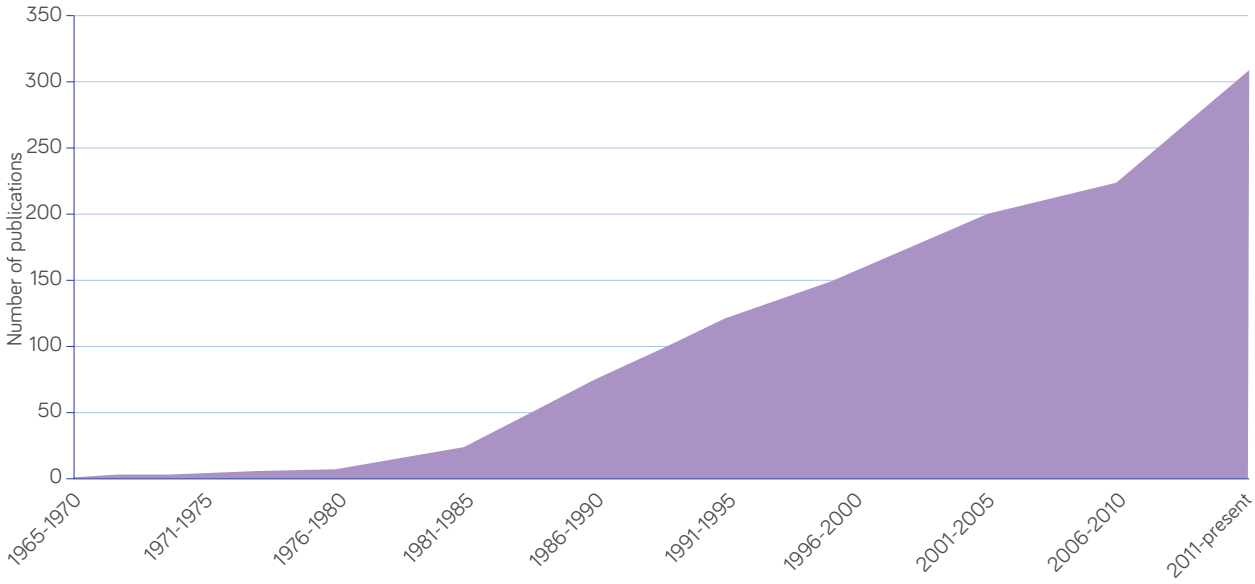


Figure 6 Nutricia's cumulative number of publications in the preterm field

The ever increasing contribution that Nutricia is making to preterm research is shown in Figure 6. These publications span preclinical and clinical research, and further the understanding of the important role of nutrition in the preterm infant, in hospital through to their discharge home. This deep understanding of preterm nutrition has led to pioneering nutritional solutions for the preterm infant and has been possible because of Nutricia's strong research base and notable collaborations with academics and leading neonatal clinicians.

PIONEERING NUTRITIONAL SOLUTIONS

Four advancements which will be discussed are:

- Development of a patented prebiotic oligosaccharide blend in 1998, which has demonstrated benefits for gastrointestinal function and immune health.
- Inclusion of LCPUFAs for the first time in a preterm infant formula in 1992; LCPUFAs are now recognised to play a critical role in brain development.
- Provision of LCPUFA as phospholipid-bound.
- Development of a fat blend that is closer to the lipid profile in breast milk.

PREBIOTICS – NON-DIGESTIBLE OLIGOSACCHARIDES

The development of Nutricia's unique and patented blend of prebiotic non-digestible oligosaccharides was the culmination of years of research. The importance of the intestinal microbiota is discussed below with reference to the preterm infant. A summary of Nutricia's preterm research on its prebiotic non-digestible oligosaccharides is provided. Nutricia's patented prebiotic is sc GOS and lcFOS in a 9:1 ratio.

The intestinal microbiota plays a key role in postnatal development of the immune system (Cebra, 1999, Kemp et al., 2003). Supporting the development of a healthy microbiota and reducing the pathogen load early in life is important to secure a stable and healthy microbiota later in life.

As reviewed by Scholtens and colleagues (Scholtens et al., 2012), gut colonization is influenced by gestational age although little is known about the colonization process in preterm infants (Mshvildadze et al., 2010a, Mshvildadze et al., 2010b). A major consequence of premature birth appears to be delayed colonisation with a limited number of species (Jacquot et al., 2011, Rouge et al., 2010). Generally, and in contrast to term infants, the first colonisers in preterm infants seem to be predominantly coliforms, enterococci, and bacteroides. In addition, preterm infants are often colonised by potentially pathogenic species, including klebsiella, enterobacter, and clostridium species, with a reduced degree of colonization by normal commensal microbiota, such as bifidobacteria and lactobacili (Butel et al., 2007, Magne et al., 2006). These circumstances place premature infants – especially those with no or little intake of breast milk – at risk to develop an unfavourable microbiota composition with a subsequently weakened immune system and increased risk of infections.

Fortunately, intestinal colonisation can be modulated by dietary intake. Although before birth the infant's gut contains few bacteria, its natural colonisation accelerates at vaginal delivery with a transfer of maternal vaginal and intestinal microbiota (Martin et al., 2010, Oozeer et al., 2013). During breastfeeding the composition of the gut microbiota develops further and within a short period becomes dominated by bifidobacteria (Harmsen et al., 2000). This colonisation with particular bacteria is in part mediated via prebiotics, which are non-digestible dietary components that promote the growth of favourable microorganisms if these reach the colon undigested. Prebiotics can be specific HMO but also other nutrients such as lactose, lcFOS or scGOS.

HMOs have been shown to promote colonisation with healthy bacteria, supporting the gut barrier, discourage colonisation by harmful bacteria, supporting the gut-associated lymphoid tissue, representing two thirds of the immune system (Harmsen et al., 2000, Knol et al., 2005) suggesting that HMO may have anti-infection roles in the intestinal, respiratory, and urinary tracts (Gewolb et al., 1999, Sakata et al., 1985).

