Sanjay Patole *Editor*

Nutrition for the Preterm Neonate

A Clinical Perspective

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Springer

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ISBN 978-94-007-6811-6 ISBN 978-94-007-6812-3 (eBook)
DOI 10.1007/978-94-007-6812-3
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2013942385

Foreword

Survival of extremely preterm neonates (gestation within 28 weeks from birth) has improved significantly following the advances in neonatal intensive care. Extra uterine growth restriction is a serious issue in this population. Nutritional exposures during this critical period in life influence the individual's risk of disease throughout life. Nutritional deficit and poor growth are associated with long term neurodevelopmental impairment, short stature and metabolic disorders in extremely preterm neonates. Optimising nutrition in the early postnatal life of the preterm neonate is therefore a priority. However this is easier said than done, considering the frequency of feed intolerance, fear of necrotising enterocolitis and the hesitancy in adopting an aggressive approach to parenteral nutrition in this population.

Some of the finest researchers in the field have come together to provide the clinical perspective on the A to Z of nutrition in the preterm neonate in simple and clear fashion in this book. I am grateful to all authors for their valuable contributions, the quality of which reflects their expertise as clinicians and researchers. The topics that are covered range from the developmental physiology of the gastrointestinal tract, to aggressive enteral and parenteral nutrition, and feeding under special conditions such as intrauterine growth restriction and chronic lung disease. I hope that with its practical approach this book will be useful for everyone involved in neonatal intensive care and also for those aiming for an academic career in this field.

I would also like to take this opportunity to thank Springer and their publishing editor Thijs van Vlijmen, for encouragement and support in getting this unique book published in a remarkably short period. I hope the readers appreciate the purpose and the passion behind this book.

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Part I
Developmental Physiology of the GIT and Feed Intolerance

Chapter 1
Developmental Physiology of the Gastrointestinal Tract and Feed Intolerance in Preterm Neonates

Sanjay Patole

Abstract Manifestations of gastrointestinal hypomotility such as large/bile stained gastric residuals, abdominal distension, and vomiting, are very common in the first few weeks of life in preterm neonates, especially those with gestation under 28 weeks at birth, and are often interpreted as "feed intolerance". Necrotising enterocolitis (NEC) is a potentially disastrous illness with significant mortality, and morbidity including long term neurodevelopmental impairment, in this population. The scientific basis for the diagnosis and interpretation of signs of feed intolerance is not clear. Inability to differentiate manifestations of ileus of prematurity from those of early (Stage I) NEC is the single most important reason for frequently withholding enteral nutrition in preterm neonates. The decision to start, continue, upgrade or stop enteral feeds in extremely preterm neonates continues to be based on poorly understood clinical parameters such as volume and colour of gastric residuals, and abdominal distension. This chapter reviews the developmental physiology of the gastrointestinal tract and the clinical studies on the significance and/or management of the various manifestations of feed intolerance in extremely preterm neonates. The need for further research on this important clinical issue is emphasised considering the fact that suboptimal nutrition due to the frequent withholding of enteral feeds increases the risk of postnatal growth restriction in extremely preterm neonates.

Key Points

- Manifestations of gastrointestinal hypomotility such as large/bile stained gastric residuals, abdominal distension, and vomiting, are very common in the first few weeks of life in extremely preterm (gestation < 28 weeks) neonates, and are interpreted as feed intolerance.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_1,

- Necrotising enterocolitis (NEC) is a potentially disastrous illness with significant mortality, and morbidity including long term neurodevelopmental impairment, in extremely preterm neonates.
- The scientific basis for the diagnosis and interpretation of signs of feed intolerance is not clear. Inability to differentiate manifestations of ileus of prematurity from those of early (Stage I) NEC is the single most important reason for frequently withholding enteral nutrition in preterm neonates.
- Understanding the developmental physiology and maturation of the gastrointestinal tract is necessary to avoid/minimise the frequent withholding of feeds based on poorly understood clinical parameters (e.g., volume and colour of gastric residuals) in extremely preterm neonates.
- Recent advances in the understanding of molecular and biochemical pathways in neonatal diseases may lead to the discovery of new biomarkers for differentiating early NEC from feed intolerance of prematurity.

Nutrition per se is known to affect the growth and development of the gastrointestinal (GI) tract at many stages of development including fetal and neonatal life [1]. At birth, the mammalian GI tract has to shift from fetal parenteral nutrition via the placenta to enteral nutrition in the neonate. The GI tract therefore undergoes enhanced growth as well as morphological and functional differentiation in the perinatal period. The fetal GIT elongates 1,000-fold from the 5th to the 40th week of gestation, doubling in length in the last 15 weeks of gestation, measuring to a mean of 275 cm at birth [2]. A complex interplay of local, systemic and luminal factors influence this programmed developmental maturation [3]. Regulatory neural-hormonal mechanisms control the expression of the genetic endowment at various stages in GI development [4]. For example the administration of glucocorticoids or thyroxine is known to cause early appearance of enzymes within the intestine. Other hormones involved in the regulation of GI development include cholecystokinin, gastrin, secretin, insulin, insulin-like growth factors, and epidermal growth factor [4]. Bryant et al. [5] have demonstrated that the secretion of gastrin, secretin, motilin, GIP, enteroglucagon, neurotensin, somatostatin, and VIP follows an adult pattern by 20 weeks of gestation. By 20 weeks of gestation, the anatomic differentiation of the fetal GI tract progresses to the extent that it resembles that of a newborn. Secretory and absorptive functions, however, develop at different rates. Active glucose transport systems are present at 17 weeks gestation [6]. Peptidase activities develop by 11 weeks and increase around 23 weeks [7]. The small intestinal villi are already formed at 16 weeks but the intestinal absorptive process is only partially available prior to 26 weeks of gestation [7]. Many different types of cells exist in the intestine including the absorptive epithelium, Paneth cells (involved in innate immunity), goblet cells (mucus secretion), and cells of the intestinal neuroendocrine and immune system. The turn over (mitotic division in the crypt-migration to the tip of the villus-differentiation to become actively absorbing cells-sloughing off from the tip of the villus into the intestinal lumen) time of the intestinal epithelial cells in infants has been proposed to be about 96 h [2]. Gastric and pancreatic secretion is only basal and can be stimulated only partially even in the full-term newborn. The first traces of gastric acid

appear at 4–5 months of gestation [8] despite the presence of parietal cells in the 11th week of gestation [9, 10]. Gastric activity is known to decrease within several hours after birth [11–14]. The secretion of gastric acid is limited in very low birth weight (VLBW) neonates with the intragastric pH remaining at ~5.5–7.0 and resistant to pentagastrin in the first 24–48 h after birth [2]. Both basal and pentagastrin stimulated acid secretion doubles from the first to the fourth week of postnatal life in preterm neonates [2].

1 Digestion and Absorption of Proteins, Lipids, and Carbohydrates

1.1 Proteins

Levels of enzymes such as pepsin which are essential for protein digestion are low and developed fully only by 3–8 months after birth [2]. Protein hydrolysis and absorption occurs in three phases: gastric, pancreatic, and intestinal. The gastric phase requires presence of an acid environment and the gastric protease-pepsin and breaks down proteins to polypeptides within-terminal amino acids. Pepsin activity increases significantly from the 28th to the 40th week of gestation [15]. It is not known whether asynchrony exists in the development of gastric acid secretion and pepsin activity; however the mean pH achieved while feeding the newborn is estimated to exceed the pH optimum for pepsin for pepsin activity [16–18]. It is therefore suggested that the ability of neonates to digest proteins may be compromised. Pancreatic proteases are added in the duodenum and have a pH optimum near neutral and depend on bicarbonate buffering of the duodenal contents for their effectiveness. Pancreatic enzyme tissue activity becomes detectable at about 12 weeks gestation and pancreatic secretion starts at the beginning of the 20th week of gestation [19]. The enzyme enterokinase is secreted after stimulation of the upper small intestine by food and catalyses the activation of trypsinogen to trypsin which further activates several other enzymes (proteases) that are essential for digestion of proteins [18, 19]. Enterokinase levels are detectable but low at 24 weeks of gestation and reach only 25 % of that in adults at term indicating a limited capacity to digest proteins, and may result in the passage of unbroken larger antigens/microorganisms in to the intestinal lumen [18, 19]. Hydrolysis of starch is limited in preterm neonates considering the limitations imposed by pancreatic deficiency. There is no clear evidence on the advantages of commercially available formulas containing hydrolysed starch. It is important to note that the osmolality of the formula rises as the degree of hydrolysis increases.

1.2 Lipids

The digestion and absorption of lipids occurs in three phrases as described by Watkins, the intraluminal phase, the mucosal phase, and the transport or the delivery

phase [20]. In the intraluminal phase, triglycerides are converted into monoglycerides and free fatty acids by lingual and pancreatic lipases. In the mucosal phase, the free fatty acids and monoglycerides reesterify to form triglycerides, which then interact with phospholipids, cholesterol esters, cholesterol, and lipoproteins to form chylomicrons and very low-density lipoproteins. In the transport phase these substances are transported from the enterocyte and diffuse to lacteals, where they travel via the thoracic duct to reach the hepatic venous system via the vena cava [20]. Several lipases like human milk secreted lipase, and lingual, gastric, pancreatic, and epithelial lipases are essential for the hydrolysis of fatty acids from glycerol [21]. Pancreatic secretion of lipase and bile acid secretion is low in preterm neonates [21]. The intraluminal lipase levels have been reported to be 5 % of those in term neonates and 5 % of those in adult [22]. Both term and preterm neonates have been reported to have a lower bile acid pool [23]. The average intraduodenal bile acid concentration of the preterm neonate is < 2 mmol/L compared with 5 and 8 mmol/L in term neonates and adults respectively [24]. The solubilisation and absorption of long chain fatty acids is most susceptible as it depends on bile acids [2]. The process of assimilation of medium chain fatty acids does not involve re-esterification and chylomicron formation as in the case of long chain fatty acids. They are also absorbed directly in to the portal venous circulation compared with the chylomicrons formed from the long chain fatty acids, which enter the lymphatic circulation [2]. Feeding with medium chain fatty acids is thus preferred in conditions such as chylothorax where lymphatic obstruction is suspected. The n-3 and n-6 essential fatty acids alpha linolenic acid and linoleic acid are the precursors of long-chain polyunsaturated fatty acids (LCPUFA) such as docosahexaenoic acid and arachidonic acid [25]. These fatty acids are found in high proportions in the structural lipids of cell membranes, particularly those of the central nervous system. Based on the fact that their accretion primarily occurs during the last trimester of pregnancy and the first year of life, LCPUFA were proposed as essential nutrients for preterm neonates who may not be able to synthesise sufficient amounts of LCPUFA to satisfy the needs of the developing brain and retina. Results of a systematic review and meta analysis however indicate that supplementation of formula with LCPUFA has no long-term beneficial or adverse effects on growth in preterm neonates [25].

1.3 Carbohydrates

The processes for digestion and absorption of carbohydrates develop in a well-defined sequence during the fetal life [2]. Mucosal enzymes involved in the digestion of carbohydrates ("disaccharides") are located in the brush border membrane of the mature enterocyte of the small intestine [26]. Disaccharidases are present by 8 weeks and their concentration increases around 14 weeks progressing steadily to term values [27, 28]. The major disaccharides in the human intestine include lactase, sucrase, and maltase. Sucrase, maltase, and isomaltase are fully active in preterm neonates [2]. However lactase activity is low and matures from 24 week onwards [2]. Lactase,

sucrase, maltase, isomaltase, and glucomylase reach mature levels at term [2]. It takes many months for pancreatic amylase to reach adult levels in preterm as well as full term neonates. Clinically significant lactose intolerance is uncommon in preterm neonates despite the low lactase levels. Early initiation of enteral feeding has been shown to increase the activity of intestinal lactase which is a marker of intestinal maturity in preterm neonates. The role of concentration of lactose in manifestations of lactose intolerance is not clear [28]. Auricchio et al. [29] have estimated that between 2 and 3 months of gestation, 2.05 g maltose, 0.02 g sucrose, 0.02 g isomaltose, and 0.01 g lactose can be hydrolysed in 24 h by the fetus and newborn. They also proposed that at term the newborn should be able to hydrolyse 107-gram maltose, 72-gram sucrose, 46-gram isomaltose, and 60-gram lactose [29]. Other investigators report that maltase and sucrase are present at 12 weeks and reach 70 % of their adult values by 14 weeks, whereas lactase is present at 50 % of adult values at 14 weeks of gestation [30]. These findings are consistent with the fact that even very preterm neonates are known to tolerate feeding with breast milk, which has high levels of lactose. Feeding non-lactose-containing formulas to preterm neonates may therefore be unnecessary especially as the volume of enteral feeds in the early postnatal life is low in preterm neonates. It is important to note that most of the lactase activity occurs at the mid to upper part of the intestinal microvillus and it usually is the first enzyme to be lost and the last to be regenerated fully [2]. Monosaccharide transport has been documented as early as the 10th week of gestation [31–33]. The transport of glucose and galactose is an active process that depends on brush border protein carriers, ATP, and intracellular sodium. Fructose transport however occurs by an energy dependent-facilitated diffusion [31–33].

1.4 Immunological Function

Spencer et al. reported that the immaturity of intestinal immunological function in preterm neonates may be due to the differences in subpopulations of T-lymphocytes in the fetal intestines and later in life [34]. The secretary component of IgA can be detected by 16 weeks. A systemic immune response to food antigen can develop only after 35 weeks gestation [35]. Peristalsis protects the small intestine from bacterial overgrowth [17]. The immaturity of the enterocyte affects the pathogenicity of the invading pathogens [36]. Neonates are thus prone to GI infections due to their relatively deficient intestinal immune function. The level of secretory IgA is known to increase slowly during the first month of life. Uptake of macromolecules may sensitise neonates to allergens may also allow for passive absorption of maternal antibodies in breast milk [37]. Gastric acid provides a barrier to microorganisms. It therefore not surprising that the incidence of sepsis has been reported to be high in critically ill patients treated with histamine blockers [38].

1.5 Motor Function

Feeding requires effective coordination of sucking, swallowing, gastric emptying, and intestinal motility. Rudimentary muscle layers are first seen at 6 weeks' gestation; the muscularis mucosa is fully developed between 17 and 20 weeks coinciding with the development of the neuronal structures of the submucosal plexus of Meissner and the myenteric plexus of Auerbach [35, 39]. Contractions of the smooth muscle of the gut are coordinated by this "enteric nervous system", which is capable of reflex control of the GIT independent of the central nervous system. The interstitial cells of Cajal located along the submucosal and myenteric surface of the gut circular muscle function as the pacemaker cells of the gut by generating a basic electrical rhythm/slow wave activity [40, 41]. Delayed maturation of the interstitial cells of Cajal has been documented as a cause of transient intestinal pseudo-obstruction in two preterm neonates [42]. Yoo et al. [43] have reported that delayed maturation of these cells could be a cause of meconium obstruction in neonates without cystic fibrosis.

1.6 Suck and Swallow

The fetus swallows about 450 ml/day of the amniotic fluid that is rich in nutrient and growth factors [44]. Sudden interruption of this process at birth may have adverse effects on further development and maturation of GI tract if postnatal nutrition is not adequate. Sucking and swallowing movements have been observed as early as 12 weeks of gestation [44]. Presence of the swallowing reflex at 31 weeks of fetal life was documented as early as in 1963 [44]. However effective sucking and swallowing is noted only around 34 weeks of gestation [45]. Early non-nutritive sucking movements may help in establishing feed tolerance and are affected by factors like maternal sedation, flow rate of milk, and length of feeding experience [46, 47]. Oesophageal peristalsis is poorly coordinated until 32 weeks of gestation and coordinated contractions are not present until several days after birth [48]. More recent studies have documented coordinated peristalsis even in preterm neonates [49]. Overall despite the somewhat immature oesophageal neuromuscular activity, peristaltic function is adequate for propulsion of a liquid bolus from the pharynx in to the stomach in preterm neonates. The high incidence of gastroesophageal reflux (GER) in preterm neonates is explained by their lower oesophageal pressure ($\sim$4 cm H_2O versus 28 cm H_2O) compared with full term neonates neonates [2]. Delayed gastric emptying is another mechanical factor that is related to GER in preterm neonates [2].