Preterm infants are able to absorb lactose and intact HMO, yet most HMO and some lactose resist digestion in the small intestine and undergo fermentation in the colon (Brand-Miller et al., 1998, Kien, 2001) suggesting potential benefits of prebiotic feeding.

There is an abundant amount of research in term infants demonstrating that scGOS/lcFOS (9:1) specifically stimulate the growth of bifidobacteria and lactobacilli and reduce the growth of pathogens. As a consequence, faecal pH values, short-chain fatty acid pattern and stool consistency become similar to those in breastfed infants. scGOS/lcFOS (9:1) also support the gut barrier, a major line of defence against infections (Arslanoglu et al., 2008, Costalos et al., 2008, Moro et al., 2006, Moro et al., 2002). This has been reviewed in extensive detail in a number of review papers (Boehm et al., 2008, Martin et al., 2010, Scholtens et al., 2012, Scholtens et al., 2014).

The use of prebiotics in preterm feeding is deemed safe (Srinivasjois et al., 2013). The most recent Cochrane meta-analysis from 2013 comprised seven studies, five of which were associated with the Nutricia prebiotic mixture scGOS/lcFOS (9:1) (Srinivasjois et al., 2013).

Clinical trials in preterm infants have shown that scGOS/lcFOS (9:1) support a healthy intestinal flora by increasing the concentration and proportion of bifidobacteria and by discouraging the growth of potential pathogens (Boehm et al., 2002, Knol et al., 2005) thus promoting a microbiota composition more similar to that of breastfed infants.

Researchers in independent studies have reported a lower pH in the prebiotic group of their interventions (Mihatsch et al., 2006, Modi et al., 2010, Westerbeek et al., 2008), which is considered to encourage colonization with bifidobacteria and is also observed in breastfed infants (Boehm and Moro, 2008). Note that in the exploratory CARROT study the prebiotic blend was scGOS/lcFOS (9:1) extended with acidic oligosaccharides (AOS) (Westerbeek et al., 2008).

scGOS/lcFOS (9:1) also strengthen innate immunity by affecting parameters of intestinal motility. Intestinal motility is an essential component of innate immunity as it enhances gastric emptying and clearing food debris, thus prohibiting pathogen accumulation, overgrowth and colonisation of the gut (Neu et al., 2012). Preterm infants fed the scGOS/lcFOS (9:1) mixture demonstrated softer stools and higher stool frequency in several studies (Dasopoulou et al., 2013, Mihatsch et al., 2006) as well as shorter gastrointestinal transit time (Mihatsch et al., 2006) and faster gastric emptying (Dasopoulou et al., 2013, Indrio et al., 2009) without affecting number of vomits and regurgitation episodes (Dasopoulou et al., 2013).

The scGOS/lcFOS (9:1) mixture strengthens the infant's immune system leading to fewer allergic symptoms when fed in a hypoallergenic formula to term infants at risk of allergies (Arslanoglu et al., 2008, Moro et al., 2006). It also reduces incidence of infections in term infants (Bruzzese et al., 2009) and reduces use of antibiotics in this population (Bruzzese et al., 2009). The studies in term infants provided evidence which suggested that a similar benefit may be observed in the preterm population. Immune parameters of clinical interest in preterm infants, such as a reduction of the incidence of NEC, (late onset) sepsis, or the modulation on inflammation were unaffected or did not reach statistical significance (Srinivasjois et al., 2013). The absence of significant effects is likely due to the prophylactic and acute use of antibiotics during hospitalisation, which is linked to the delayed colonisation (Scholtens et al., 2012). In line with this hypothesis, the extended use of antibiotics has been linked to the incidence of atopic dermatitis at six years of age in the CARROT study (Carstens et al., 2016).

Given the evidence-base for scGOS/lcFOS (9:1) positively impacting on the gut microflora and immune system in term infants, Nutricia's preterm formulae contain the same prebiotic oligosaccharide blend as found in their term formulae.

LONG CHAIN POLYUNSATURATED FATTY ACIDS

LCPUFA can be divided in two families, the omega-6 (or n-6) and the omega-3 (or n-3) family, of which linoleic acid (LA) and α-linolenic acid (ALA) are the precursors, respectively. These essential fatty acids can be modified to a series of longer-chain fatty acids such as gamma-linolenic acid (GLA, C18:3 n-6), eicosapentaenoic acid (EPA, C20:5 n-3), docosahexaenoic (DHA) (C22:6 n-3), and arachidonic acid (ARA) (C20:4 n-6) showing in these forms most of their biological activity (Sprecher et al., 1999). By further modification, GLA, EPA, DHA and ARA give rise to metabolically active mediators. These bioactive metabolites are important regulators of bodily function such as pain pathways, inflammation, thrombosis and vasoconstriction by serving as signalling molecules and transcription factors (Uauy et al., 2015).

The DHA and ARA status of preterm infants is positively correlated to their neurocognitive development including visual acuity and ARA status is additionally positively correlated with first year growth (Carlson et al., 1996).

However, although preterm infants are able to elongate and desaturate LA and ALA, the process has not yet reached full efficacy (Carnielli et al., 1996, Makrides et al., 2000, Sauerwald et al., 1997) so preterm infants are dependent on dietary LCPUFA supplementation.

Nutricia (as Milupa), was the first to include LCPUFA in infant formula and added LCPUFA to preterm formula in 1992 which was a major innovation at the time. Since then LCPUFA provision has been included in expert guidance and since 2006 ESPGHAN recommends supplying all formula-fed preterm infants after hospital discharge with LCPUFA (Aggett et al., 2006).

PHOSPHOLIPID-BOUND LONG-CHAIN POLYUNSATURATED FATTY ACIDS

Fats naturally occur as triglycerides (TG) or phospholipids (PL). Breast milk consists mainly of triglycerides i.e. three fatty acids attached to a glycerol backbone.

LCPUFA in PL–bound form are more closely related to the form in which they occur in the body's structural tissue, such as eye and brain (Bitman et al., 1983). About 98% of BM lipids are TG; only 0.5- 0.7% of BM lipids are in PL-form and 0.5% are cholesterol. However, in BM, LCPUFA are about ten times more often bound to PL-molecules than to TG, meaning that in BM, the PL fraction provides a relatively large portion of ARA and DHA to infants (Harzer et al., 1983).

Both the brain and retina contain phospholipids that are rich in DHA and ARA. About 25% of the brain's total fatty acid content, mostly in the form of phospholipids, contain DHA and ARA and their accumulation in the brain has been linked to cognitive benefits, as reviewed in Hadley et al. (2016) (Hadley et al., 2016).

Nutritionally, TG and PL forms of LCPUFA are not equivalent: dietary PL-DHA is more efficiently absorbed by preterm infants than TG-DHA as shown by Carnielli et al. 1998. This group compared uptake from breast milk to formula containing LCPUFA -PL and formula with LCPUFA-TG in preterm infants. Although the intestinal absorption of ARA was not different between the groups, the DHA-PL was more efficiently absorbed than DHA-TG (Carnielli et al., 1998). Carnielli et al. also showed that absorption of all n-3 but not n-6 LCPUFA was better from PL than from TG or even preterm breast milk.

Tissue development, especially of brain and eye, are supported by provision of LCPUFA. These include LA, ALA, EPA, gamma-(dihomo) linolenic acid (DGLA), and DHA and ARA. Even more so, it is considered that formulae with a mixture of TG- and PL-bound LCPUFA – as found in BM – could be beneficial to the infant as reviewed by Hadley et., 2016 (Hadley et al., 2016).

In ongoing research since 1983, Nutricia has demonstrated that lipid form is important for the functional benefits of LCPUFAs. Since the introduction of PL-bound LCPUFA in the Nutricia (Milupa), fat blend, many studies have been published investigating its positive effects in preterm infants (Boehm et al., 1996, Chirouze et al., 1994, Damli et al., 1996, Faldella et al., 1996, Koletzko et al., 1995, Koletzko et al., 1989)

FAT BLEND CLOSER TO THE PROFILE OF LIPIDS IN BREAST MILK

The predominant fatty acids (FA) in BM are oleic acid (C18:1 n-9, 35.5%) and palmitic acid (C16:0, PA, 26.5%). 60-70% of the palmitic acids in breast milk are presented in sn-2, the beta (β) position, compared to only 39% in cow's milk. Palmitic acid from vegetable oil, such as palm oil, is usually positioned at the sn1, the alpha (α) position.

The position of palmitic acid within the glycerol molecule has been found to be relevant for the efficacy of lipid assimilation. For instance, it was demonstrated that plasma levels of breastfed infants contain a higher proportion of C16:0 in the sn-2 position compared with non-breastfed infants (Innis et al., 1994). Adding anhydrous milk fat to infant feeding also improves fat absorption and digestion in term and preterm infants (Carnielli et al., 1995a, Carnielli et al., 1995b).

Milk fat is considered an alternative in replacing palm oil in infant formula milk, because it provides a different FA profile with more βpalmitic acid, which is more comparable to breast milk (Innis et al., 1994).

The middle position of the triglyceride in breast milk is known as the sn-2 position and a fatty acid on the sn-2 position is said to be β -positioned e.g. β -palmitate in the case of palmitic acid. This structure of fat is broken down and absorbed very efficiently by infants and has been shown to be well tolerated, result in softer stools and improve calcium absorption in infants when compared with vegetable fats (Bar-Yoseph et al., 2013, Kennedy et al., 1999, Quinlan et al., 1995).

This contrasts to vegetable fat and traditional infant formula when the palmitate is in the sn-1 and sn-3 position (Figure 7).

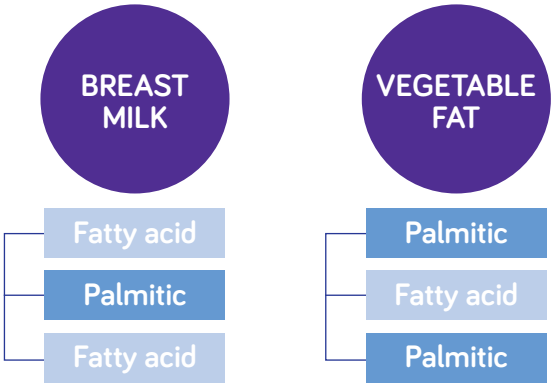


Figure 7 Differences between breast milk and vegetable fat structure

From 2017, Nutricia's Preterm Hospital Formula (PHF) and Post Discharge Formula (PDF) consist of an upgraded milk fat blend that contains sn-2 palmitate. This upgrade is a testament to Nutricia's ongoing study of breast milk, and commitment to optimise solutions to provide superior nutrition to preterm infants. This milk fat blend has benefits for three clinical benefit areas:

- **Growth and metabolism** – Breast and bovine milk lipids with the sn-2 palmitate structure are digested, absorbed and utilised efficiently by infants. The reduction of calcium soap formation seen in milk fat compared to vegetable lipids means that calcium is absorbed more efficiently which is important for bone development.
- **Brain** – LCPUFAs are essential for brain development and PL-bound LCPUFA have shown to be readily incorporated into the brain. Preclinical trials showed that an increased palmitic acid on the sn-2 position contributes to improved fatty acid absorption (Liu et al., 2014). Preclinical studies have demonstrated that an increased availability of DHA is important for the neuroplasticity of the brain (Delplanque et al., 2013, Dinel et al., 2016)
- **GI function** – vegetable lipids with palmitate in the sn-1 or sn-3 position create calcium soaps when metabolised. Breast and bovine milk fat with the sn-2 palmitate structure do not create calcium soaps and result in softer stools.

  A study showed that infants fed with a high sn-2 infant formula had (Kennedy et al., 1999)
  - Lower proportion of palmitic acid in stool fats
  - Reduced stool hardness
  - Stool characteristics and biochemistry intermediate between that of breastfed infants and those fed a standard infant formula.