1.7 Gastric Emptying

Gastric emptying has been shown to be slower in preterm neonates, developing appropriately from 32 week onwards [50]. Preterm neonates at 27–28 weeks' gestation

have been shown to generate only 20–25 % of the pressure that the term neonate can generate at the gastric antrum [51]. However data on extremely preterm neonates is not available. The gastric emptying half time in stable neonates weighing around 2 kg has been reported to be 17–100 min (mean: 56 min) [52]. The estimated gastric volume emptied/body surface area per hour ranged from 8.4 to 39.2 ml/0.1m^2 × h (mean: 19.1 ml/0.1m^2 × h) in this study [52]. In term neonates, 10 % dextrose feedings empty in a pattern similar to adults, with an initial rapid phase followed by more gradual emptying [53]. A similar pattern of gastric emptying is also observed in preterm neonates fed human breast milk at 33–38 weeks' postconceptual age. However a more linear pattern is observed when these neonates are fed a formula [53]. In term neonates glucose-containing solutions empty more slowly than equivalent volumes of water. Starch feedings however empty at a rate similar to water, [55, 56] probably due to the slow hydrolysis related to the low amylase concentrations in neonates [57]. Increasing caloric density (from 0.2 to 0.66 cal/ml) has also been shown to decrease gastric emptying [58–60]. Results of the studies by Siegel et al. [58–60] show that the quantity of calories delivered into the duodenum from the stomach increased with concentrated formula despite the reduced gastric emptying at higher caloric density. Medium chain triglycerides have been shown to have a less inhibitory effect on gastric emptying in adults compared with long chain triglycerides. Siegel et al. [58] have reported similar results in preterm neonates. Other investigators using more reliable methods have not confirmed these results. Stimulation of duodenal receptors by acid, fat, carbohydrates, tryptophan, or increasing osmolality is known to affect gastric emptying [61, 62]. Rise in osmolality from 279-to-448 mOsm/kg however has been reported to result in no significant change in gastric emptying time compared with isocaloric formulas [60]. No data is available on the ability of duodenal feedback mechanism to control the rate of gastric emptying in VLBW neonates. Other factors such as formula temperature, [63] phototherapy [63], position [53], and non-nutritive sucking [64] may also have an effect on gastric emptying. Gastric emptying is known to decrease in presence of bilirubin levels of 233–332 mmol/L, improving after resolution of the jaundice [65]. Nasojejunal feeds, which bypass the stomach, may provide only a temporary solution to reduced gastric emptying in preterm neonates as the intestinal motility is also immature [66, 67]. Prokinetic agents such as metoclopramide [68, 69], and low dose erythromycin [70, 71] have been shown to increase the gastric emptying in preterm neonates.

1.8 Antral and Duodenal Motility

A cyclical pattern of antral and intestinal contractile activity, called as migrating motor complex (MMC), progresses from the antrum to the ileum during fasting [72, 73]. In adult humans these MMCs consist of four phases. Phase I consists of no contractile activity, which is sequentially interrupted by periods of irregular contractions (phase II), followed by regular contractions at a rate of 3/min in the atrium or 12/min in the duodenum (phase III), and a brief period of irregular contractions before the

return of quiescence (phase IV). The cycle repeats every 45–180 min. The MMC has been described as housekeeper of the bowel, removing indigestible solids and bacteria from the upper GIT [73]. This cyclic activity is interrupted by a meal, with an indistinct pattern of irregular contractions appearing until hours after the meal. By 34 weeks these complexes are of variable length, with clear intervals and being increasingly propagated. The mature MMC at this stage has a periodicity of 20–40 min and is interrupted by feeding [5, 74].

Small intestinal peristaltic activity has been documented in the 3–5 month old human fetus [75]. The degree of antroduodenal coordination improves simultaneously towards term [76]. Thus except for the markedly shorter periodicity the MMC at term gestation is similar to that in adults.

Enteral nutrients have a positive feedback mechanism on intestinal function by stimulating hormonal production and motor activity. Berseth et al. [77] have documented that preterm neonates can respond to feeding with an increase in duodenal pressure wave that is equivalent to term neonates. Berseth et al. [78, 79] have also documented that early feeding promotes the motor activity in preterm neonates. Preterm neonates may therefore be able to tolerate enteral feeds despite their immature fasting motor patterns [77]. Neonates with feed intolerance have been shown to have less mature patterns of motor activity, with decreased episodes of motor quiescence and a lack of pattern change after feeding in manometry studies [78, 79]. Feed tolerant neonates on the other hand have more mature and organised motor pattern with a clear increase in motor activity after feeding [79, 79]. Preterm neonates given hypocaloric feeds have been shown to exhibit a more mature intestinal motility pattern sooner than their counterparts given water or no enteral feeds [78, 79]. It is important to note that although these differences vanished once caloric feeds were established, the early fed neonates had less feed intolerance and were advanced to full feeds sooner than later-fed neonates. Observations of small intestinal contractile patterns have also been shown to be useful in predicting feed readiness in term, asphyxiated neonates [80]. Birth asphyxia has been shown to delay the maturation of intestinal motility in term neonates [81]. It is also known that intestinal motility patterns are disturbed in conditions with mucosal damage such as enteritis [82].

1.9 Antroduodenal Motility After Feeding

Gryboski has reported that feeding causes an abrupt onset of contractions in the duodenum in the term neonate [49]. It was noted that unlike in the adult, clusters of non-propagating contractions may be observed after feeding, and they have the same frequencies as those of the gastric (3/min) and duodenal (12/min) MMC [49]. It was shown that the number of antral pressure waves and their amplitude are both diminished after feeding in preterm and term neonates, and this was in contrast to adults where these are reported to increase after a feed [49]. It is important to note that these differences could relate to the differences in methodology including the type of calories, and method of delivery in adult and neonatal studies.

Prokinetics like erythromycin may not be effective in very preterm neonates considering that motilin receptors are not present until 32 weeks' gestation. Positive results from clinical trials however indicate that non-motilin mediated action/s of erythromycin may be responsible for the enhanced GI motility in neonates at such earlier gestation [79]. The ability of even small amounts of enteral feeds to enhance the motor maturity of the GIT could also be additive or responsible for such results at early gestational ages [79].

Defecation occurs when the fecal bolus passes into the rectum and the internal sphincter relaxes allowing stool passage [83]. The baseline sphincter pressures are lower than the adult range in the preterm neonates. The gestational age at which the relaxation reflex can be documented reliably is controversial. Using a relatively insensitive sleeve device, Ito et al. demonstrated it in neonates at > 39 weeks' gestation [83]. Bowes et al. [84] using a standard perfused catheter method, have demonstrated it in neonates as young as 27 weeks' gestation.

2 Feed Intolerance in Preterm Neonates

Manifestations of ileus of prematurity such as large/bile stained gastric residuals, abdominal distension and vomiting, are very common in the first few weeks of life, and interpreted as feed intolerance in preterm neonates, especially in extremely preterm neonates with gestation under 28 weeks at birth. Necrotising enterocolitis (NEC) is a potentially disastrous illness with significant mortality, and morbidity including long term neurodevelopmental impairment, in this population. Significant progress has been made in the field of NEC over last few years but the scientific basis for the diagnosis and interpretation of signs of feed intolerance continues to be unclear. Inability to differentiate manifestations of ileus of prematurity from those of early (Stage I) NEC is the commonest reason for frequently withholding enteral nutrition in preterm neonates. The approach to enteral feeding in extremely preterm neonates continues to be based on poorly understood clinical parameters such as volume and colour of gastric residuals, and abdominal distension.

2.1 Gastric Aspirates

The definition and significance of a large/bile stained gastric aspirate is not clear. Neonatologists therefore often arbitrarily consider different volumes and colours of gastric aspirate as markers of feed intolerance and/or early NEC. Mihatsch et al. have studied whether the mean gastric residual volume (GRV) and green gastric residuals (GR) were significant predictors of feed intolerance in the early enteral feeding advancement (12 ml/kg/day increments, 12 feeds a day) in extremely low birth weight (ELBW) neonates [85]. GR were checked before each feeding, and a GRV up to 2 or 3 ml in infants ≤ 750 g or > 750 g was tolerated respectively. Feeds

were reduced or withheld when GRV increased. The colour of GR was assessed as clear, milky, green-clear, green-cloudy, bloodstained, or hemorrhagic. The median volume of feeds reached on day 14 (V14) was 103 ml/kg/day (0–166). V14 increased with an increasing percentage of milky GR, whereas the mean GRV and green colour did not have a significant effect. The critical GRV seemed to be above 2 ml/3 ml because there was no significant negative correlation between the mean GRV and V14. Green GR was not negatively correlated with V14. A GRV of 2 ml failed to identify neonates who subsequently had delayed times to reach full enteral feeds (FEF). The authors concluded that green gastric residues should not slow down the increments of feed volumes in absence of other clinical signs and symptoms [85]. Other investigators also recommend that gastric residuals are very common in the early days of life but virtually never associated with NEC, and should not be allowed to interfere with feeding [85–87]. The safety of such recommendations in relation to NEC remains to be proven. Researchers have used a volume of 50 % of a total 3-h bolus feed as a marker of bowel pathology, in an attempt to study the significance of the volume of gastric residuals. However others have not validated these findings [88–91]. Cobb et al. [92] have assessed gastric residuals and their relationship to NEC in very low birth weight (VLBW) neonates. Their retrospective case-control study compared 51 cases of proven NEC with 102 controls matched for gestation (median: 26 weeks), birth weight (median: 822 g), race, and sex. The median postnatal age at diagnosis of NEC was 24 days. Feeding characteristics were recorded for the previous 6 days for both groups for the corresponding time period of birth. Feeds were started on the 5th day and aimed to increase to the full quota over 10 days (median) in both groups. The median time to FEF was 13 days in both groups. Neonates who developed NEC had more gastric residuals. The total residuals as percentage of feeds (primary outcome) and the average of maximum residuals increased in the NEC group from the first 3 days to the 3 days before diagnosis of NEC. A similar increase was not noted for control subjects. The maximum gastric residual [median (25th–75th centiles)] seemed to be the best predictor for NEC. [Control: 2 ml/feed (0.5–3.5) or 14 % of a feed (4–33); NEC: 4.5 ml/feed (1.5–9.8) or 40 % of a feed (24–61)] Neonates with NEC showed an increase in maximum residuals only on the day before the diagnosis of NEC, indicating that the residuals are probably an early sign of NEC rather than a predisposing or risk factor. The authors cautioned that the clinical utility of their observations was limited due to the overlap of study variables with those in the control group [92]. Other researchers have also advised caution in interpreting these results [93]. This was mainly in view of the method of selection of the control group that attempted to exclude neonates who had stage I NEC, and the fact that many preterm neonates have initial GI dysmotility and feed intolerance due to various reasons but do not necessarily develop NEC [93]. It was pointed out that by ensuring that controls had no "feed intolerance", many neonates who could have had their feeds held for reasons other than NEC were eliminated thus increasing the reported differences in gastric residual volumes artificially. This was also the likely reason for the higher percentage of control group neonates achieving FEF compared with the NEC group neonates (91 vs 69 %; $p = 0.01$) [93].

Bertino et al. [94] have reported gastric residuals as a marker of feed intolerance, and bloody residuals as possibly the best predictor for NEC. In their case-control

study, neonates with NEC (cases) were compared with gestation and birth weight matched controls without NEC. Feed tolerance was assessed by maximum gastric residual volume, maximum residual as percentage of previous feeding, and residual appearance. A total of 844 VLBW neonates were admitted during the study period, with an overall mortality before discharge of 14.6 %. NEC frequency was 2 %. Patent ductus arteriosus (PDA) was significantly associated with NEC. Mean maximum residual from birth to NEC onset, maximum residual as percentage of the corresponding feed volume, and the percentage of neonates with hemorrhagic residuals were significantly higher in cases compared with controls [94]. Shulman et al. [95] have recently studied the relationships of reaching FEF and feed volume with clinical measures in 50 preterm neonates. Daily total gavage feed intake, gastric residual volumes (GRVs; mls/day, number of GRVs > 50 % of the previous feed volume, and number of GRVs > 2 ml/kg), and abdominal distension were monitored. Repeated measurements of lactase activity, GI permeability, fecal calprotectin concentration, and gastric emptying were performed. The number of GRVs > 2 ml/kg tended to decrease with postnatal age ($p = 0.06$). Lactase activity and feed volume (ml/kg/day) before achieving FEF were correlated ($p = 0.007$, $\beta = 0.164$). There was no correlation between feeding outcomes and GRV, GRV > 50 %, GRV > 2 ml/kg, small bowel/colonic/whole bowel permeability, fecal calprotectin concentration, gastric emptying, or abdominal distension. They concluded that GRV is unreliable in predicting attainment of FEF, and that lactase activity is related to feed volume [95]. Christensen et al. [96] have studied the antecedents of Bell stage III NEC using data collected over a period of 7 years. Stage III NEC occurred in 118 neonates. The earliest recognized antecedents were nonspecific for NEC (apnea /bradycardia, skin mottling and irritability). These were recorded at 2.8 ± 2.1, 4.5 ± 3.1 and 5.4 ± 3.7 (Mean $\pm$ S.D.) h, respectively, before NEC was diagnosed. The most commonly identified antecedents were blood in the stools, increased abdominal girth and elevated pre-feed gastric residuals or vomiting, identified 2.0 ± 1.9, 2.8 ± 3.1 and 4.9 ± 4.0 h before NEC was diagnosed respectively [96].

It is recommended that gastric residuals be re-fed prior to gavage feeds in preterm neonates. In a survey of neonatal nurses ($N = 75$, 26 Respondents) only 2/26 indicated that they did this at least half the time. Many (54 %) indicated that they knew refeeding residuals benefited preterm neonates, and 58 % selected "doctors in this hospital do not order" as their rationale for not refeeding [97]. The effect of returning or discarding gastric residuals, on gastric emptying, feeding, electrolyte, and patient comfort, has been studied in critically ill adults [98]. In this RCT 125 critically ill patients were assigned to the "return" or "discard" gastric residual volume (GRV) group. Feed intolerance indicators, feeding delays and potential complications were studied. Fluid and electrolyte measures included serum potassium, glycaemia control and fluid balance. Discomfort was identified by significant changes in vital signs. Patients in both groups presented similar mean GRV with no significant differences found ($p = 0.111$), but patients in the intervention arm showed a lower incidence and severity of delayed gastric emptying episodes ($p = 0.001$). Except for hyperglycaemia there were no significant differences in other outcomes [98]. Hurt and McClave report that the use of GRVs is more of a tradition based on flawed assumptions, which unfortunately guides the delivery of enteral nutrition in critically ill adult patients

[99]. They have reminded that clinicians should not assume that GRVs are an inexpensive "poor man's test" for determining tolerance of enteral nutrition. Large scale prospective studies are required to develop an evidence based policy for managing GRVs in preterm neonates [99].

2.2 Abdominal Girth

The definition and interpretation of abdominal distension in preterm neonates is not clear. Malhotra et al. have measured the gastric residual (GR) volume in 50 healthy preterm neonates, 38 appropriate-for-dates (AFD), and 12 small-for-dates (SFD) with 28–36 weeks' gestation [100]. No linear correlation was found between increase in abdominal girth and GR volume. However, if the increase in abdominal girth was at least ≥ 2 cm, a GR of ≥ 23 % was observed. They recommended that such an increase in abdominal girth should be taken as warnings to reduce/withhold oral feeds [100]. Bhatiya et al. [101] have serially measured the abdominal circumference in 27 preterm neonates 1 h before feeding, immediately before and after feeding, and 1 and 2 h after feeding. In some neonates these measurements were repeated for 2 ($n = 18$) or 3 ($n = 3$) consecutive days. All 258 measurements were performed by the same examiner; 124 were also repeated by another examiner (masked to previous results) to test interobserver reliability. The coefficient of variation of repeated measurements of any one neonate by the same examiner on a single day was 2.7 %; 90 % of repeated measurements agreed within 1.5 cm, and the interobserver reliability was 0.99. Abdominal circumference positively correlated with birth weight ($p = 0.0001$) and time from last defecation ($p = 0.0001$) and negatively correlated with time from last feeding ($p = 0.04$). They concluded that increase in abdominal circumference of < 1.5 cm occurs normally and, in the absence of other clinical signs, should not be considered indicative of disease [101].

The significance of abdominal distension with or without bowel loops visible through the abdominal wall (without other features of intra-abdominal disease) is unclear, especially in the modern era when early and prolonged use of continuous positive airway pressure (CPAP) results in intestinal gaseous distension. Currently there is no evidence to indicate that such benign abdominal distension (CPAP belly) is a risk factor for NEC in preterm neonates. Whether or not it adversly affects feed tolerance is not clear [102, 103].