A review of sn-2 palmitate oil (C16:0) showed that the progressive enrichment of triglycerides at the sn-2, rather than sn-1or sn-3 positions, led to a dose response increase in both calcium and palmitic acid absorption. The reduction in faecal calcium and palmitic acid as calcium soaps is accompanied by a decrease in the incidence of harder stools (Bar-Yoseph et al., 2013)

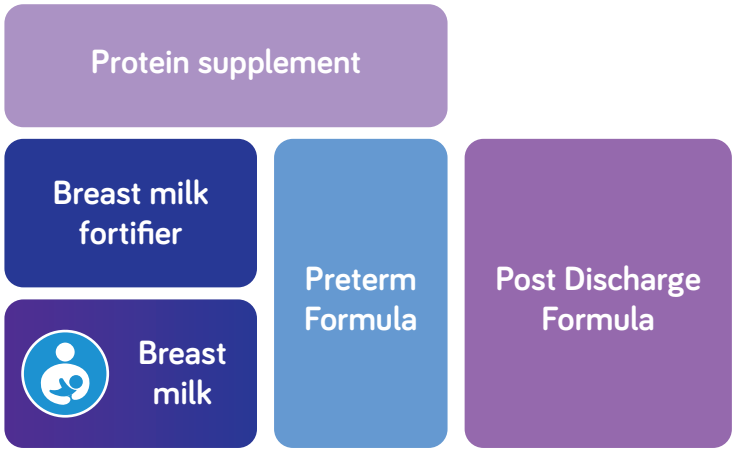
KEY POINTS

- Nutricia is committed to supporting breastfeeding
- Nutricia is inspired by, and actively involved in advancing the science of, breast milk
- Nutricia has a long-standing and proven track record in preterm nutrition research in a number of areas of clinical benefit: growth, metabolism, brain development, gastrointestinal function and immune function.
- Nutricia's research has led to unique advancements in the provision of preterm nutrition:
  - The first company to include LCPUFA in preterm formulae in the early 1990s.
  - Development of a unique patented blend of prebiotic oligosaccharides that mimics the natural prebiotic effect of human milk oligosaccharides.
  - Provision of LCPUFA that are phospholipid bound.
  - Development of a milk fat blend that is closer to the lipid profile found in breast milk.

NUTRICIA'S **PRETERM SOLUTIONS**

Nutricia recognises that a personalised and tailored approach is required to meet the nutritional needs of each preterm, which is reflected in the range of products for use in hospital and after discharge. Nutricia's Hospital products all comply with the ESPGHAN 2010 recommendations for composition (Agostoni et al., 2010), and the extensive range of personalised nutrition supports optimal growth, brain development, immune health, GI function and metabolic development for every stage of preterm development.

NUTRICIA'S SOLUTION PORTFOLIO



Nutricia's extensive range of personalised nutrition supports optimal growth, brain development, immune health, GI function and metabolic development for every stage of preterm development.

Figure 8 Nutricia's preterm solutions

At all stages of the preterm infant's nutrition journey, breast milk is the preferred nutrition and human milk fortifier and protein supplement are critical solutions to ensuring breast milk can meet the high nutritional needs of the preterm infant in a personalised manner.

Premature Infants <1000g (ELBW)
Nutricia's Human Milk Fortifier (HMF) and Protein Supplement added to BM and/or Protein Supplement added to Nutricia's Preterm Formula

Premature Infants <1800g (VLBW)
Nutricia's HMF is indicated and/or
Nutricia's Preterm Formula

The Preterm Formula and PDF are nutritionally complete products that can meet the needs of the preterm infant entirely when breast milk is not available or when an additional source of nutrition is needed when the breast milk supply is not sufficient.

NUTRICIA'S NUTRITIONAL SOLUTIONS IN SUPPORT OF BREASTFEEDING

HUMAN MILK FORTIFIER

Nutricia recognises that the composition of BM is influenced by the diet of the mother and by the phase in lactation; BM in the first week postpartum has more protein than in later weeks for instance. The calculated nutritional impact of adding the HMF is based on 'mean values' for the composition of BM, which in turn have been based on the scientific literature and recommendations (Arslanoglu et al., 2010, Corvaglia et al., 2010, Klein, 2002a, Tsang, 2005).

Nutricia's HMF is a nutritional supplement to fortify expressed breast milk for preterm infants. It is a mixture of extensively hydrolysed protein, carbohydrates, vitamins and minerals. Nutricia's Human Milk Fortifier contains a mixture of 50% casein and 50% whey protein hydrolysates.

The HMF contains hydrolysed proteins, as recommended by experts consulted during the development of the HMF. Furthermore, hydrolysed protein dissolves better in breast milk which is important for tube fed infants to prevent blocking of the enteral feeding tube.

The HMF contains a range of vitamins, minerals and electrolytes.

Osmolarity and osmolality

Osmolarity is the concentration of a solution in terms of osmoles of solute per kilogram of solvent (mOsmol/l); it is influenced by temperature and pressure and is difficult to measure adequately. Osmolality is the preferred measure (Pearson et al., 2013) but both terms are used in the literature which can be confusing.

Breast milk has about the same osmolality as serum and varies between 280-310 mOsm/kg water depending on the hydration status of the mother. Adding HMF increases the osmolality of breast milk. Recent feeding guidelines for preterm infants do not include an upper recommended level of feed osmolality/osmolarity (Agostoni et al., 2010; Koletzko et al., 2014; AAP, 2014), however in 1976 the American Academy of Pediatrics recommended that formulae for all infants should have an osmolarity no greater than 400 mOsm/l (approximately 450 mOsm/kg) (Nutrition, 1976). This recommendation remains without clear substantiation based on relevant trials, however it has led to high feed osmolality being associated with adverse events – particularly gastrointestinal dysfunctions and NEC – in preterm infants.

A review in 2013 concluded that there is no causal relationship between the osmolality of feeds and the development of necrotising enterocolitis (Pearson et al., 2013). However, the review did not indicate how studies were selected or the search strategy and the most recent study included was from 2008. Therefore a second more complete systematic review of the literature on human and animal studies was conducted to investigate any link between high milk feed osmolality and adverse gastrointestinal events, including feeding intolerance and NEC (Ellis et al., 2018). For this review all relevant studies on the topic that measured feed osmolality regardless of differences in formula composition were included. Based on 10 included human studies with 618 subjects there was no consistent evidence that differences in feed osmolality in the range 300-500 mOsm/kg are associated with adverse gastrointestinal symptoms (Ellis et al., 2018).

KEY POINTS

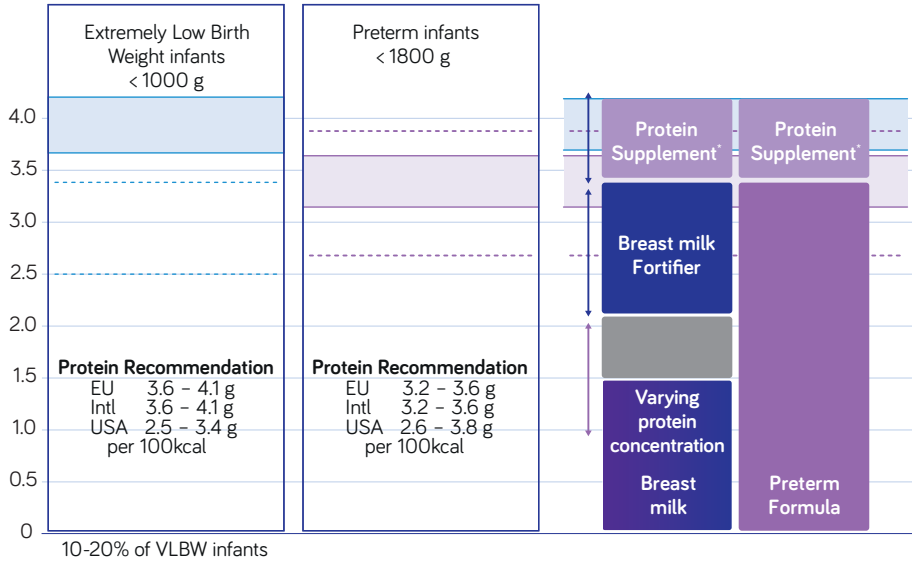
Nutricia's Human Milk Fortifier helps meet the increased energy, protein and nutritional requirements in preterm infants being fed breast milk.

PROTEIN SUPPLEMENT

As discussed in section 3 protein requirements of ELBW preterm infants are particularly increased and this increase is greater in the lower weight infants (Figure 9).

Nutricia's Protein Supplement allows protein requirements to be met in a personalised approach according to the infant's weight, as shown in the Figure 9. In clinical practice protein concentrations can be adapted to the unique needs of each preterm infant. Furthermore, if BM analysis by bed-side apparatus is available, the Protein Supplement can be utilised to achieve an individual protein target.

HMF allows BM enrichment to flexibly meet protein requirements for extremely or very low birth weight infants independent of the protein variation in maternal or donor milk. Preterm Formula meets the require-ments of VLBW infants; For those in special need of protein (ELBW), the protein supplement can be added as needed to either fortified BM or preterm formula.



**The Protein Supplement and Human Milk Fortifier allow flexible adjustment to infant needs according to different globally accepted recommendations for preterm infant feeding*

Figure 9 Nutricia's unique approach to meeting the protein needs of ELBW preterm infants

The Nutricia Protein Supplement contains a mixture of 50% casein and 50% whey protein hydrolysates, similar to that used for the HMF. It consists of 82.1% of protein equivalents and adds 3.38 kcal/g to breast milk or formula. To add 1 g protein, 1.2 g of Protein Supplement should be added. The composition of the Protein Supplement is given in Appendix 2. Experiments in Nutricia laboratories showed that the addition of 1 g of Protein Supplement to 100ml water increased the osmolality by 40 mOsm/kg.

KEY POINTS

- Nutricia's Protein Supplement is intended for ELBW preterm infants.
- Nutricia's Protein Supplement meets ESPGHAN recommendations.
- It may be added to breast milk fortified with HMF or to Preterm Formula.
- It permits meeting nutritional requirements for protein at an individual level.

PRETERM FORMULA

Nutricia's Preterm Formula has been designed to provide tailored nutrition for the growth and development of the preterm infant during hospitalisation. It is indicated when breast milk is not available or insufficient supply. The composition of Nutricia's Preterm Formula complies with the ESPGHAN recommendations to ensure optimal nutrition for formula-fed preterm infants (Agostoni et al., 2010). Moreover, the composition of Nutricia Preterm Formula also complies with the Life Sciences Research Office (LSRO) report on composition of preterm infant formulas (Klein, 2002b, Klein, 2002a).

IN SUPPORT OF GROWTH AND METABOLISM

Energy

Intrauterine growth rate is used to calculate the energy needs of preterm infants but the extrauterine environment may require extra fat deposition for thermal or mechanical protection. An energy intake of 110–135 kcal/kg/day is recommended (Agostoni et al., 2010). For ELBW infants on enteral nutrition 130–150 kcal/kg/day is recommended (Tsang, 2005). The Nutricia Preterm Formula provides 80 kcal/100 ml. Thus, a fluid intake of 150-180 ml/kg/d, which is recommended by ESPGHAN will result in an energy intake (120-144 kcal/kg/d) complying with the ESPGHAN recommendations (110-135 kcal/kg/d).

Lipids

Dietary lipids provide preterm infants with a large proportion of their energy, with essential fatty acids and lipid soluble vitamins (Koletzko et al., 2014). The amount and composition of dietary lipids can have direct impact on the quality of growth and body composition (Koletzko et al., 2014). The quantity and quality of the dietary lipid supply is of particular importance to preterm infants especially since their endogenous lipid stores are very limited (Agostoni et al., 2010, Klein, 2002a, Tsang, 2005). Nutricia's Preterm Formula contains 4.8 g/100 kcal of fat in a fat blend as described in section 4.4.