2.3 Passage of Meconium

Experts have commented that apart from poor gastroduodenal coordination and excessive quiescence in motor activity it is likely that delayed and slow colonic motility also plays a role in feed intolerance in preterm neonates [104–106]. A delayed stooling pattern, delayed/altered stooling pattern preceding decreased gastroduodenal

motility and gastric emptying, and vomiting in bowel obstruction/functional ileus that is similar to gastric residuals support this assumption [104–106]. Mihatsch et al. [107] have evaluated the correlation between the timings of the first and the last stool and feed intolerance in ELBW neonates. Forty one ELBW neonates were fed following a standardised protocol (day3–14). Bolus feeds were started at 48 h of age (12 mg/kg/day increments, 12 feeds a day). Gastric residuals up to 2 ml or up to 3 ml were tolerated for neonates with birth weight ≤ 750 g and > 750 g respectively. No enemas or laxatives were given during the study. The impact of the time until the passage of the first (M-1) and the last (M-last) meconium on the feeding volume on day 14 (V14) was assessed by linear regression analysis. The median (range) V14, M-1, and M-last were as follows: V14: 99 (0–156) ml/kg, M-1: 31(0.5–77) h, M-last: 6 (1.4–22) days. The results indicated a significant correlation between feed tolerance and the time for the last and not the first evacuation of meconium (ie. V14 increased with decreasing M-last, $p < 0.001$). These findings were interpreted as the passage of the first meconium only indicates terminal large bowel function and total evacuation of the meconium is a far better parameter of feed intolerance. The investigators suggested that passage of meconium should be considered when decisions on feeding ELBW neonates are made, and hypothesised if V14 could be advanced by accelerating passage of meconium [107]. Their hypothesis indicates a potential role of therapeutic agents accelerating passage of meconium in facilitating feed tolerance in preterm neonates [108]. Use of glycerine suppositories, prokinetics, small volume enemas, and gastrograffin upper GI series for evacuating meconium, meconium plugs is not uncommon in preterm neonates. A survey of enteral feeding practices for neonates < 32 weeks' gestation has shown that glycerin suppositories, and prokinetics were prescribed ("sometimes", "often", and "always") by (30.9 %, 9.1 %, 3.6 %), and (25.9 %, 5.6 %, 0 %) of the Australian neonatologists respectively [109]. Of those prescribing glycerine suppositories 57.7 and 70.6 % believed that the suppositories never/rarely helped in establishing regular bowel movements or diminishing feed intolerance, respectively [109]. These findings are not unexpected as total evacuation of meconium and feed tolerance requires normal function of both, the upper as well as lower gastrointestinal tract, and glycerin suppositories do not have any effect on the right colon and the small bowel [104, 105].

2.4 Vomiting

Vomiting is commonly interpreted as a sign of feed intolerance. However the frequency, amount, and colour of vomiting that indicates feed intolerance in preterm neonates is not well specified in the literature [110]. Many researchers have included vomiting as one of the key measurements of feed intolerance [14, 15, 17–19, 25, 26, 28, 30] but only Rüdiger et al. [111] have specified it as severe and Oei et al. [112] required it to occur more than once [110].

2.5 Blood in Stools

The presence of occult blood in stools is often interpreted as a significant finding with regards to early NEC. Abramo et al. have analysed the relationship of occult blood in stools with the development of NEC in enterally fed neonates ($N = 95$) with birth weight under 1800 g [113]. Daily stool specimens were tested for occult blood during the first 6 weeks of life. Fifty-four (58 %) of the 95 neonates had one or more blood-positive stools. Six (6.3 %) developed NEC. NEC occurred in only 2/54 neonates with one or more blood-positive stools vs 4/41 neonates with blood-negative stools. The presence of occult blood in the stools did not correlate with development of NEC [113]. Gralton et al. have reported the frequency of occult blood in stools in neonates hospitalized for a medical problem other than a GI disorder [114]. A total of 180 neonates (Ages: 2 days to 1 year), participated in the study. The majority (77.2 %), had guiac negative stools during the entire hospitalization, while 22.8 % had guiac positive stools during part or all of the hospitalization. Since most of those who tested positive were not receiving a milk-based formula or breast milk, a cause other than allergic sensitivity or milk-induced enterocolitis was suggested [114]. Pinheiro et al. [115] have reviewed the routine testing for occult blood and reducing substances in the stools in neonates. They reported that neither the performance characteristics of these tests with respect to NEC, nor their indirect impact, were evaluated formally before widespread adoption into clinical care. The published evidence suggested that these tests are not useful as diagnostic or screening tools. There was no evidence that such routine testing predicts NEC or decreases its rate or severity. They pointed out that the direct costs of these tests are significant, and of a greater concern was their potential unintended consequences, including the cost of secondary tests, restricted nutritional intake, and accumulation of distracting, useless data [115].

2.6 Imaging

Abdominal x-rays are often ordered in presence of persistent large and/or bile stained gastric residuals and abdominal distension, to "rule out NEC" in otherwise well preterm neonates. The utility of x-rays under such situation is questionable. Di Napoli et al. have reported the inter-observer reliability (Kappa values) of radiological signs of NEC. A total of 297 X-rays from 57 neonates were reported independently by 3 paediatric radiologists without having any clinical information about the patients [116]. The reproducibility of radiographic signs was as follows: 0.55 ($p < 0.01$) for diffuse gaseous intestinal distention, 0.22 ($p < 0.01$) for bowel wall thickening, 0.10 ($p < 0.01$) for the presence of portal venous gas, and 0.29 ($p < 0.01$) for pneumatosis intestinalis. The agreement for radiographic diagnosis suspected/confirmed of NEC was 0.31 ($p < 0.01$). Among the 23 possible combinations of radiographic signs, the radiologists indicated four profiles that produced a diagnosis of NEC containing, respectively, 2, 3, 4, and 5 signs. It was concluded that clinical information

and the presence of more than one radiological sign can reduce the margin of observer's error that inevitably exists when dealing with a diagnosis as difficult as NEC [116.]

The Duke Abdominal Assessment Scale (DAAS) is a 10-point numerical scale of plain film bowel gas pattern findings designed to reflect progression and increase the certainty of the diagnosis of NEC [117]. Coursey et al. [117] have validated it as a tool for predicting the severity of disease in neonates with suspected NEC. For every 1-point increase in the DAAS score, patients were statistically significantly more likely to have severe disease as measured by need for surgical intervention [117]. Hollingworth et al. agree that DAAS provides an objective method for improving clinical decision making in NEC considering the inherent variability and lack of consistency in reporting of X-rays [118]. Further research is important to assess the utility of such scales in minimising the misinterpretation of feed intolerance of prematurity as early NEC [118]. Investigators have reported the superiority of abdominal ultrasound (AUS) over x-rays for the diagnosis and monitoring of NEC [119–123]. However the benefits of AUS seem to be more for the diagnosis and monitoring of definite (Stage II/III) rather than suspected (Stage I) NEC [124].

2.7 Laboratory Markers

Laboratory parameters such as C-reactive protein (CRP), white cell count, plasma glucose levels are often used in conjunction with clinical and radiological findings to diagnose early NEC. Hallstrom et al. [125] have reported that a persistent metabolic acidosis, decreasing platelet count, and increasing blood glucose level on several successive days might predict a developing NEC, and leukocyte values above 30×10^9/L, pH under 7.25, and a blood glucose rise by 1.5 mmol/L or more within 24 h predict NEC with intestinal perforation. Pourcyrous et al. reported that a persistently normal CRP makes a diagnosis of NEC unlikely, and that antibiotics should be stopped and feeds resumed early in such cases [126]. CRP becomes abnormal in stage II and stage III NEC. In those with NEC, persistently elevated CRP after initiation of appropriate medical management suggests complications, which may require surgical intervention [126]. The risk of NEC is highest, and the manifestations of ileus of prematurity most frequent in extremely preterm neonates with gestation < 28 week sat birth. This is also the population where a borderline (otherwise insignificant) rise in CRP, drop in platelets, or rise in plasma glucose is not uncommon. The task of differentiating feed intolerance from early NEC has been difficult so far considering the unreliability of the clinical, radiological and conventional laboratory parameters in diagnosing the illness. However recent advances in the understanding of molecular and biochemical pathways in neonatal diseases are expected to lead to the discovery of new biomarkers that may help in resolving these issues [127–129]. As we wait for such advances, an improved understanding of the development and maturity of the GI tract in the preterm neonate should help in minimising the frequent withholding of feeds in extremely preterm neonates due to the fear of NEC, thereby reducing the risk of postnatal growth restriction [130].

References

1. Klurfeld DM (1999) Nutritional regulation of gastrointestinal growth. Front Biosci 4:D299–D302
2. Neu J (2003) The neonatal gastrointestinal tract: developmental anatomy, physiology and clinical implications. NeoReviews 4:e7–e13
3. Trahair JF, Sangild PT (1997) Systemic and luminal influences on the perinatal development of the gut. Equine Vet J Suppl 24:40–50
4. Lebenthal A, Lebenthal E (1999) The ontogeny of the small intestinal epithelium. JPEN J Parenter Enteral Nutr 23:S3–S6
5. Bryant MG, Buchan AM, Gregor M, Ghatei MA, Polak JM, Bloom SR (1982) Development of intestinal regulatory peptides in the human fetus. Gastroenterology 83:47–54
6. Eggermont E (1991) Problems of transfer of carbohydrates at the level of the intestinal mucosa. In: Schaub J, Van Hoof F, Vis HL (eds) Inborn errors of metabolism. Nestle nutrition, workshop series, vol 24. Raven Press, New York, pp 197–205
7. Veereman-Wauters G (1996) Neonatal gut development and postnatal adaptation. Eur J Pediatr 155:627–632
8. Keene MFL, Hewer EE (1929) Digestive enzymes of the human fetus. Lancet 1:767–769
9. Salenius P (1962) On the ontogenesis of the human gastric epithelial cells. Acta Anat 50:1–76
10. Numura Y (1966) On the submicroscopic morphogenesis of parietal cells in the gastric gland of the human fetus. Z Anat Entwicklungsgesch 125:316–356
11. Hess AF (1913) The gastric secretion of infants at birth. Am J Dis Child 6:264–284
12. Ebers DW, Smith DI, Gibbs GE (1956) Gastric acidity on the first day of life. Pediatrics 18:800–802
13. Ahn CI, Kim YJ (1963) Acidity and volume of gastric contents in the first week of life. J Korean Med Assoc 6:948–950
14. Avery GB, Randolph JG, Weaver T (1966) Gastric acidity in the first day of life. Pediatrics 37:1005–1007
15. Werner B (1948) Peptic and tryptic capacity of the digestive glands on newborns. Acta Paediatr Scand 35:1–80
16. Mason S (1962) Some aspects of gastric function in the newborn. Arch Dis Child 37:387–391
17. Klumpp TG, Neale AV (1930) The gastric and duodenal contents of normal infants and children. Am J Dis Child 40:1215–1229
18. Lieberman J (1966) Proteolytic enzyme activity in fetal pancreas and meconium. Gastroenterology 50:183–190
19. Koshtoyants CS (1931) Beitrag zur physiololgie des embryos (Embryosecretin). Pfluegers Arch Gesamte Physiol 227:359–360
20. Watkins JB (1975) Mechanisms of fat absorption and the development of gastrointestinal function. Pediatr Clin North Am 22:721–730
21. Carey MC, Hernall O (1992) Digestion and absorption of fat. Semin Gastrointes Dis 3:189–208
22. Zoppi G, Andreotti G, Pajno-Ferrara F, Njai DM, Gaburro D (1972) Exocrine pancreas function in premature and full term neonates. Pediatr Res 6:880–886
23. Watkins JB, Ingall D, Szczepanik P, Klein PD, Lester R (1973) Bile-salt metabolism in the newborn. Measurement of pool size and synthesis by stable isotope technic. N Engl J Med 288:431–434
24. Watkins JB, Szczepanik P, Gould JB, Klein P, Lester R (1975) Bile salt metabolism in the human premature infant. Preliminary observations of pool size and synthesis rate following prenatal administration of dexamethasone and phenobarbital. Gastroenterology 69:706–713
25. Schulzke SM, Patole SK, Simmer K (2011) Long-chain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev 2:CD000375
26. Kenny AJ, Maroux S (1982) Topology of microvillar membrance hydrolases of kidney and intestine. Physiol Rev 62:91–128

27. Eggermont E (1969) The hydrolysis of the naturally occurring alfa-glucosides by the human intestinal mucosa. Eur J Biochem 9:483–487
28. Schmitz J (1991) Digestive and absorptive function. In: Walker WA (ed) Pediatric gastrointestinal disease, 2nd volume, vol 1. B. C. Decker, Philadelphia, pp 266–280
29. Auricchio S, Rubino A, Muerset G (1965) Intestinal glycosidase activities in the human embryo, fetus, and newborn. Pediatrics 35:944–954
30. Lacroix B, Kedinger M, Simon-Assmann P, Haffen K (1984) Early organogenesis of human small intestine: scanning electron microscopy and brush border enzymology. Gut 25:925–930
31. Koldovsy O, Heringova A, Jirsova V et al (1965) Transport of glucose against a concentration gradient in everted sacs of jejunum and ileum on human fetuses. Gastroenterology 48:186–187
32. Jirsova V, Koldovsy O, Heringova A et al (1968) The development of the functions of the small intestine of the human fetus. Biol Neonate 9:44–49
33. Levin RJ, Koldovsky O, Hoskova J, Jirsova V, Uher J (1968) Electrical activity across human foetal small intestine associated with absorption processes. Gut 9:206–213
34. Spencer J, Isaacson PG, Walker-Smith JA, MacDonald TT (1989) Heterogeneity in intraepithelial lymphocyte subpopulations in fetal and postnatal human small intestine. J Pediatr Gastroenterol Nutr 9:173–177
35. Weaver LT (1991) Development of the gastrointestinal tract and accessory organs. Anatomy and embryology. In: Walker WA (ed) Pediatric gastrointestinal disease, 2nd volume, vol 1. B. C. Decker, Philadelphia, pp 195–216
36. Gluck L, Kulovich MV, Borer RC Jr, Brenner PH, Anderson GG, Spellacy WN (1971) Diagnosis of the respiratory distress syndrome by amniocentesis. Am J Obstet Gynecol 109:440–445
37. Udall JN, Watson RR (1991) Development of immune functions. In: Walker WA (ed) Pediatric gastrointestinal disease, 2nd volume, vol 1. B. C. Decker, Philadelphia, pp 300–311
38. Kantorova I, Svoboda P, Scheer P, Doubek J, Rehorkova D, Bosakova H, Ochmann J (2004) Stress ulcer prophylaxis in critically ill patients: a randomized controlled trial. Hepatogastroenterology 51:757–761
39. Bisset WM (1988) The development of motor control system in the gastrointestinal tract of the preterm infant. In: Milla PJ (ed) Disorders of gastrointestinal motility in childhood. John Wiley, Chichester, pp 17–27
40. Christensen J (1988) The enteric nervous system. In: Kumar D, Gustavsson S (eds) An illustrated guide to gastrointestinal motility. John Wiley, New York, pp 9–31
41. Gershon MD, Wade PR (1994) New developments in the enteric nervous system. Curr Opin Gastroenterol 10:183–192
42. Kenny SE, Vanderwinden JM, Rintala RJ et al (1998) Delayed maturation of the interstitial cells of Cajal: a new diagnosis for transient neonatal pseudo obstruction. Report of two cases- J Pediatr Surg 33:94–98
43. Yoo SY, Jung SH, Eom M, Kim IH, Han A (2002) Delayed maturation of interstitial cells of Cajal in meconium obstruction. J Pediatr Surg 37:1758–1761
44. De Vries JIP, Visser GHA, Prechtl HFR (1982) The emergence of fetal behaviour. I Qualitative aspect. Early Hum Dev 7:301–322
45. Bisset WM (1988) Intestinal motor activity in the preterm infants. In: Milla PJ (ed) Disorders of gastrointestinal motility in childhood. John Wiley, Chichester, pp 17–27
46. Casaer P, Daniels H, Devlieger H, De Cock P, Eggermont E (1982) Feeding behaviour in preterm neonates. Early Hum Dev 7:331–346
47. Milla PJ (1991) Feeding tasting and sucking. In: Walker WA (ed) Pediatric gastrointestinal disease: pathophysiology, diagnosis, management, 2nd volume, vol 1. B. C. Decker, Philadelphia, pp 217–223
48. Gryboski J (1969) Suck and swallow in the premature infant. Pediatrics 43:96–110
49. Dumont RC, Rudolph CD (1994) Development of the gastrointestinal motility in the infant and child. Gastroenterol Clin N Am 23:655–671
50. Gupta M, Brans YW (1978) Gastric retention in neonates. Pediatrics 62:26–29