Medium-chain fatty acids

Medium-chain fatty acids (MCFA) are fatty acids with a carbon chain length of C6 to C12. In the form of mid-chain triglycerides (MCT) oils, predominantly C8:0 and C10:0 are provided. MCFA are useful energy provider, since they are water soluble, readily absorbed even when intraluminal bile salts and pancreatic lipase concentrations are low. MCFA are rapidly cleaved by serum lipases and –after absorption - released directly into the blood stream and reach the liver quickly for β-oxidation (energy production and heat generation). The energy carried in these molecules is therefore available faster than those of long-chain triglycerides. MCFA also seem to protect LCPUFA from oxidation, which could enhance LCPUFA availability for tissue (brain) incorporation and further elongation and desaturation into membrane components (Rodriguez et al., 2003).

Fatty acids from MCFA are less prone to calcium and magnesium soap formation than long-chain fatty acids (LCFA). Addition of MCFA to preterm formula can therefore increase not only LCPUFA but also calcium and magnesium absorption (Tsang, 2005).

ESPGHAN recommends that MCT should not exceed 40% of total lipids (Agostoni et al., 2010). Nutricia's preterm formula contains 16% of total fatty acids as MCFA.

Protein and other nitrogen components

The goal for protein intake in preterm infants is to supply the correct quality and quantity of protein to achieve sufficient growth and nitrogen accretion. However, accumulations of potentially harmful end products of protein degradation are to be avoided. The protein utilisation is affected by protein and non-protein nitrogen intakes, the biological value of ingested proteins, nutritional status, catch-up growth, hormonal environment, clinical status and the PER (Agostoni et al., 2010). The protein supply needs to compensate for the accumulated protein deficit observed in almost all preterm infants, and this varies depending on the weight of the infant (Agostoni et al., 2010).

The Nutricia Preterm Formula is based on intact proteins with a whey:casein ratio of 60:40, similar to that found in breast milk. Nutricia's Preterm Formula contains 3.3g protein/100kcal (2.7g/100ml) in line with recommendations of ESPGHAN 2010 (Agostoni et al., 2010) which meet the needs of all preterm infants weighing more than 1000g.

Carbohydrates

Carbohydrates are a major source of energy. Glucose is the primary source of energy for the brain and is an important carbon for the de novo synthesis of fatty acids and several amino acids. The guidelines from ESPGHAN recommend 10.5-12.0 g carbohydrate/100kcal in Preterm Formula (glucose or nutritionally equivalent di-, oligo-, or polysaccharides). Nutricia's Preterm Formula contains 10.5 g/100kcal (Agostoni et al., 2010).

Lactose is the major carbohydrate in breast milk (6.5-7 g/100ml). In the fetus, lactase expression and activity are measurable from 10 to 12 weeks gestation on, but they remain low until about 36 weeks gestation when they reach the activity seen in full-term neonates (Kien et al., 1992a). However, this does not seem to impact lactose digestion in preterm infants, as large lactose proportions are digested; around 35% is fermented by the gut microbiota in the colon and serves as a natural prebiotic (Kien et al., 1992b, Kien et al., 1987, Tsang et al., 2005).

Nutricia's Preterm Formula contains lactose concentration of 5 g/100 ml (6.3 g/100 kcal). Additional carbohydrate is added in the form of glucose-polymers. Glucose polymers are well tolerated and absorbed by preterm infants and have a low osmotic load.

Nucleotides

Nucleotides represent up to 20% of the non-protein nitrogen in breast milk. They are assumed to play an important role in carbohydrate, lipid, protein and nucleic acid metabolism. Dietary nucleotides have been reported to modify intestinal microflora, blood lipids and immune responses. Nucleotides can be synthesized de novo. Therefore, the ESPGHAN committee concluded that there is not sufficient evidence to recommend addition of nucleotides to preterm formula but if they are added, the total amount should not exceed 5 mg/100 kcal (Agostoni et al., 2010). A total of 4.0 mg/100 kcal of nucleotides is contained in Nutricia's Preterm Formula represented by cytidine-5-monophosphate, uridine-5-monophosphate, adenosine-5-monophosphate, guanosine-5-monophosphate and inosine-5-monophosphate.

IN SUPPORT OF GUT FUNCTION AND IMMUNE HEALTH

Oligosaccharides

As discussed in section 4, clinical data provide compelling evidence for the beneficial role of oligosaccharides in the nutritional care of the preterm infant (Agostoni et al., 2010). Nutricia's Preterm Formula contains prebiotic scGOS/LcFOS (9:1) at concentrations of 0.8 g / 100 ml similar to oligosaccharide concentrations found in breast milk.

IN SUPPORT OF BRAIN DEVELOPMENT

Long-chain polyunsaturated fatty acids (LCPUFA)

The accumulation of body fat stores occurs predominantly in the last trimester of pregnancy and is particularly active in the last 3 weeks prenatally. Consequently, body stores of essential fatty acids (EFA) and fat deposits are significantly lower in preterm than term infants (Makrides et al., 2000). LCPUFA are important structural components of cell membrane PLs especially those of the central nervous system and of retinal tissue, and function as precursors for the synthesis of eicosanoids (Koletzko et al., 1995); LCPUFA are important for brain development.

A dietary source of LCPUFAs is crucial for preterm infants. Clinical trials have demonstrated that supplementing preterm formulas with LCPUFA benefits visual and cognitive functions (Clandinin et al., 2005, SanGiovanni et al., 2000, Simmer, 2000).

ESPGHAN and Koletzko et al. which represent the most up to date expert guidance recommend that preterm infant formulas contain at least 0.4% ARA and 0.35% DHA of total fatty acids (Agostoni et al., 2010, Koletzko et al., 2014).