51. Bisset WM, Watt JB, Rivers RP, Milla PJ (1988) Ontogeny of fasting small intestinal motor activity in the human infant. Gut 29:483–488
52. Veereman-Wauters G, Ghoos Y, van der Schoor S et al (1996) The 13C-octanoic acid breath test: a noninvasive technique to assess gastric emptying in preterm infants. J Pediatr Gastroenterol Nutr 23:111–117
53. Blumenthal I, Pildes RS (1979) Effect of posture on the pattern of stomach emptying in the newborn. Pediatrics 63:532–536
54. Cavell B (1979) Gastric emptying in preterm infants. Acta Paediatr Scand 68:725–730
55. Costalos C, Russell G, Al Rahim Q, Blumenthal I, Hanlin S, Ross I (1980) Gastric emptying of Caloreen meals in the newborn. Arch Dis Child 55:883–885
56. Hyman PE, Napolitano JA, Diego A et al (1990) Antroduodenal manometry in the evaluation of chronic functional gastrointestinal symptoms. Pediatrics 86:39–44
57. Zoppi G, Andreotti G, Pajno-Ferrara F, Njai DM, Gaburro D (1972) Exocrine pancreas function in premature and full term neonates. Pediatr Res 6:880–886
58. Siegel M, Krantz B, Lebenthal E (1985) Effect of fat and carbohydrate composition on the gastric emptying of isocaloric feedings in premature infants. Gastroenterology 89:785–790
59. Siegel M, Lebenthal E, Krantz B (1984) Effect of caloric density on gastric emptying in premature infants. J Pediatr 104:118–122
60. Siegel M, Lebenthal E, Topper W, Krantz B, Li PK (1982) Gastric emptying in prematures of isocaloric feedings with differing osmolalities. Pediatr Res 16:141–147
61. Lin HC, Kim BH, Elashoff JD, Doty JE, Gu YG, Meyer JH (1992) Gastric emptying of solid food is most potently inhibited by carbohydrate in the canine distal ileum. Gastroenterology 102:793–801
62. Read NW, McFarlane A, Kinsman RI et al (1984) Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon. Gastroenterology 86:274–280
63. Blumenthal I, Lealman GT, Shoesmith DR (1980) Effect of feed temperature and phototherapy on gastric emptying in the neonate. Arch Dis Child 55:562–564
64. Szabo JS, Hillemeier AC, Oh W (1985) Effect of non-nutritive and nutritive suck on gastric emptying in premature infants. J Pediatr Gastroenterol Nutr 4:348–351
65. Costalos C, Russell G, Bistarakis L, Pangali A, Philippidou A (1984) Effects of jaundice and phototherapy on gastric emptying in the newborn. Biol Neonate 46:57–60
66. Laing IA, Lang MA, Callaghan O, Hume R (1986) Nasogastric compared with nasoduodenal feeding in low birthweight infants. Arch Dis Child 61:138–141
67. Caillie MV, Powell GK (1975) Nasoduodenal versus nasogastric feeding in the very low birthweight infant. Pediatrics 56:1065–1072
68. Hyman PE, Abrams CE, Dubois A (1988) Gastric emptying in infants: response to metoclopramide depends on the underlying condition. J Pediatr Gastroenterol Nutr 7:181–184
69. Sankaran K, Yeboah E, Bingham WT, Ninan A (1982) Use of metoclopramide in preterm infants. Dev Pharmacol Ther 5:114–119
70. Tomomasa T, Miyazaki M, Koizumi T, Kuroume T (1993) Erythromycin increases gastric antral motility in human premature infants. Biol Neonate 63:349–352
71. Oei J, Lui K (2001) A placebo-controlled trial of low-dose erythromycin to promote feed tolerance in preterm infants. Acta Paediatr 90:904–908
72. Code CF, Marlett JA (1975) The interdigestive myo-electric complex of the stomach and small bowel of dogs. J Physiol 246:289–309
73. Vantrappen G, Janssens J, Hellemans J, Ghoos Y (1977) The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine. J Clin Invest 59:1158–1166
74. Berseth CL (1989) Gestational evolution of small intestine motility in preterm and term infants. J Pediatr 115:646–651
75. Bryant MG, Buchan AM, Gregor M, Ghatei MA, Polak JM, Bloom SR (1982) Development of intestinal regulatory peptides in the human fetus. Gastroenterology 83:47–54

76. Ittmann PI, Amarnath R, Berseth CL (1992) Maturation of antroduodenal motor activity in preterm and term infants. Dig Dis Sci 37:14–19
77. Berseth CL (1990) Neonatal small intestinal motility: motor responses to feeding in term and preterm infants. J Pediatr 117:777–782
78. Berseth CL (1992) Effect of early feeding on maturation of the preterm infant's small intestine. J Pediatr 120:947–953
79. Berseth CL, Nordyke C (1993) Enteral nutrients promote postnatal maturation of intestinal motor activity in preterm infants. Am J Physiol 264:G1046–G1051
80. Berseth CL, Nordyke CK (1992) Manometry can predict feeding readiness in preterm infants. Gastroenterology 103:1523–1528
81. Berseth CL, McCoy HH (1992) Birth asphyxia alters neonatal intestinal motility in term neonates. Pediatrics 90:669–673
82. Milla PJ (1986) Intestinal motility and its disorders. Clin Gastroenterol 15:121–136
83. Ito Y, Donahoe PK, Hendren WH (1977) Maturation of the rectoanal response in premature and perinatal infants. J Pediatr Surg 12:477–482
84. Bowes KL, Kling S (1979) Anorectal manometry in premature infants. J Pediatr Surg 14:533–535
85. Mihatsch WA, von Schoenaich P, Fahnenstich H et al (2002) The significance of gastric residuals in the early enteral feeding advancement of extremely low birth weight infants. Pediatrics 109:457–459
86. Ziegler EE, Thureen PJ, Carlson SJ (2002) Aggressive nutrition of the very low birthweight infant. Clin Perinatol 29:225–244
87. Thureen PJ, Hay WW Jr (2001) Early aggressive nutrition in preterm infants. Semin Neonatol 6:403–415
88. Akintorin SM, Kamat M, Pildes RS et al (1997) A prospective randomized trial of feeding methods in very low birth weight infants. Pediatrics 100:E4
89. Rayyis SF, Ambalavanan N, Wright L, Carlo WL (1999) Randomized trial of "slow" versus "fast" feed advancements on the incidence of necrotizing enterocolitis in very low birth weight infants. J Pediatr 134:293–297
90. Dollberg S, Kuint J, Mazkereth R, Mimouni FB (2000) Feeding intolerance in preterm infants: a randomized trial of bolus and continuous feeding. J Am Coll Nutr 19:797–800
91. Schanler RJ, Shulman RJ, Lau C, Smith EO, Heitkemper M (1999) Feeding strategies for premature infants: randomized trial of initiation and method of feeding. Pediatrics 103:434–439
92. Cobb BA, Carlo WA, Ambalavanan N (2004) Gastric residuals and their relationship to necrotizing enterocolitis in very low birth weight infants. Pediatrics 113:50–53
93. Kenton AB, Fernandes CJ, Berseth CL (2004) Gastric residuals in prediction of necrotizing enterocolitis in very low birth weight infants. Pediatrics 113:1848–1849
94. Bertino E, Giuliani F, Prandi G, Coscia A, Martano C, Fabris C (2009) Necrotizing enterocolitis: risk factor analysis and role of gastric residuals in very low birth weight infants. J Pediatr Gastroenterol Nutr 48:437–442
95. Shulman RJ, Ou CN, Smith EO (2011) Evaluation of potential factors predicting attainment of full gavage feedings in preterm infants. Neonatology 99:38–44
96. Christensen RD, Wiedmeier SE, Baer VL, Henry E, Gerday E, Lambert DK, Burnett J, Besner GE (2010) Antecedents of Bell stage III necrotizing enterocolitis. J Perinatol 30:54–57
97. Hodges C, Vincent PA (1993) Why do NICU nurses not refeed gastric residuals prior to feeding by gavage? Neonatal Network. 12:37–40
98. Juvé-Udina ME, Valls-Miró C, Carreño-Granero A et al (2009) To return or to discard? Randomised trial on gastric residual volume management. Intensive Crit Care Nurs 25:258–267
99. Hurt RT, McClave SA (2010) Gastric residual volumes in critical illness: what do they really mean? Crit Care Clin 26:481–490, viii–ix
100. Malhotra AK, Deorari AK, Paul VK, Bagga A, Singh M (1992) Gastric residuals in preterm babies. J Trop Pediatr 38:262–264
101. Bhatia P, Johnson KJ, Bell EF (1990) Variability of abdominal circumference of premature infants. J Pediatr Surg 25:543–544

102. Chauhan M, Henderson G, McGuire W (2008) Enteral feeding for very low birth weight infants: reducing the risk of necrotising enterocolitis. Arch Dis Child Fetal Neonatal Ed 93:F162–F166
103. Aly H, Massaro AN, Hammad TA, Narang S, Essers J (2009) Early nasal continuous positive airway pressure and necrotizing enterocolitis in preterm infants. Pediatrics 124:205–210
104. Jadcherla SR, Kliegman RM (2002) Studies of feeding intolerance in very low birth weight infants: definition and significance. Pediatrics 109:516–517
105. Amarnath RP, Berseth CL, Malagelada J et al (1989) Postnatal maturation of small intestinal motility in preterm and term infants. J Gastrointes Motil 1:138–143
106. Palazzolo V, Meetze W, Burchfield D et al (1992) Meconium passage in the micropremie: effect of hypocaloric enteral priming. Pediatr Res 31:192
107. Mihatsch WA, Franz A, Lindener W, Pohlandt F (2001) Meconium passage in extremely low birthweight infants and its relation to very early enteral nutrition. Acta Paediatr 90:409–411
108. Brunton LL (1996) Agents affecting gastrointestinal water flux and motility; emesis and antiemetis, bile acids and pancreatic enzymes. Section VA, Chapter 38 In: Hardman JG, Limbird LE, Molinoff PB et al (eds) The pharmacological basis of therapeutics. McGraw Hill, New York, pp 917–936
109. Patole S, Muller R (2004) Enteral feeding of preterm neonates: a survey of Australian neonatologists. J Matern Fetal Neonatal Med 16:309–314
110. Moore TA, Wilson MW (2012) Feeding intolerance- A concept analysis. Adv Neonatal Care 11:149–154
111. Rüdiger M, Herrmann S, Schmalisch G, Wauer RR, Hammer H, Tschirch E (2008) Comparison of 2-h versus 3-h enteral feeding in extremely low birth weight infants, commencing after birth. Acta Paediatr 97:764–769
112. Oei J, Lui K (2001) A placebo-controlled trial of low-dose erythromycin to promote feed intolerance in preterm infants. Acta Paediatr 90:904–908
113. Abramo TJ, Evans JS, Kokomoor FW, Kantak AD (1988) Occult blood in stools and necrotizing enterocolitis. Is there a relationship? Am J Dis Child 142:451–452
114. Gralton KS (1999) The incidence of guiac positive stools in newborns and infants. Pediatr Nurs 25:306–308
115. Pinheiro JM, Clark DA, Benjamin KG (2003) A critical analysis of the routine testing of newborn stools for occult blood and reducing substances. Adv Neonatal Care 3:133–138
116. Di Napoli A, Di Lallo D, Perucci CA, Schifano P, Orzalesi M, Franco F, De Carolis MP (2004) Inter-observer reliability of radiological signs of necrotising enterocolitis in a population of high-risk newborns. Paediatr Perinat EP 18:80–87
117. Coursey CA, Hollingsworth CL, Wriston C, Beam C, Rice H, Bisset G 3rd (2009) Radiographic predictors of disease severity in neonates and infants with necrotizing enterocolitis. AJR Am J Roentgenol 193:1408–1413
118. Hollingsworth CL, Rice HE (2010) The Duke Abdominal Assessment Scale: initial experience. Expert Rev Gastroenterol Hepatol 4:569–574
119. Bohnhorst B, Kuebler JF, Rau G, Gluer S, Ure B, Doerdelmann M (2011) Portal venous gas detected by ultrasound differentiates surgical NEC from other acquired neonatal intestinal diseases. Eur J Pediatr Surg 21:12–17
120. Dördelmann M, Rau GA, Bartels D, Linke M, Derichs N, Behrens C, Bohnhorst B (2009) Evaluation of portal venous gas detected by ultrasound examination for diagnosis of necrotising enterocolitis. Arch Dis Child Fetal Neonatal Ed 94:F183–F187
121. Silva CT, Daneman A, Navarro OM, Moore AM, Moineddin R, Gerstle JT, Mittal A, Brindle M, Epelman M (2007) Correlation of sonographic findings and outcome in necrotizing enterocolitis. Pediatr Radiol 37:274–282
122. Epelman M, Daneman A, Navarro OM, Morag I, Moore AM, Kim JH, Faingold R, Taylor G, Gerstle JT (2007) Necrotizing enterocolitis: review of state-of-the-art imaging findings with pathologic correlation. Radiographics 27:285–305
123. Faingold R, Daneman A, Tomlinson G, Babyn PS et al (2005) Necrotizing enterocolitis: assessment of bowel viability with color Doppler US. Radiology 235:587–594

124. Dilli D, Suna Oğuz S, Erol R, Ozkan-Ulu H, Dumanl H, Dilmen U (2011) Does abdominal sonography provide additional information over abdominal plain radiography for diagnosis of necrotizing enterocolitis in neonates? Pediatr Surg Int 27:321–327
125. Hällström M, Koivisto AM, Janas M, Tammela O (2006) Laboratory parameters predictive of developing necrotizing enterocolitis in infants born before 33 weeks of gestation. J Pediatr Surg 41:792–798
126. Pourcyrous M, Korones SB, Yang W, Boulden TF, Bada HS (2005) C-reactive protein in the diagnosis, management, and prognosis of neonatal necrotizing enterocolitis. Pediatrics 116:1064–1069
127. Young C, Sharma R, Handfield M, Mai V, Neu J (2009) Biomarkers for infants at risk for necrotizing enterocolitis: clues to prevention? Pediatr Res 65:91R–97R
128. Mussap M, Noto A, Cibecchini F, Fanos V (2013) The importance of biomarkers in neonatology. Semin Fetal Neonatal Med 18:56–64. doi:10.1016/j.siny.2012.10.006
129. Fanos V, Van den Anker J, Noto A, Mussap M, Atzori L (2013) Metabolomics in neonatology: fact or fiction? Semin Fetal Neonatal Med 18:3–12. doi: 10.1016/j.siny.2012.10.014
130. Ruth VA (2008) Extrauterine growth restriction: a review of the literature. Neonatal Netw 27:177–184

Part II
Enteral Nutrition

Chapter 2
Minimal Enteral Feeding

Olachi Mezu-Ndubuisi and Akhil Maheshwari

Abstract In preterm infants, enteral feeding is often delayed by hours to days after birth for fear of feeding intolerance due to immaturity, to avoid the accentuation of hypoxic/ischemic intestinal injury that might have been sustained *in utero* due to maternal risk factors such as pre-eclampsia, placental inusufficiency, or chorioamnionitis, or after birth due to the presence of cardio respiratory compromise in the early neonatal period, and as a protective strategy to reduce the risk of necrotizing enterocolitis. However, some degree of luminal nutrient exposure is essential to prevent intestinal mucosal atrophy. Minimal enteral feeding is a clinical compromise where small volumes of maternal milk or formula, typically 12–24 mL/kg/day, are provided to avoid complete enteral fasting for prolonged periods. Although preclinical and observational human studies indicate that minimal enteral feeding is likely to be beneficial through maturation of gut motility, induction of gut hormones, and prevention of adverse effects of enteral fasting and parenteral nutrition on the mucosa, randomized clinical trials conducted thus far have not provided conclusive evidence to confirm these benefits. Current clinical evidence suggests that minimal enteral feeding is relatively safe and does not increase incidence of NEC. However, the amount, duration, and the rate of advancement of minimal enteral feeding remain controversial. There is a need for a large, multi-centric study with pre-defined statistical and clinical definitions to draw strong conclusions. In this chapter, we review the physiological rationale and appraise the quality of existing evidence to support minimal enteral feeding in the neonatal intensive care unit.