Nutricia's Preterm Formula has a fat blend which provides a source of both the essential fatty acids (EFAs) (linoleic acid and alpha-linolenic acid), coupled with the preformed LCPUFAs (ARA and DHA). The ratio of LA:ALA is 6.5:1. Nutricia's Preterm Formula contains the following amounts of LCPUFAs:

- LA: 599 mg/100 kcal 13.1% of total fat
- ARA: 25 mg/100 kcal 0.55% of total fat
- DHA: 25 mg/100 kcal 0.55% of total fat
- ALA: 89.4 mg/100 kcal 1.95% of total fat

Nutricia's Preterm Formula provides predominantly TG-bound but also PL-bound LCPUFA of which PL-DHA and PL-ARA concentrations are similar to those that can be found in BM. Nutricia's Preterm Formula provides the concentrations and quality of LCPUFA in line with the ESPGHAN and Tsang guidelines (Agostoni et al., 2010, Tsang, 2005).

OTHER KEY NUTRIENTS

Nutricia's Preterm Formula contains a complete range of vitamins, minerals and electrolytes in line with ESPGHAN recommendations (Agostoni et al., 2010).

KEY POINTS

- The Nutricia Preterm Formula meets ESPGHAN recommendations for the increased energy and protein needs of the preterm infant
- The high energy intake is achieved through a combination of lactose and glucose polymers which does not exert a high osmotic load
- The Nutricia Preterm Formula contains prebiotic oligosaccharides which provide beneficial effects: stimulation of healthy gut microbiota, softer stools, increased stool frequency
- Nutricia's Preterm Formula contains optimal lipid concentrations to meet energy requirements and provide essential fatty acids and lipid soluble vitamins
- Preterm infants have limited ability to synthesize LCPUFA, a dietary supply is of crucial importance for their physical, visual and cognitive development.
- Nutricia Preterm Formula provides the concentrations and quality of LCPUFA in line with the ESPGHAN recommendations.
- Nutricia Preterm Formula also contains MCTs as they can enhance the availability of LCPUFA and are less prone to calcium soaps formation.

HOME CARE

Preterm infants are usually growth restricted at hospital discharge (Young et al., 2012), and an enriched diet may be indicated after discharge depending on growth pattern (Aggett et al., 2006). The continuation of breastfeeding during hospital and after discharge for as long as possible is the desired goal of feeding the preterm infant. A systematic review investigated the effect of feeding preterm infants following hospital discharge with multi-nutrient fortified breast milk versus unfortified BM on growth and development (Young et al., 2012). Only two small relevant trials were found and it was concluded that the limited data do not provide convincing evidence for feeding preterm infants with multi-nutrient fortified breast milk (Young et al., 2012).

NUTRITIONAL RATIONALE OF A POST-DISCHARGE FORMULA

Clear recommendations for feeding during the period after discharge are lacking. Although ESPGHAN put forward a commentary on the topic in 2006, recommendations of intake with concentrations or concentration ranges for nutrients to be contained in feedings have been and are still lacking (Aggett et al., 2006). Table 1 of that publication is often erroneously considered as recommendation; however, this table only lists the composition of formulae tested by RCT at the time of publication, which should no longer serve as reference as recommendations for feeding preterm infants have been updated since then (Agostoni et al., 2010). The recommendation of ESPGHAN is a text statement and calls for “a special post-discharge formula with high contents of protein, minerals and trace elements as well as long-chain polyunsaturated fatty acids” (LCPUFA) for formula-fed preterm infants “with suboptimal weight at discharge and thus with increased risk of long-term growth failure” (Agostoni et al., 2010).

Nutricia has developed its PDF after consideration of the scientific knowledge about the nutrition of preterm infants post-discharge and any recommendations that do exist (Aggett et al., 2006, Carlson, 2005, Cooke et al., 2001, Cooke et al., 1998, Cooke et al., 1999, Lapillonne, 2014). Breast milk composition also provides a reference to guide the composition of the PDF. Furthermore the legislative frameworks for infant formula and foods for special medical purposes (FSMPs) provided guidance.

POST-DISCHARGE FORMULA

In 2006 ESPGHAN published post-discharge recommendations (Aggett et al., 2006), and in 2014 the AAP published recommendations for this period too (Nutrition, 2014). A PDF has a nutritional composition between a hospital preterm formula and an infant formula designed for a term baby.

INDICATIONS FOR A POST-DISCHARGE FORMULA

The aim of the PDF is to support growth of non-breastfed or partially breastfed preterm infants after hospital discharge under medical supervision, until a standard term formula serves to sustain healthy growth.

Given the population heterogeneity, nutritional support approaches should be individualised (Lapillonne, 2014). Medical history and existing nutritional deficiencies should be considered in the decision of the healthcare professional (HCP) (Lapillonne, 2014).

Current weight may be a consideration; infants who weigh less than 1,800 or 2,000g at discharge may be a candidate for a PDF at discharge (Aggett et al., 2006, Nutrition, 2014). In addition, current feed volume may be another indicator e.g. intakes greater than 180 ml/kg/d may be moved to a PDF at discharge.

The following preterm infants may particularly benefit form additional nutritional support:

- Preterm infants born less than 1000 g body weight (Lapillonne, 2014).
- Preterm infants discharged with less than 2,000 g body weight (Lapillonne, 2014, Nutrition, 2014).
- Preterm infants experiencing growth faltering and those in need of growth recovery, prevailing effects of intrauterine growth retardation and/or accumulated extrauterine growth retardation (Aggett et al., 2006, Haiden et al., 2012).
- Preterm infants with feeding difficulties, i.e. those that have uncoordinated suck swallow (Lapillonne, 2014).
- Preterm infants with persistent co-morbidities (Lapillonne, 2014) and subsequent growth faltering.

Timing

Formula-fed preterm infants in need of a PDF should be transitioned at least within a week prior to discharge so that feeding difficulties can be identified quickly. Ideally, a complete feeding assessment should be completed within the first week of discharge (Lapillonne, 2014).

GROWTH MONITORING

Growth monitoring should take place within 48 hours of discharge, at expected term date, and every 2-4 weeks after discharge until at least one year of age (Lapillonne, 2014, Nutrition, 2014).