Key points

- Minimal Enteral Feeding is a way to provide luminal nutrient stimulation to the immature or vulnerable neonatal gastrointestinal tract to prevent the adverse effects of prolonged enteral fasting

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_2,

- Evidence from animal and human studies strongly suggests that minimal enteral feeding can be beneficial by promoting physiologic gut function, maturation of gut motility, induction of gut hormones, and prevention of adverse effects of enteral fasting and TPN dependence on the mucosa
- Evidence strongly suggests that minimal enteral feeding is relatively safe, and does not increase incidence of NEC
- Amount, duration, and speed of advancement of minimal enteral feeding remains controversial
- There is a need for well-designed research with pre-set statistical and clinical measures to draw strong conclusions with minimal heterogeneity

1 Introduction

The introduction of enteral feedings is often delayed in very low birth weight (VLBW) infants due to the fear of poor tolerance in the presence of multi-system dysfunction, immaturity of the gastrointestinal tract, and the risk of necrotizing enterocolitis (NEC). However, concerns also remain that gut 'disuse' during extended periods of enteral fasting could delay or alter the postnatal adaptation of the premature intestine and prolong the need for parenteral nutrition [9, 102]. Minimal enteral feeding is a compromise alternative where small volumes of maternal milk or formula, typically 12–24 mL/kg/day, are provided to avoid complete enteral fasting [30]. Minimal enteral feeding has been described in the literature by various synonyms such as 'minimal enteral nutrition', 'gut priming' for stimulation of gastrointestinal function, 'trophic feedings' for promotion of gut growth, and 'hypocaloric feedings' as a reminder that minimal enteral feedings are not intended to be the primary or sole source of nutrient supply.

2 Historical Perspective

Minimal enteral feeding seems to have first appeared in the literature in animal studies in the 1950's. In the clinical setting, minimal enteral feeds first found favor in adult patients after bowel surgery and were used with an intention to promote tolerance to feeding [16, 35]. Studies in critically-ill and preterm infants started to arise in the 1970's and 80's as an intervention to promote gut maturation. The term "minimal enteral nutrition" was first used in the mid 1980's by Lucas et al. [14], who showed that enteral administration of very small quantities of human milk in term and preterm infants was associated with higher plasma concentrations of gut hormones than in enterally-fasted infants on parenteral nutrition. Cumulative feeding volumes (since birth) as small as 12 mL/kg body weight were associated with increased plasma enteroglucagon, gastrin, and gastric inhibitory peptide, and maximal responses were obtained with an average total intake of 50 mL/kg. Although larger enteral volumes

(still lower than full enteral feeds) were needed to produce a neurotensin or motilin surge, these findings suggested that minimal enteral feeding could help maintain mucosal integrity and possibly promote gut maturation in enterally-fasted infants dependent on parenteral nutrition [14]. In another study at about the same time, Slagle et al. [59] randomized 46 VLBW infants receiving parenteral nutrition to be either enterally-fasted or to receive minimal enteral feeding (12 mL/kg/day) from postnatal day 8 through day 18. After day 18, feedings were increased by 15 mL/kg/day in both groups. The minimal enteral feeding group showed improved tolerance to feedings, manifested by fewer days when feedings were withheld or when gastric residuals totaled more than 10 % of feedings. More infants in the minimal enteral feeding group achieved enteral intakes of 120 kcal/kg/day by 6 weeks than in the delayed feeding group (94 % *vs*. 64 % infants, respectively; $p < 0.05$). In other early studies, [2, 41] enteral feedings of 12–24 mL formula/kg/day (4–20 kcal/kg/day) during the first 8 days in ill VLBW infants was associated with better weight gain, faster decline in serum bilirubin levels, reduced cholestasis, better tolerance to subsequent larger-volume feedings, and faster attainment of full enteral feeds than infants who were enterally-fasted during the same period [64, 79].

The terms 'gut priming', 'trophic feedings', and 'hypocaloric feedings' became established in the 1990's as use of minimal enteral feeding was favorably reviewed in the nutritional management of critically-ill preterm neonates [25]. In the last 2 decades, there has been a gradual paradigm shift from avoiding enteral feeds to widespread acceptance of minimal enteral feeding as a preferred mode of initiation of feeding in critically-ill VLBW and extremely low birth weight (ELBW) infants. However, the volume, duration, methods and frequency of feedings vary considerably between individual centers and with limited evidence, there are no clear guidelines for practice.

3 Physiological Considerations in Early Introduction of Enteral Feedings

The developing gastrointestinal tract handles large volumes of amniotic fluid in utero Starting at 8–11 weeks, the fetus ingests increasing amounts of amniotic fluid during mid- and later gestation. In the 3rd trimester, the fetus swallows nearly 550 mL/day (range 210–840mL/day) of amniotic fluid [80, 81]. Although amniotic fluid is largely comprised of water (nearly 98–99 %), its composition varies with gestation [19]. In the 1st trimester, the osmolality of amniotic fluid is 290 mOsm/kg and is isotonic to maternal serum. However, as the fetal skin becomes keratinized and the renal function matures near term, the osmolality of amniotic fluid falls to 255 mOsm/kg. Despite its low caloric density and nutrient content (protein content ~1 % weight/volume), amniotic fluid is an important source of nutrition for the 3rd trimester fetus who swallows large volumes (up to 20 % of body weight per day) that may provide for up to 10–20 % of the daily energy needs [86].

Adverse effects of enteral feedings, real and presumed Following preterm birth, enteral feedings are withheld for a variety of pre- and postnatal reasons. Feedings are frequently withheld to allow the gastrointestinal tract to recover from actual/presumed ischemic insults that might have occurred *in utero* due to maternal pre-eclampsia, chorioamnionitis, placental insufficiency (indicated by the absence or reversal of umbilical arterial or aortic blood flow on Doppler studies), and fetal infection. Feedings may also be withheld for postnatal issues, if the infant 'looks unwell', has respiratory distress, persistent patency of the ductus arteriosus, or has had perinatal hypoxic-ischemia, events that could cause hypoxemia and/or hypotension and thereby trigger the 'diving' reflex, redirecting oxygenated blood away from the gut and towards vital organs such as the brain, heart, and adrenal glands. Although some infants with one or more of the above conditions may have truly sustained intestinal ischemia, most infants who receive presumptive treatment do not show any clinical signs of intestinal injury. In the absence of reliable biomarkers of intestinal ischemia, the care-provider is often left with no choice but to presume the worst-case scenario in all 'at-risk' infants that the gut mucosa needs time to recover from ischemic injury before enteral feedings can be initiated safely.

Developmental constraints to enteral feeding in the preterm infant Several studies have investigated the ontogeny of intestinal peristalsis and digestive function. Although not quite as well-developed as in the term infant, nutrient absorption in preterm infants is adequate to sustain normal growth [43, 60]. Similarly, with the exception of lactase activity that matures at about 34 weeks gestation, most digestive functions are in place by the end of the 2nd trimester [74]. Gastric acid output, bile synthesis, and exocrine pancreatic function are also considered adequate for digestion [12, 28, 65, 87]. Preterm infants can also increase their splanchnic blood flow after feeds, although the immature autoregulatory mechanisms can become overwhelmed under stress related to hypoxemia, shock, anemia, and transfusions [34, 51, 68].

Immaturity of motor function is a major limitation to successful enteral feeding in preterm infants. Readiness for oral feeding requires suck-swallow coordination, which develops at about 32 weeks gestation [56]. Infants born earlier than 32 weeks are at risk of aspiration of gastric contents into the trachea and lungs during oral feeding. To avoid recurrent overt or micro-aspirations, most clinicians prefer gavage as the modality of choice for VLBW infants.

In the gastrointestinal tract, effective propulsion of nutrients requires anterograde peristaltic contractions that are organized in time and location, and are synchronized with a relaxation response in segments immediately distal to the contraction wave. The motor activity of the gastrointestinal tract is regulated by inputs from the extrinsic nervous system, which includes the parasympathetic and sympathetic systems, and also from the intrinsic nervous system that is comprised of nerves that reside solely in the gastrointestinal tract [103]. Although major neural elements are in place by 15–18 weeks gestation, [96] the motor activity of the gastrointestinal tract continues to show signs of immaturity until late in the 3rd trimester such as laxity of the lower esophageal sphincter, delayed gastric emptying, and slow duodenal-anal transit [7, 97, 103, 109].

Prolonged enteral fasting can cause gut mucosal atrophy The absence of food in the gastrointestinal tract produces mucosal and villous atrophy and decreased expression of enzymes necessary for digestion and substrate absorption [29, 30]. In experimental animals, prolonged fasting can clearly cause small intestinal atrophy, loss of villus height and crypt depth, decreased intestinal weight, and enterocyte apoptosis [26]. The effects of enteral fasting vary with species and are most prominent in rodents, which can lose up to 50 % of the mucosal mass. Loss of mucosal mass is also seen in suckling pigs, but is less striking at about 20 %. In humans, the data are less clear. In critically-ill adults, enteral fasting for as few as 4 days was associated with decreased villus height and with abnormalities in lactulose-mannitol absorption [98]. In other studies, children with inflammatory bowel disease who were dependent on parenteral nutrition for 9–12 months showed relatively modest (about 10 %) mucosal atrophy [1]. The effects of enteral fasting have not been studied in neonates. However, ingestion of both amniotic fluid *in utero* as well as feeding after birth are required for the development of the crypt-villus histoarchitecture, [4, 27, 76, 94] and one can safely infer that the effects of enteral fasting are not likely to be less pronounced in infants than in older children and adult subjects.

Enteral fasting is also associated with decreased gut hormonal responses, including the hormones and trophic peptides produced in the oral cavity, stomach and the intestine in response to enteral feeding [32]. A variety of immune deficits can also develop, such as decreased mucosal IgA, increased expression of adhesion molecules, and leukocyte recruitment, which may increase the risk of mucosal inflammation [4, 32]. Fasting-related mucosal atrophy may also be directly associated with bacterial translocation from the lumen to mesenteric lymph nodes in rodents, although these findings need confirmation in humans [69].

Association between enteral feedings and necrotizing enterocolitis (NEC) Observational studies indicate that more than 90 % cases of NEC occur in infants who have received enteral feedings; many cases have a history of recent volume advancement or re-initiation of enteral feedings after a period of enteral fasting [20, 44]. The association may have an element of biological plausibility because enteral feeding, particularly with formula, could alter splanchnic perfusion and increase the risk of ischemic injury, [108] cause osmotic injury to the mucosa, and in the presence of undigested substrate in the gut lumen, promote bacterial overgrowth [57, 63]. In support of these data, in some studies, delayed introduction of enteral feeds beyond the first few days after birth protected against NEC [44]. Other studies showed that adoption of standardized, cautious feeding regimens where feeding volume was increased by < 24 mL/kg body weight each day lowered the risk of NEC [95, 105]. In the neonatal research network of the National Institute of Child Health and Development, Centers where enteral feedings were introduced at an earlier postnatal age and were advanced rapidly showed a higher incidence of NEC than institutions with more conservative feeding practices [55]. Based on data from these and other observational studies, most care-providers in neonatology adopted a very conservative approach to enteral feeding [105].

In contrast to observational/anecdotal data, early introduction or rapid advancement of feedings has not been shown to increase the incidence of NEC in randomized studies. In meta-analysis [70] of 4 randomized controlled trials (RCTs) [2, 12, 41, 52] comparing infants who received slow advancement of feedings (daily increments of 15–20 mL/kg) *vs*. those who were advanced rapidly (30–35 mL/kg/day), there was no difference in the incidence of NEC (typical relative risk (RR): 0.91, 95 % confidence interval (CI): 0.47–1.75) or all-cause mortality (RR: 1.43, 95 % CI: 0.78–2.61). Infants who had slow rates of feed volume advancement took longer to regain birth weight (median difference 2–6 days) and to establish full enteral feeding (median difference 2–5 days). Similarly, the protective effects of delayed introduction of enteral feedings, if any, were not detected in RCTs. In meta-analysis [67] of 5 RCTs, [24, 57, 58, 66, 88] delayed feedings did not reduce the risk of NEC [RR: 0.89, 95 % CI: 0.58–1.37] or all-cause mortality (RR: 0.93, 95 % CI: 0.53–1.64). Infants who had delayed introduction of enteral feeds took longer to establish full enteral feeding (reported median difference 3 days).

Minimal enteral feeding as an alternative to enteral fasting in premature infants Based on data from physiological and pre-clinical studies, minimal enteral feeding can stimulate gut motility and gastrointestinal hormone release, reduce the incidence of dysmotility and feeding intolerance, and thereby reduce the time taken to reach full enteral feeds. Enteral feedings may also reduce the incidence of complications associated with enteral fasting such as hyperbilirubinemia (related to increased enterohepatic circulation of bilirubin) or with parenteral nutrition such as infections and metabolic complications [107].

In most nurseries, minimal enteral feeding is defined as enteral administration of 12–24 mL/kg/day of expressed breast milk or formula. If more than 25 % of the patient's nutritional needs are administered enterally, the feeding is no longer considered 'trophic'. In a 1-kg infant, 20 mL/kg/day of enteral feedings represent about 5 % of the total volume of amniotic fluid that a gestational age-matched fetus would ingest each day *in utero*. Although the preterm gut may be able to handle larger volumes, minimal feeding strategies limit the feed volumes in view of the higher osmolality and protein/lipid concentration in milk/formula than amniotic fluid, which may affect the tolerance to enteral feeding.

Preclinical studies on the effects of enteral fasting Animal studies emphasize the importance of the first few postnatal weeks as a critical time for the growth and development of the gastrointestinal tract. In fetal pigs, the small intestine of responds rapidly to the introduction of oral colostrum or milk formula with large increases (50–75 %) in intestinal weight, similar to those in preterm and term newborn pigs receiving sow's colostrum [18]. This gastrointestinal growth response is not seen in enterally-fasted newborn piglets maintained on total parenteral nutrition. These findings are consistent with rapid growth of the small intestine seen in human infants during the early neonatal period [5, 99].

Kansagra et al. [42] showed that lack of enteral nutrition in piglets led to gut mucosal atrophy with decreased jejunal mass (34.8 %), villus height (44.4 %), and villus area (56.1 %) of TPN-fed piglets compared to enterally-fed newborn piglets.

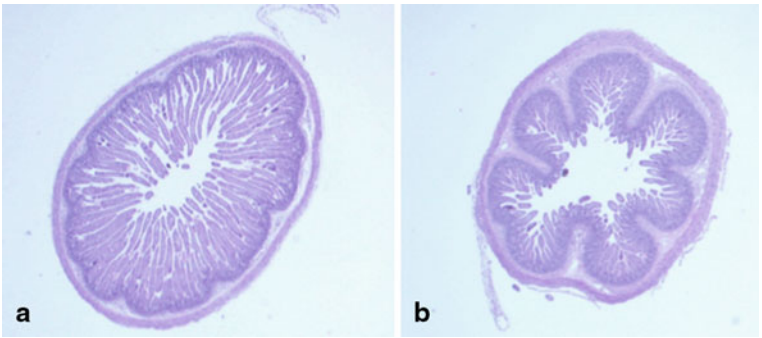

Fig. 2.1 Photomicrographs of jejunal tissue sections (hematoxylin and eosin; magnification 62.5 ×) from (**a**) enterally-fed newborn piglets; and (**b**) enterally-fasted piglets maintained on parenteral nutrition. Enteral fasting was associated with mucosal atrophy and loss of villus height (images courtesy Dr. Douglas Burrin, Baylor College of Medicine, Houston, Texas)

The absence of luminal nutrition in the intestine has also been associated with abnormal permeability to macromolecules, compromised barrier function, and eventually, loss of mucosal integrity [42, 106]. These changes, in turn, increase the risk of bacterial translocation and gut-derived sepsis [82]. In newborn piglets, gut mucosal atrophy ensues in the setting of partial/total absence (< 60 % total caloric intake) of enteral nutrition and is characterized by reduced villus height (Fig. 2.1), decreased crypt cell proliferation, and increased enterocyte apoptosis [8, 40]. TPN-induced mucosal atrophy is also associated with lymphocyte activation, [83] increased expression of adhesion molecules, [23, 48, 53] recruitment of neutrophils, and increased expression of inflammatory cytokines [13, 29, 89].

Studies from preterm animals show that early initiation of feeds from birth with animal colostrum results in an enhanced resistance to NEC [29]. Early introduction of minimal enteral feeding have been shown to promote intestinal motility, peristalsis, and enzymatic activity, augment intestinal blood flow, maintaining intestinal barrier function, reduction of infections and promoting development of beneficial gut microflora [11, 39, 54, 100].