Growth monitoring should not only document weight, length, head circumference and their gain over time, but also include feed intake, weight-for-age, length-for-age, and head circumference-for-age, and indexes of body proportionality (Lapillonne, 2014). If possible, body composition assessments should be included in growth monitoring (Nutrition, 2014).

Growth monitoring should also make use of validated growth curves for length and weight (and head circumference), such as Fenton or Intergrowth 21 in alignment with WHO growth charts or locally applicable ones considering sex and corrected age (see section 3 for information on growth charts).

DURATION

Providing nutritional support does not mean that growth recovery needs to take place within the first months after discharge. Nutritional support according to fetal requirements should be provided until expected term age in order to avoid nutritional deficiencies; However, this strategy would apply to children that have shown steady growth and/ or growth recovery during hospitalisation where there is no further need to compensate for nutritional or growth deficits after discharge (Lapillonne, 2014).

Children in need of growth recovery may receive nutritional support as recommended by ESPGHAN 2006 until three months corrected age (Aggett et al., 2006). This period may be extended as several studies have provided enriched feeding until six months CA with improved body composition (Teller et al., 2016). Other guidance has suggested that enriched feeding was safe to apply until 12 months CA (Aggett et al., 2006, Carlson, 2005, Tsang, 2005). The decision for the duration of enriched feeding is the responsibility of the HCP and should be based on regular growth monitoring of the individual child.

Weight and length gain

PDF may be continued until at least weight is at and above the 2nd percentile (-2 SD) of sex appropriate growth charts adjusted for gestational age (Lapillonne, 2014). The HCP could consider whether the formula should be continued until the 9th or 16th weight (and length) percentiles have been reached (1.34 SD or 1.0 SD, respectively) yet preferably not beyond reaching of the 25th weight percentile (0.67 SD), unless otherwise decided by the HCP.

It is not recommended to provide the PDF after the 50th weight percentile has been reached.

Proportional weight gain

HCPs and parents should carefully observe both weight and length gain. If weight gain is disproportionally faster than length gain, the child is at risk of depositing unfavourable fat. Assessment of specific nutrient deficits may be undertaken. Switching to a standard formula for term infants - possibly in combination with supplementation of specific nutrients - could be considered.

Low birth weight (LBW) infants of unknown gestational age, i.e. possibly being term SGA, may have different nutritional needs than premature infants after hospital discharge, which have not been defined. The decision to use the PDF for these infants lies in the responsibility of the HCP.

IN SUPPORT OF GROWTH AND METABOLISM

Energy and protein

The PER is of crucial importance within preterm infant feeding as it has shown to be a component in lean mass accretion (Ziegler, 2015). Studies have shown that a high(er) PER during discharge results in improved recovery of growth and body composition (Teller et al., 2016).

Preterm infants weighing between 1800 - 2200 g require ~2.7 g protein per 100 kcal (Ziegler, 2011). The energy of the PDF is 75 kcal/100 ml; protein 2.1 g/100 ml and PER 2.7 g/100 kcal.

Carbohydrate

The total carbohydrate concentration in the PDF is 7.5 g/100 ml (10 g/100 kcal), which is in line with directive (EC)2006/141_IFFOF (9.0 - 14.0 g/100 kcal) and expert opinion for preterm infants after discharge from hospital (Carlson, 2005) . The carbohydrate fraction is 80% lactose and 20% glucose syrup (maltodextrin DE32). The lactose: maltodextrin ratio is 80:20 compared to 70:30 in the preterm formula which serves as a transition to 100% lactose found in Nutricia's Term Formula.

IN SUPPORT OF BRAIN DEVELOPMENT

Long-chain polyunsaturated fatty acids

Since 2006, ESPGHAN recommends supplying all formula-fed preterm infants after hospital discharge with LCPUFA (Aggett et al., 2006). Nutricia's PDF contains LCPUFA in concentrations and molecular forms that have been shown to be readily absorbed in support for proper visual, brain and cognitive development.

Nutricia uses a fat blend that provides LCPUFA in the form of both essential fatty acids LA, ALA, GLA, as well as EPA, ARA, DHA, the latter fatty acids bound as TG and as PL. The levels comply with latest recommendations for levels in hospital preterm formula (Agostoni et al., 2010, Koletzko et al., 2014). The omega-6 to omega-3 fatty acid ratio is not addressed in any recommendations; the PDF offers a ratio of 5.5:1.

Nutricia's PDF contains the following amounts of LCPUFAs:

- LA: 658 mg/100 kcal 13.2% of total fat
- DHA: 25 mg/100 kcal 0.5% of total fat
- ARA: 25 mg/100 kcal 0.5% of total fat
- ALA: 100 mg/100 kcal 2% of total fat

Nutricia's PDF provide predominantly TG-bound but also PL-bound LCPUFA of which PL-DHA and PL-ARA concentrations are similar to those that can be found in BM.

IN SUPPORT OF GUT FUNCTION AND IMMUNE HEALTH

Prebiotic oligosaccharides

The Nutricia PDF also contains the patented prebiotic mix scGOS/lcFOS (9:1) to further support a more BM-like gut colonisation that may have been impaired and/ or delayed by prophylactic or acute and extended antibiotic use during the infant's hospital stay.

OTHER KEY NUTRIENTS

The vitamin, mineral and electrolyte levels of the PDF comply with the EC regulation for infant formula composition or the EC regulation for Foods for Specific Medical Purposes (FSMPs)

KEY POINTS

- PDF is indicated for infants in need of recovery growth at discharge.
- Breast milk and breastfeeding should be encouraged for as long as possible after discharge.
- Ongoing growth monitoring is crucial post discharge to ensure weight gain is appropriate.
- PDF has a nutritional composition between a hospital preterm formula and a term infant formula.
- Expert bodies recognise the important role of PDF in infants weighing less than 1,800-2,000g
- Nutricia's PDF meets the compositional regulations in the EC directive for infant formula and/or the EC directive for FSMPs

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