4 Clinical Studies on Minimal Enteral Feeding

Minimal enteral feeding vs. enteral fasting in the first week after birth Several observational studies and clinical trials have examined minimal enteral feeding (Table 2.1). In 2005, Tyson and Kennedy [46] reviewed 11 studies of minimal enteral feeding. In 10 studies that compared minimal enteral feeding *vs.* enteral fasting, [15, 33, 45, 49, 50, 59, 61, 79, 84, 91] they noted that the minimal feeding group took fewer days to reach full enteral feeds (weighted mean difference (WMD) = 2.6 days), had fewer days when feedings were held (WMD = 3.1 days), and a shorter length of hospital stay (WMD = 11.4 days). There was no effect on

Table 2.1 Minimal enteral feeding: quality of evidence

Authors, Year	Description of study	Outcome/Results	Comments on study design and quality of evidence
Leaf, 2012 [24]	Infants < 35 weeks gestational age, birth weight < 10 percentile, and abnormal antenatal umbilical artery Doppler randomized to early feeding on day 2 ($n = 201$) *vs*. delayed on day 6 ($n = 201$).	Full feeds achieved earlier in early feeding group (median age18 days *vs*. 21 days) (hazard ratio: 1.36 [95 % CI 1.11–1.67]). No difference in the incidence of NEC.	Randomized, blinded, all subjects included in analysis.
Mosqueda, 2008 [21]	Minimal enteral feeding of expressed breast milk or formula 2 mL every 4 hrs from day 2–7 ($N = 41$) *vs*. enteral fasting (N = 43). Both groups received progressive enteral feeds from day 8, increasing by 10 mL/kg/day.	No difference in growth patterns, feeding tolerance, mortality, length of stay, incidence of sepsis and NEC between groups.	Randomized, but not blinded and incomplete follow-up.
Van Elburg, 2004 [104]	Minimal enteral feeding 0.5–1 mL every 2 h with breast milk/preterm formula from day 2–5 ($N = 28$) *vs*. enteral fasting ($N = 28$). Both groups received progressive enteral feeds from day 8, increasing by 10 mL/kg/day.	No difference in feeding tolerance, growth, incidence of NEC, and postnatal maturation of gut mucosal permeability as measured by lactose: mannitol absorption test.	Randomized, but not blinded and incomplete follow-up.
de Pipaon, 2003 [93]	Minimal enteral feeding of 10 mL/kg/day on day 1, then 20 mL/kg/day through until day 7 ($N = 24$) *vs*. enteral fasting for 7 days ($N = 12$).	Intestinal maturation measured as leucine uptake by splanchnic tissues. Minimal enteral feeding increasedleucine uptake by the splanchnic tissue.	Randomization unbalanced (larger minimal enteral feeding group). Not all infants included in analysis. Biochemical outcome.
Mclure, 2000 [84]	Minimal enteral feeding group received 0.5–1 mL/h breast milk/preterm formula from day 3 until discontinuation of assisted ventilation ($N = 48$). Control group was enterally-fasted ($N = 52$). After ventilation was stopped, feeds were started at 1 mL/kg/h and increased by 1 mL/kg/h every 8–12 h	Minimal enteral feeding group had better weight gain and increase in head circumference, fewer episodes of sepsis, decreased duration of parenteral nutrition, oxygen use, and length of hospital stay.	Randomized, blinded and all subjects included in analysis.

Table 2.1 (continued)

Authors, Year	Description of study	Outcome/Results	Comments on study design and quality of evidence
Schanler, 1999 [31]	Infants 26–30 weeks. Minimal enteral feeding ($N = 82$) 20 mL/kg/day of expressed breast milk or half-strength preterm formula from day 4–14 after birth *vs.* enteral fasting ($N = 89$). Minimal enteral feeding and fasting groups randomized to continuous *vs.* bolus feeds.	Time to full oral feeding similar in both groups. Minimal enteral feeding associated with better mineral retention, and shorter intestinal transit times. Bolus feeding associated with less feeding intolerance and better weight gain than the continuous method.	Randomized with complete follow-up but not blinded.
Becerra et al. 1996 [50]	Minimal enteral feeding with breast milk or preterm formula at 20–25 mL/kg/day ($n = 96$) *vs.* enteral fasting until 6–8 days post-natal ($N = 94$). Also included an arm with "healthy" VLBW infants	Minimal enteral feeding group had higher early weight gain, less hours of NPO, and less hyperglycemia than controls. No difference in the number of days to regain birth weight, weight on postnatal day 60, NEC, sepsis, and overall mortality.	Although all subjects were included in the analysis, study was non-blinded and the methods of randomization were not defined.
Troche, 1995 [72]	Infants 25–30 wks. Minimal enteral feeding group received expressed breast milk/standard formula from 24 h after birth at a rate of 0.5–1 mL/h until umbilical artery catheter was removed ($N = 16$) *vs.* enteral fasting ($N = 13$). Both groups received parenteral nutrition starting from day 3.	Minimal enteral feeding group required fewer days to reach full enteral feeds and had better weight gain. Minimal enteral feeding was well-tolerated in critically-ill VLBW nfants requiring mechanical ventilation.	Randomization unclear, not blinded and not all subjects included in analysis.
Davey, 1994 [45]	Minimal enteral feeding group received 2–5 mL every 2 hrs of 1/4 strength formula from 2 days ($N = 31$) *vs.* late enteral group which reeived feedings from day 5 ($N = 31$). Both groups had same volume of feeds and rate of advancement.	Minimal enteral feeding group had fewer days on parenteral nutrition, fewer interruptions in feedings, fewer sepsis evaluations, and fewer central lines. No difference in weight gain, NEC, mortality, or age at discharge.	Randomized and blinded in radiologic assessment but not blinded in clinical assessment. Not all patients were included in analysis.
Meetze, 1992 [62]	Minimal enteral feeding group received preterm formula from day 3 at 2.5 mL/kg/day and advancing to 22 mL/kg/day on day 14 ($N = 22$) *vs.* enteral fasting (N = 25). Both groups received progressive enteral feeds from day 15.	Minimal enteral feeding group had improved feeding tolerance after day 20 and a faster rise in serum gastrin. No difference in weight gain, frequency of feeding complications.	Randomization unclear, not blinded and not all subjects included in analysis.

Table 2.1 (continued)

Authors, Year	Description of study	Outcome/Results	Comments on study design and quality of evidence
Berseth, 1992 [61]	Infants 28–32 weeks on mechanical ventilation. Minimal enteral feeding group received a standard formula at 24 mL/kg/day from day 3–5 until day 10–14 ($N = 14$) *vs*. enteral fasting group ($N = 13$) that remained NPO until day 10–14. Both groups received 150 mL/kg/day total fluids.	Minimal enteral feeding group tolerated full oral feeds sooner, had fewer days of feeding intolerance, and had shorter length of hospital stay. These infants also showed more mature motor patterns and higher plasma levels of gastrin and gastric inhibitory peptide.	Although all subjects included in analysis, study was non-blinded; methods of randomization not defined.
Dunn, 1988 [14]	Minimal enteral feeding group ($N = 19$) from 48 hrs of life at 15–20 mL/kg/day using diluted preterm formula *vs*. enteral fasting ($N = 20$) until 9 days after birth.	Minimal enteral feeding group took fewer days to reach full enteral feedings, spent less time under phototherapy, had less cholestasis, and lower peak direct bilirubin levels.	Not randomized, not blinded, and not all subjects included in analysis.
Slagle, 1988 [16]	Infants 500–1500g, < 33 weeks. Minimal enteral feeding group ($N = 22$) received 12 mL/kg/d breast milk feedings from day 8 to day 18 *vs*. enteral fasting ($N = 24$).	Minimal enteral feeding group tolerated feeds better with fewer days when feedings were withheld. More infants reached full enteral feedings by 6 weeks.	Randomized, but not blinded and not all subjects included in analysis.
Khayata, 1987 [67]	N = 12, VLBW infants. Minimal enteral feeding group received standard formula starting at < 96 hrs of age at 12 mL/kg/day, increased to 24 mL/kg/day on day 2, 36 mL/kg/day on day 3–5. Late group remained NPO until day 10 and then fed using same schedule.	No difference in weight gain during the first six weeks after birth	Methodological details missing in abstract; unclear if subjects randomized; not blinded.
Ostertag, 1986 [52]	Infants < 32 weeks and < 1500 g. Minimal enteral feeding group ($N = 18$) fed enterally at 1 mL/h, starting with sterile water on day 1 and progressing to 2.5 % dextrose, 1/2 strength formula, and full strength formula over 7 days. Control group stayed NPO for 7 days followed by progressive enteral feeds increasing by 10 mL/kg/day ($N = 20$).	No difference in incidence of NEC	Unstated randomization, not blinded, but all subjects included in analysis.

NEC (RR = 1.16, 95 % CI = 0.75, 1.79); risk difference = 0.02 [−0.03, 0.06]). In 1 study [62] comparing early minimal feeding to progressively increasing feeds, the minimal feeding group showed a marginally significant reduction in NEC (total 8 cases of NEC; RR = 0.14 [0.02, 1.07]; risk difference = −0.09 [−0.16, −0.01]. The minimal feeding group took longer to reach full enteral feeds (WMD = 13.4 days) and tended to have longer hospital stay (WMD = 11.0).

In 2009, Bombell and McGuire [31] updated the earlier meta-analysis by Tyson and Kennedy [46]. They included all RCTs of early minimal enteral feeding (milk volumes up to 24 mL/kg/day introduced before 96 h postnatal age and continued for until at least one week after birth) *vs*. a comparable period of enteral fasting in VLBW infants. Of the 16 trials identified, [6, 21, 33, 49, 50, 59, 61, 62, 72, 73, 79, 84, 88, 91, 101] they excluded 7 studies, [33, 45, 59, 91, 62, 72, 109] some of which were a part [33, 45, 50, 59, 61] of the previous review by Tyson and Kennedy [46]. They reviewed data from 9 eligible trials [6, 15, 21, 49, 50, 61, 73, 79, 84] with a total of 754 infants but did not find strong evidence for benefit from early minimal enteral feeding. Eight trials examined time to establish full enteral feeding. In 3 studies, minimal enteral feeding was associated with less time to full enteral feeds [50, 79, 84]. However, no difference was detected in meta-analysis of 6 trials that reported means and standard deviation [WMD −0.97 (95 % CI −2.47, 0.53) days] or in 2 studies [21, 73] reporting median and ranges.

NEC was examined as an outcome measure in all 9 studies. Meta-analysis did not show a significant effect [RR: 1.07 (95 % CI: 0.67, 1.70); typical risk difference: 0.01 (95 % CI: −0.03, 0.05)]. Two trials reported data for sepsis. McClure et al. [50] noted fewer episodes of culture-positive sepsis in the minimal feeding group, whereas Mosqueda et al. [73] did not detect a difference. There was also no effect on overall mortality (RR: 0.77 (95 % CI: 0.46, 1.30)). Minimal enteral feeding also did not change the length of hospital stay. No difference was detected in meta-analysis of 3 trials that reported means and standard deviation [WMD −3.8 (95 % CI: −12.2, 4.5) days] or in 1 trial that reported data as median and range [73].

None of the 9 trials included in the meta-analysis reported a significant difference in the time to regain birth weight. McClure et al. [61] reported a marginally-significant increase in weight gain and head circumference in the minimal enteral feeding group. Similarly, Troche et al. [84] detected a greater increase in weight to day 30. However, no difference was detected in meta-analysis of 5 trials that reported means and standard deviation [WMD −0.01 (95 % CI −0.96, 0.95) days] or in 2 trials [21, 73] reporting median and ranges. Long-term growth parameters or neurodevelopmental outcome were not reported by any of the trials.

In their meta-analysis, Bombell and McGuire [31] detected considerable heterogeneity, which may limit the validity of their findings. A major limitation was the variability in data collection; for instance, it was not clear whether the included trials used pre-specified definitions of “feed intolerance” that mandated interrupting or ceasing feed volume advancement. Furthermore, the results may be biased by the exclusion from analysis of infants who developed complications [79, 84]. Other limitations were lack of clear information on the type of first feeding, formula *vs*. breast milk, and low number of extremely low birth weight infants. Although the benefits of early minimal enteral feeding were not detected in this meta-analysis, an

important conclusion was that minimal enteral feeding did not increase the risk of NEC as compared to the enteral fasting group.

Timing of initiation of minimal enteral feeding The ideal time of initiation of minimal enteral feeding is controversial with significant differences between individual Centers. Whereas 'early' initiation usually refers to starting feeds on the first postnatal day, 'delayed' initiation typically indicates that feedings may be started on postnatal day 4–8, or sometimes after day 10, after clinical stabilization of the infant. Clinical care-providers are often reluctant to start minimal enteral feeding earlier than 6 h after birth in VLBW infants, particularly when there is a history of perinatal depression and low Apgar scores, respiratory distress syndrome, hemodynamic instability, and/or persistent patency of the ductus arteriosus with diversion of blood away from the gastrointestinal tract. Although a majority of clinicians are now comfortable feeding with an umbilical arterial catheter in place, [75, 93] most are reluctant to start minimal enteral feeding while the infant is receiving indomethacin as prophylaxis against intra-ventricular hemorrhage or to treat a patent ductus arteriosus [9].

The benefits of early *vs*. late initiation of minimal enteral feeding remain unclear. As discussed in the previous sections, delayed introduction of enteral feedings does not protect against feeding intolerance, NEC, abnormal gut mucosal permeability, or prolonged length of hospital stay [21, 39, 44, 58, 66, 67, 72, 104]. The safety of early, aggressive feeding in high-risk infants was noted in a recent multicentre RCT by Leaf et al. [66], who enrolled 404 preterm small-for-gestation infants at increased risk of NEC in view of documented absence/reversal of diastolic blood flow in the umbilical artery/aorta on antenatal Doppler studies [36]. Subjects were randomized to an early (started feeding between 24–48 h) or late (between 120 and 144 h after birth) enteral feeding group. Early enteral feeding group achieved full enteral feeds at median 18 days (inter-quartile range (IQR) 15–24), compared to median 21 (IQR 19–27) days in the late feeding group; $p = 0.003$. There was no difference in the incidence of NEC (all-stage NEC 18 % in early *vs*. 15 % in the late group; RR: 1.20, 95 % CI: 0.77–1.87; $p = 0.42$; incidence of NEC stages 2 and 3 was 8 % in both groups) [66].

Bolus vs. continuous feeding The ideal method for minimal enteral feeding is not known. There is insufficient evidence to support one style of feeding over another. Whereas bolus feeding is widely accepted to be more physiological as it mimics the cyclic release of gut hormones, continuous feeds resemble fetal ingestion of amniotic fluid [37].

Using open-circuit respiratory calorimetry, Grant and Denne [85] measured energy expenditure in preterm infants ($n = 11$) fed the same volume of feeds during and after either intermittent (5 min) or continuous feeding (over 2–3 h). Energy expenditure was significantly increased after intermittent compared with continuous feeding (2.18 ± 0.07 kcal/kg/h *vs*. 2.09 ± 0.05 kcal/kg/h; $p < 0.05$) [85]. However, this increased energy expenditure with intermittent feeding has not been shown to have an adverse effect on growth [3].

Premji and Chessell [78] reviewed data from 7 randomized trials [3, 15, 37, 47, 77, 78, 90, 92] involving 511 infants and found no difference in the time to achieve full enteral feeds between feeding methods (WMD 2 days; 95 % CI −0.3–3.9), in somatic

growth, or in the incidence of NEC. One study [15] noted a trend toward more apneas in infants fed by the continuous tube feeding method compared to the intermittent feeding group [mean difference (MD) 14 apneas during study period; 95 % CI −0.2–28.2]. When data were compared by weight groups, 1 study [77] suggested that infants with birth weights < 1000 g and 1000–1250 g gained weight faster when fed by the continuous nasogastric method than by intermittent nasogastric method (MD 2.0 g/day; 95 % CI: 0.5–3.5; MD 2.0 g/day; 95 % CI: 0.2–3.8, respectively). A trend toward earlier discharge was noted in ELBW infants fed by the continuous feeding method than intermittent nasogastric feeding (MD −11 days; 95 % CI −21.8 −0.2).

Type of feeds for minimal enteral feeding The question about the ideal trophic feed needs examination in carefully-designed studies. Theoretically, the ideal trophic feed should have low osmolality and casein content, and should not increase absorption of precipitable minerals such as calcium and phosphate until appropriate meconium clearance [10]. Maternal milk has the added advantage of protective components such as immunoglobulins, lactoferrin, lysozyme, glycoconjugates, oligosaccharides, white blood cells, and antibodies that reflect the antigenic repertoire of the mother's intestine and respiratory tract [71].

Scahnler et al. [15] showed that early initiation of minimal enteral feeding with maternal milk, given as intermittent boluses, and in maximum volumes was associated with lower morbidity. Although the protective effects of human milk feeds over formula are well-documented, the maternal milk and formula need rigorous comparison in the context of the small volumes in minimal enteral feeding. In a large prospective RCT of early enteral feeding in preterm infants, Lucas and Cole [22] demonstrated the protective effect of breast milk against NEC (OR: 10.6, 95 % CI: 3.0–37.3 for confirmed cases, OR: 3.5, 95 % CI: 1.5–8.1 for all cases). They also showed a protective effect of delaying the introduction of formula feeding. The importance of exclusive human milk-based diet has been emphasized by two recent studies. Sullivan et al. randomized preterm infants fed their own mothers' milk to receive either (1) pasteurized donor human milk-based human milk fortifier when the enteral intake was 100 mL/kg and pasteurized donor human milk if no mother's milk was available; (2) pasteurized donor human milk-based human milk fortifier when the enteral intake was 40 mL/kg and pasteurized donor human milk if no mother's milk was available; or (3) bovine milk-based human milk fortifier when the enteral intake was 100 mL/kg/d and preterm formula if no mother's milk was available. Outcomes included duration of parenteral nutrition, morbidity, and growth. The groups receiving an exclusively human milk diet had significantly lower rates of NEC ($p = 0.02$) and NEC requiring surgical intervention ($p = 0.007$). In another study, Jirapaet et al. [38] studied the effect of human milk *vs*. formula on NEC and sepsis in VLBW infants. They started human milk feedings within 24 h of life using a standardized algorithm to identify infants with feeding intolerance. NEC was noted in 3.9 % infants in the human milk group and in 20 % in the formula group ($p = 0.04$). There was no difference in the incidence of late-onset sepsis between the two groups. However, in VLBW infants, the incidence of late-onset sepsis was 2.1 % (1 in 47) *vs*. 28.5 % (2 in 7) in human milk group and the formula group, respectively.

Advancement of feeding A consensus approach for advancement of enteral feeds in preterm infants remains elusive. As discussed in a previous section of this chapter, several well-designed randomized trials [2, 12, 41, 52] have shown that slow *vs*. rapid advancement of feeds may be equally safe [70]. The findings of Berseth et al. [62] merit careful consideration in this context. They randomized 144 infants to either receive a trophic feeding regimen (20 mL/kg/day of unfortified human milk or standard premature formula for 10 days) or receive advancing feedings (fed 20 mL/kg on postnatal day 1 and advanced 20 mL/kg/day on each day thereafter until 140/mL/kg was achieved). The advancing feed group showed a cluster of cases of NEC (10 % versus 1.4 % in minimal enteral feeding group), which prompted the study to close early. Although the minimal enteral feeding group reached full feeds later than the advancing volume group, the maturation of intestinal motor patterns and the incidence of late-onset sepsis and feeding intolerance was similar in both groups.

Overall, current data do not provide evidence that slow advancement of enteral feed volumes reduces the risk of NEC in VLBW infants. Increasing the volume of enteral feeds at slow rather than faster rates results in several days' delay in regaining birth weight and establishing full enteral feeds but the long term clinical importance of these effects is unclear. There is a need for additional RCTs to determine how the rate of daily increment in enteral feed volumes affects clinical outcomes in VLBW infants. An excellent discussion on this issue can be found in a recent review by Fallon et al. [17]

Current feeding practices Nutritional practices in preterm infants vary not only between neonatal units, but also between practitioners within the same unit. In a recent web-based survey of 127 tertiary neonatal intensive care units in different countries on different continents, Klingenberg et al. [102] sought to evaluate enteral feeding practices. Out of 124 units that responded, there was considerable variation in initiation of enteral feeding within the first 24 h after birth: 43/124 (35 %) if gestational age (GA) < 25 weeks, 53/124 (43 %) if GA 25–27 weeks and 88/124 (71 %) if GA 28–31 weeks. For infants below 28 weeks' gestation, breast milk feedings were initiated early and continuous feedings in Scandanavian units. In contrast, continuous feedings were rarely used in Australia/New Zealand. Minimal enteral feeding for 4–5 days was common in Canada, but rare in Scandinavia. Target enteral feeding volume in a 'stable' preterm infant was 140–160 mL/kg/day in most Canadian units and 161–180 mL/kg/day or higher in units in the other regions. The survey also revealed marked regional differences in criteria for use and timing when human milk fortifier was added. There was a wide variation in feeding practices across units around the world, indicating a need for RCTs to examine key issues and promote standardization of feeding regimens.

5 Directions for Further Research

There is a need for well-designed, multi-centred RCTs to clarify specific concerns about minimal enteral feeding. To account for the variability in the current-day NICU patient populations, only a sufficiently large cohort with adequate representation

of high-risk and ELBW infants is likely to be meaningful for variables such as death or NEC requiring surgical resection before discharge, death before discharge, prolonged hospital stay, growth restriction, or other major co-morbidities like short bowel syndrome, and severe neurodevelopmental disability assessed at $\geq$ 18 months adjusted age. A well designed large multi-centered RCT should also be able to address key issues such as the optimal time to start, role of co-morbidities, volumes suitable for minimal enteral feeding, timing for advancement of feedings, slow *vs*. rapid advancement, choice of feed, route of feeding, and so forth. There is a need for clear definition of outcome variables, particularly for feeding intolerance.

In their systematic review, Tyson et al. [11] identified difficulties in interpretation of currently published trials of trophic feedings, and proposed several ways to design a large multicenter trial to answer the deficits in current literature. They argued that with the sample sizes achievable in major neonatal networks, Bayesian analyses are likely to provide clear and clinically-useful assessments of the probability of benefit for all important clinical outcomes resulting from initial feeding regimens for ELBW infants. They further suggested that the ideal RCT should compare three feeding regimens: enteral fasting *vs*. trophic feeding, enteral fasting *vs*. rapid advancement of feedings, and slow *vs*. rapid advancement of feedings in ELBW infants [11]. An appropriate primary question in these studies would be to identify the feeding regimen that results in the lowest proportion of infants with a composite outcome of either NEC or death before discharge. The authors estimated that the proposed sample size needed for such a study would be as large as 3960 patients enrolled over 3 years with outcomes assessed at 2 years for 90 % of infants discharged home.

In summary optimizing nutrition is a vital part of caring for the preterm infant, but is affected by various innate and external challenges, such as gut and intestinal immaturity, critical illness and infection, feeding intolerance, and NEC. Minimal enteral feeding is a promising approach to avoid the adverse effects of prolonged enteral fasting in critically-ill infants. It is supported by strong physiological and pre-clinical data but requires further evaluation in the clinical setting with carefully-designed RCTs.

References

1. Adan D, La Gamma EF, Browne LE (1995) Nutritional management and the multisystem organ failure/systemic inflammatory response syndrome in critically ill preterm neonates. Crit Care Clin 11:751–784
2. Akintorin SM, Kamat M, Pildes RS, Kling P, Andes S, Hill J, Pyati S (1997) A prospective randomized trial of feeding methods in very low birth weight infants. Pediatrics 100:E4
3. Alpers DH (2002) Enteral feeding and gut atrophy. Curr Opin Clin Nutr Metab Care 5:679–683
4. Antonowicz I, Chang SK, Grand RJ (1974) Development and distribution of lysosomal enzymes and disaccharidases in human fetal intestine. Gastroenterol 67:51–58
5. Atkinson SA, Bryan MH, Anderson GH (1981) Human milk feeding in premature infants: protein, fat, and carbohydrate balances in the first two weeks of life. J Pediatr 99:617–624
6. Bastian L, Weimann A (1999) Practical aspects of early enteral feeding. Anaesthesiol Reanim 24:95–100

7. Becerra M, Ambiado S, Kuntsman G, Figueroa A, Balboa P, Fernandez P, Uauy R (1996) Feeding VLBW infants: effect of early enteral stimulation (EES). Pediatr Res 39:304A
8. Berg RD (1995) Bacterial translocation from the gastrointestinal tract. Trends Microbiol 3:149–154
9. Berseth CL (1990) Neonatal small intestinal motility: motor responses to feeding in term and preterm infants. J Pediatr 117:777–782
10. Berseth CL (1992) Effect of early feeding on maturation of the preterm infant's small intestine. J Pediatr 120:947–953
11. Berseth CL, Nordyke C (1993) Enteral nutrients promote postnatal maturation of intestinal motor activity in preterm infants. Am J Physiol 264:G1046–G1051
12. Berseth CL, Nordyke CK, Valdes MG, Furlow BL, Go VL (1992) Responses of gastrointestinal peptides and motor activity to milk and water feedings in preterm and term infants. Pediatr Res 31:587–590
13. Berseth CL, Bisquera JA, Paje VU (2003) Prolonging small feeding volumes early in life decreases the incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics 111:529–534
14. Bhatia AM, Feddersen RM, Musemeche CA (1996) The role of luminal nutrients in intestinal injury from mesenteric reperfusion and platelet-activating factor in the developing rat. J Surg Res 63:152–156
15. Bjornvad CR, Schmidt M, Petersen YM, Jensen SrK, Offenberg H, Elnif J, Sangild PT (2005) Preterm birth makes the immature intestine sensitive to feeding-induced intestinal atrophy. American J Physiolo Regul Integr Compara Physiol 289:R1212–R1222
16. Bombell S, McGuire W (2008) Delayed introduction of progressive enteral feeds to prevent necrotising enterocolitis in very low birth weight infants. Cochrane Database Syst Rev:CD001970
17. Bombell S, McGuire W (2009) Early trophic feeding for very low birth weight infants. Cochrane Database Syst Rev:CD000504
18. Brace RA (1997) Physiology of amniotic fluid volume regulation. Clin Obstet Gynecol 40:280–289
19. Burrin DG, Stoll B, Jiang R, Chang X, Hartmann B, Holst JJ, Greeley GH, Jr., Reeds PJ (2000) Minimal enteral nutrient requirements for intestinal growth in neonatal piglets: how much is enough? Am J Clin Nutr 71:1603–1610
20. Cavell B (1981) Gastric emptying in infants fed human milk or infant formula. Acta Paediatr Scand 70:639–641
21. Caple J, Armentrout D, Huseby V, Halbardier B, Garcia J, Sparks JW, Moya FR (2004) Randomized, controlled trial of slow versus rapid feeding volume advancement in preterm infants. Pediatrics 114:1597–1600
22. Condino AA, Barleycorn AA, Lu W, Maheshwari A, Christensen RD, Calhoun DA (2004) Abnormal intestinal histology in neonates with congenital anomalies of the gastrointestinal tract. Biol Neonate 85:145–150
23. Conour JE, Ganessunker D, Tappenden KA, Donovan SM, Gaskins HR (2002) Acidomucin goblet cell expansion induced by parenteral nutrition in the small intestine of piglets. Am J Physiol Gastrointest Liver Physiol 283:G1185–G1196
24. Davey AM, Wagner CL, Cox C, Kendig JW (1994) Feeding premature infants while low umbilical artery catheters are in place: a prospective, randomized trial. J Pediatr 124:795–799
25. Dollberg S, Kuint J, Mazkereth R, Mimouni FB (2000) Feeding tolerance in preterm infants: randomized trial of bolus and continuous feeding. J Am Coll Nutr 19:797–800
26. Dorling J, Kempley S, Leaf A (2005) Feeding growth restricted preterm infants with abnormal antenatal Doppler results. Arch Dis Child Fetal Neonatal Ed 90:F359–F63
27. Dsilna A, Christensson K, Alfredsson L, Lagercrantz H, Blennow M (2005) Continuous feeding promotes gastrointestinal tolerance and growth in very low birth weight infants. J Pediatr 147:43–49
28. Dunn L, Hulman S, Weiner J, Kliegman R (1988) Beneficial effects of early hypocaloric enteral feeding on neonatal gastrointestinal function: preliminary report of a randomized trial. J Pediatr 112:622–629

29. Fallon EM, Nehra D, Potemkin AK, Gura KM, Simpser E, Compher C, Puder M (2012) A.S.P.E.N. Clinical guidelines: nutrition support of neonatal patients at risk for necrotizing enterocolitis. JPEN J Parenter Enteral Nutr
30. Ganessunker D, Gaskins HR, Zuckermann FA, Donovan SM (1999) Total parenteral nutrition alters molecular and cellular indices of intestinal inflammation in neonatal piglets. JPEN J Parenter Enteral Nutr 23:337–344
31. Gershon MD, Chalazonitis A, Rothman TP (1993) From neural crest to bowel: development of the enteric nervous system. J Neurobiol 24:199–214
32. Grant J, Denne SC (1991) Effect of intermittent versus continuous enteral feeding on energy expenditure in premature infants. J Pediatr 118:928–932
33. Gregory KE, Connolly TC (2012) Enteral feeding practices in the NICU: results from a 2009 neonatal enteral feeding survey. Adv Neonatal Care 12:46–55 10.1097/ANC.0b013e3182425aab
34. Gupta M, Brans YW (1978) Gastric retention in neonates. Pediatrics 62:26–29
35. Harkness L (2002) The history of enteral nutrition therapy: from raw eggs and nasal tubes to purified amino acids and early postoperative jejunal delivery. J Am Diet Assoc 102:399–404
36. Havranek T, Johanboeke P, Madramootoo C, Carver JD (2007) Umbilical artery catheters do not affect intestinal blood flow responses to minimal enteral feedings. J Perinatol 27:375–379
37. Hay WW, Jr (2008) Strategies for feeding the preterm infant. Neonatology 94:245–254
38. Hernandez G, Velasco N, Wainstein C, Castillo L, Bugedo G, Maiz A, Lopez F, Guzman S, Vargas C (1999) Gut mucosal atrophy after a short enteral fasting period in critically ill patients. J Crit Care 14:73–77
39. Herbst JJ, Sunshine P (1969) Postnatal development of the small intestine of the rat. Changes in mucosal morphology at weaning Pediatr Res 3:27–33
40. Hsueh W, Caplan MS, Qu XW, Tan XD, De Plaen IG, Gonzalez-Crussi F (2003) Neonatal necrotizing enterocolitis: clinical considerations and pathogenetic concepts. Pediatr Dev Pathol 6:6–23
41. Hyman PE, Clarke DD, Everett SL, Sonne B, Stewart D, Harada T, Walsh JH, Taylor IL (1985) Gastric acid secretory function in preterm infants. J Pediatr 106:467–471
42. Jirapaet K, Jirapaet V, Sritipsukho S (2010) Safety of initiating early enteral feeding with slow volume advancement in preterm infants. J Med Assoc Thai 93:1177–1187
43. Kansagra K, Stoll B, Rognerud C, Niinikoski H, Ou CN, Harvey R, Burrin D (2003) Total parenteral nutrition adversely affects gut barrier function in neonatal piglets. Am J Physiol Gastrointest Liver Physiol 285:G1162–G1170
44. Karagianni P, Briana DD, Mitsiakos G, Elias A, Theodoridis T, Chatziioannidis E, Kyriakidou M, Nikolaidis N (2010) Early versus delayed minimal enteral feeding and risk for necrotizing enterocolitis in preterm growth-restricted infants with abnormal antenatal Doppler results. Am J Perinatol 27:367–373
45. Kempley ST, Sinha AK, Thomas MR (2005) Which milk for the sick preterm infant? Current Paediatrics 15:390–399
46. Kennedy KA, Tyson JE, Chamnanvanikij S (2000) Early versus delayed initiation of progressive enteral feedings for parenterally fed low birth weight or preterm infants. Cochrane Database Syst Rev:CD001970
47. Khayata S, Gutcher G, Bamberger Jea (1987) Early versus Late Feeding of low birth weight (LBW) infants: Effect on growth and hyperbilirubinemia. Pediatr Res 21:431 A
48. Kiristioglu I, Antony P, Fan Y, Forbush B, Mosley RL, Yang H, Teitelbaum DH (2002) Total parenteral nutrition-associated changes in mouse intestinal intraepithelial lymphocytes. Dig Dis Sci 47:1147–1157
49. Kliegman RM, Fanaroff AA (1981) Neonatal necrotizing enterocolitis: a nine-year experience. Am J Dis Child 135:603–607
50. Klingenberg C, Embleton ND, Jacobs SE, O'Connell LA, Kuschel CA (2012) Enteral feeding practices in very preterm infants: an international survey. Arch Dis Child Fetal Neonatal Ed 97:F56–F61

51. Krimmel GA, Baker R, Yanowitz TD (2009) Blood transfusion alters the superior mesenteric artery blood flow velocity response to feeding in premature infants. Am J Perinatol 26:99–105
52. Krishnamurthy S, Gupta P, Debnath S, Gomber S (2010) Slow versus rapid enteral feeding advancement in preterm newborn infants 1000–1499 g: a randomized controlled trial. Acta Paediatr 99:42–46
53. Kudsk KA (2002) Current aspects of mucosal immunology and its influence by nutrition. Am J Surg 183:390–398
54. Kuzma-O'Reilly B, Duenas ML, Greecher C, Kimberlin L, Mujsce D, Miller D, Walker DJ (2003) Evaluation, development, and implementation of potentially better practices in neonatal intensive care nutrition. Pediatrics 111:e461–470
55. LaGamma EF, Ostertag SG, Birenbaum H (1985) Failure of delayed oral feedings to prevent necrotizing enterocolitis. Results of study in very-low-birth-weight neonates. Am J Dis Child 139:385–389
56. Lau C, Smith EO, Schanler RJ (2003) Coordination of suck-swallow and swallow respiration in preterm infants. Acta Paediatr 92:721–727
57. Leaf A, Dorling J, Kempley S, McCormick K, Mannix P, Linsell L, Juszczak E, Brocklehurst P (2012) Early or delayed enteral feeding for preterm growth-restricted infants: a randomized trial. Pediatr 129:e1260–8
58. Levine GM, Deren JJ, Steiger E, Zinno R (1974) Role of oral intake in maintenance of gut mass and disaccharide activity. Gastroenterology 67:975–982
59. Lucas A, Cole TJ (1990) Breast milk and neonatal necrotising enterocolitis. Lancet 336:1519–1523
60. Lucas A, Bloom SR, Aynsley-Green A (1980) Development of gut hormone responses to feeding in neonates. Arch Dis Child 55:678–682
61. Lucas A, Bloom SR, Aynsley-Green A (1986) Gut hormones and 'minimal enteral feeding'. Acta Paediatr Scand 75:719–23
62. Macdonald PD, Skeoch CH, Carse H, Dryburgh F, Alroomi LG, Galea P, Gettinby G (1992) Randomised trial of continuous nasogastric, bolus nasogastric, and transpyloric feeding in infants of birth weight under 1400 g. Arch Dis Child 67:429–431
63. Maheshwari A, Corbin LL, Schelonka RL (2011) Neonatal necrotizing enterocolitis. Res Report Neonatol 1:39–53
64. McClure RJ, Newell SJ (2000) Randomised controlled study of clinical outcome following trophic feeding. Arch Dis Child Fetal Neonatal Ed 82:F29–F33
65. McKeown RE, Marsh TD, Amarnath U, Garrison CZ, Addy CL, Thompson SJ, Austin JL (1992) Role of delayed feeding and of feeding increments in necrotizing enterocolitis. J Pediatr 121:764–770
66. Meetze W, Valentine C, McGuigan Jea (1991) Gastrointestinal (GI) priming prior to full enteral nutrition in very low birthweight (VLBW) infants. Pediatr Res 29:300 A
67. Meetze W, Valentine C, McGuigan J, Conlon M, Sacks N, Neu J (1992) Gastrointestinal priming prior to full enteral nutrition in very low birth weight infants. J Pediatr Gastroenterol Nutr 15:163–170
68. Morgan J, Young L, McGuire W (2011) Slow advancement of enteral feed volumes to prevent necrotising enterocolitis in very low birth weight infants. Cochrane Database Syst Rev:CD001241
69. Morgan J, Young L, McGuire W (2011) Delayed introduction of progressive enteral feeds to prevent necrotising enterocolitis in very low birth weight infants. Cochrane Database Syst Rev:CD001970
70. Mosqueda E, Sapiegiene L, Glynn L, Wilson-Costello D, Weiss M (2008) The early use of minimal enteral nutrition in extremely low birth weight newborns. J Perinatol 28:264–269
71. Mulvihill SJ, Stone MM, Debas HT, Fonkalsrud EW (1985) The role of amniotic fluid in fetal nutrition. J Pediatr Surg 20:668–672
72. Neu J (2007) Gastrointestinal maturation and implications for infant feeding. Early Hum Dev 83:767–775

73. Neu J (2007) Gastrointestinal development and meeting the nutritional needs of premature infants. Am J Clin Nutr 85:629−634 S
74. Neu J, Douglas-Escobar M, Lopez M (2007) Microbes and the developing gastrointestinal tract. Nutr Clin Pract 22:174–182
75. Ostertag SG, LaGamma EF, Reisen CE, Ferrentino FL (1986) Early enteral feeding does not affect the incidence of necrotizing enterocolitis. Pediatrics 77:275–280
76. Premji SS, Chessell L (2011) Continuous nasogastric milk feeding versus intermittent bolus milk feeding for premature infants less than 1500 g. Cochrane Database Syst Rev:CD001819
77. Pritchard JA (1966) Fetal swallowing and amniotic fluid volume. Obstet Gynecol 28:606–610
78. Ramji S (2002) Enteral feeding of low birth weight infants. Indian J Pediatr 69:401–404
79. Rayyis SF, Ambalavanan N, Wright L, Carlo WA (1999) Randomized trial of "slow" versus "fast" feed advancements on the incidence of necrotizing enterocolitis in very low birth weight infants. J Pediatr 134:293–297
80. Rossi TM, Lee PC, Young C, Tjota A (1993) Small intestinal mucosa changes, including epithelial cell proliferative activity, of children receiving total parenteral nutrition (TPN). Dig Dis Sci 38:1608–1613
81. Saenz de Pipaon M, VanBeek RH, Quero J, Perez J, Wattimena DJ, Sauer PJ (2003) Effect of minimal enteral feeding on splanchnic uptake of leucine in the postabsorptive state in preterm infants. Pediatr Res 53:281–287
82. Salhotra A, Ramji S (2004) Slow versus fast enteral feed advancement in very low birth weight infants: a randomized control trial. Indian Pediatr 41:435–441
83. Sangild PT, Petersen YM, Schmidt M, Elnif J, Petersen TK, Buddington RK, Greisen G, Michaelsen KF, Burrin DG (2002) Preterm Birth affects the intestinal response to parenteral and enteral nutrition in newborn pigs. J Nutr 132:2673–2681
84. Schanler RJ, Rifka M (1994) Calcium, phosphorus and magnesium needs for the low-birth-weight infant. Acta Paediatr Suppl 405:111–116
85. Schanler RJ, Shulman RJ, Lau C, Smith EO, Heitkemper MM (1990) Feeding strategies for premature infants: randomized trial of gastrointestinal priming and tube-feeding method. Pediatrics 103:434–439
86. Schmidt M, Sangild PT, Blum JW, Andersen JB, Greve T (2004) Combined ACTH and glucocorticoid treatment improves survival and organ maturation in premature newborn calves. Theriogenology 61:1729–1744
87. Shin ED, Estall JL, Izzo A, Drucker DJ, Brubaker PL (2005) Mucosal adaptation to enteral nutrients is dependent on the physiologic actions of glucagon-like peptide -2 in mice. Gastroenterology 128:1340–1353
88. Siggers J, Sangild PT, Jensen TK, Siggers RH, Skovgaard K, Stoy AC, Jensen BB, Thymann T, Bering SB, Boye M (2011) Transition from parenteral to enteral nutrition induces immediate diet-dependent gut histological and immunological responses in preterm neonates. Am J Physiol Gastrointest Liver Physiol 301:G435–G445
89. Siggers RH, Siggers J, Thymann T, Boye M, Sangild PT (2011) Nutritional modulation of the gut microbiota and immune system in preterm neonates susceptible to necrotizing enterocolitis. J Nutr Biochem 22:511–521
90. Silvestre MA, Morbach CA, Brans YW, Shankaran S (1996) A prospective randomized trial comparing continuous versus intermittent feeding methods in very low birth weight neonates. J Pediatr 128:748–752
91. Slagle TA, Gross SJ (1988) Effect of early low-volume enteral substrate on subsequent feeding tolerance in very low birth weight infants. J Pediatr 113:526–531
92. Szabo JS, Mayfield SR, Oh W, Stonestreet BS (1987) Postprandial gastrointestinal blood flow and oxygen consumption: effects of hypoxemia in neonatal piglets. Pediatr Res 21:93–98
93. Thompson AM, Bizzarro MJ (2008) Necrotizing enterocolitis in newborns: pathogenesis, prevention and management. Drugs 68:1227–38
94. Tiffany KF, Burke BL, Collins-Odoms C, Oelberg DG (2003) Current practice regarding the enteral feeding of high-risk newborns with umbilical catheters in situ. Pediatrics 112:20–23

95. Toce SS, Keenan WJ, Homan SM (1987) Enteral feeding in very-low-birth-weight infants. A comparison of two nasogastric methods. Am J Dis Child 141:439–444
96. Trahair JF (1989) Remodeling of the rat small intestinal mucosa during the suckling period. J Pediatr Gastroenterol Nutr 9:232–237
97. Trahair JF, Harding R (1995) Restitution of swallowing in the fetal sheep restores intestinal growth after midgestation esophageal obstruction. J Pediatr Gastroenterol Nutr 20:156–161
98. Troche B, Harvey-Wilkes K, Engle WD NHC, Frantz ID 3rd, Mitchell ML HRJ (1995) Early minimal feedings promote growth in critically ill premature infants. Biol Neonate 67:172–181
99. Thureen PJ, Hay WW, Jr (2001) Early aggressive nutrition in preterm infants. Semin Neonatol 6:403–415
100. Tyson JE, Kennedy KA (2000) Minimal enteral nutrition for promoting feeding tolerance and preventing morbidity in parenterally fed infants. Cochrane Database Syst Rev:CD000504
101. Tyson JE, Kennedy KA, Lucke JF, Pedroza C (2007) Dilemmas initiating enteral feedings in high risk infants: how can they be resolved? Semin Perinatol 31:61–73
102. Uauy RD, Fanaroff AA, Korones SB, Phillips EA, Phillips JB, Wright LL (1991) Necrotizing enterocolitis in very low birth weight infants: biodemographic and clinical correlates. National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr 119:630–638
103. van Elburg RM, van den Berg A, Bunkers CM, van Lingen RA, Smink EW, van Eyck J, Fetter WP (2004) Minimal enteral feeding, fetal blood flow pulsatility, and postnatal intestinal permeability in preterm infants with intrauterine growth retardation. Arch Dis Child Fetal Neonatal Ed 89:F293–F296
104. Weiler HA, Fitzpatrick-Wong SC, Schellenberg JM, Fair DE, McCloy UR, Veitch RR, Kovacs HR, Seshia MM (2006) Minimal enteral feeding within 3 d of birth in prematurely born infants with birth weight $<$ or $=$ 1200 g improves bone mass by term age. Am J Clin Nutr 83:155–162
105. Wolf R, Moore T (2006) Amniotic Fluid and Nonimmune Hydrops Fetalis. In: Martin R, Faranoff A, Walsh M, eds. Neonatal-Perinatal Medicine. Volume 1. Mosby Elsevier, Philadelphia:409–428
106. Yang H, Finaly R, Teitelbaum DH (2003) Alteration in epithelial permeability and ion transport in a mouse model of total parenteral nutrition. Crit Care Med 31:1118–1125
107. Yanowitz TD, Yao AC, Pettigrew KD, Werner JC, Oh W, Stonestreet BS (1999) Postnatal hemodynamic changes in very-low-birthweight infants. J Appl Physiol 87:370–380
108. Ziegler EE, Thureen PJ, Carlson SJ (2002) Aggressive nutrition of the very low birthweight infant. Clin Perinatol 29:225–244
109. Zoppi G, Andreotti G, Pajno-Ferrara F, Njai DM, Gaburro D (1972) Exocrine pancreas function in premature and full term neonates. Pediatr Res 6:880–886

Chapter 3
Strategies for Managing Feed Intolerance in Preterm Neonates

Sanjay Patole

Abstract Optimisation of enteral nutrition in extremely preterm neonates (gestation under 28 weeks) has become a priority considering that postnatal growth restriction is a major and almost universal issue in this population. Majority of protein and energy deficit associated with postnatal growth restriction occurs within the first two weeks of life. Manifestation of feed intolerance due to ileus of prematurity (e.g., abdominal distension, bile stained and/or increased gastric residuals) are also very common during this critical period in extremely preterm neonates.

Necrotising enterocolitis (NEC) is a potentially disastrous illness in preterm very low birth weight neonates with significant mortality, and morbidity. The outcomes of NEC are worse in extremely preterm neonates with higher mortality, need for surgery, and risk of long-term neurodevelopmental impairment after surviving surgery for the illness.

The inability to differentiate feed intolerance of prematurity from a potentially disastrous illness like NEC frequently leads to stoppage of enteral feeds during a critical period of life in extremely preterm neonates. The significant variation in clinical practice reflects the fact that evidence for many of the enteral feeding strategies for extremely preterm neonates have either inadequate or no sound scientific basis.

Evidence base for current enteral feeding practices for preterm neonates is reviewed. The proven benefits of well established strategies such as antenatal glucocorticoids and preferential use of breast milk are emphasised. Newer options for facilitating feed tolerance such as probiotics and prebiotics are discussed. Areas for further research are suggested.

Key points

- Postnatal growth restriction (PGR) is a major and almost universal issue in extremely preterm (EP) neonates.
- Majority of protein and energy deficit associated with PGR occurs within the first two weeks of life when stoppage of enteral feeds is very common because of the

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_3,

fear of necrotising enterocolitis (NEC) in presence of manifestations of ileus of prematurity.
- Many of the enteral feeding practices for EP neonates have either inadequate or no sound scientific basis.
- Balancing the benefits and risks of aggressive enteral and parenteral nutrition to optimise growth and development of EP neonates is a difficult task.
- Further research is necessary for finding the optimal method for enteral feeding of EP neonates, and to evaluate newer options such as probiotics and prebiotics for facilitating feed tolerance in this high-risk population.

Survival of extremely preterm neonates has increased following the advances in neonatal intensive care. Optimisation of enteral nutrition in this population has become a priority after the realisation that postnatal growth restriction and failure to thrive is a major, and almost universal issue in preterm extremely low birth weight (ELBW: Birth weight < 1,000 g) neonates [1, 2]. It is important to note that the majority of protein and energy deficit associated with postnatal growth failure occurs within the first two weeks of life [3, 4].

Necrotising enterocolitis (NEC) is a potentially disastrous illness in preterm very low birth weight (VLBW) neonates with significant mortality, and morbidity including protracted feed intolerance, complications of parenteral nutrition, multiple episodes of sepsis, need for surgery, and survival with short bowel syndrome [5–8]. The incidence (10–12 % vs. 4–6 %) and outcomes (Need for surgery: 20–30 % vs. 40–45 %, Mortality: 25 % vs. 40–45 %) are worse in ELBW compared with VLBW neonates [9, 10]. Long term neurodevelopmental impairment (NDI) after surviving surgery for the illness is a serious concern in extremely preterm (gestation < 28 weeks) neonates [11]. Considering the prolonged hospital stay due to complications, the economic burden of ≥ Stage II NEC is significant [12]. The fear of NEC is perhaps justified considering its potentially disastrous consequences and lack of a reliable marker for early diagnosis. This fear, and the inability to differentiate early NEC from the almost universal manifestations of "feed intolerance" due to ileus of prematurity in the first few weeks of life makes it very difficult to optimise enteral nutrition in extremely preterm neonates [13]. Significant variation in feeding practices reflects the lack of a clear understanding of the pathophysiology of both, feed intolerance and NEC. Considering the importance of evidence-based practice it is necessary to know the spectrum of current strategies for preventing/minimising feed intolerance in preterm neonates and the evidence supporting them.

1 Antenatal Glucocorticoids

The beneficial effects of antenatal glucocorticoids on gastrointestinal maturation and function include reduced uptake of macromolecules from the mucosa, reduced hepatic bacterial translocation [14–18], and increased activity of enzymes like lactase, maltase, and sucrase [19–21]. A significant reduction in the incidence of NEC following antenatal glucocorticoid therapy was first reported by Bauer et al. [22]. The

effect was more striking on NEC than on respiratory distress syndrome (RDS)—the desired primary outcome. Later a systematic review of randomised controlled trials (RCT) by Crowley et al. [23] and the RCT by Halac et al. [24] confirmed that the risk of NEC was reduced significantly by antenatal glucocorticoid therapy. Results of the updated (2007) Cochrane systematic review of 21 RCTs (3,885 women and 4,269 infants) confirm that antenatal corticosteroids reduce the risk of neonatal death, and morbidity including RDS, intraventricular haemorrhage (IVH), and NEC [25]. Current guidelines recommend antenatal corticosteroids for mothers in preterm labour from 24 to 34 weeks' gestation, but not before 24 weeks due to lack of data [26]. Carlo et al. recently conducted a multicentre ($N = 23$) cohort study to determine if antenatal corticosteroid treatment is associated with improved major outcomes for infants ($N = 10{,}541$) born at 22–23 weeks' gestation [27]. Their results indicate that the neonatal benefits of antenatal glucocorticoid treatment for death and morbidity including NEC, likely occur even at limits of viability [27].

2 Breast Milk

Human milk has been reported to reduce the incidence of NEC by up to seven fold compared with formula milk [28]. In this multicenter trial the benefits of breast milk were noted even as a supplement [28]. The protective effect of breast milk has been correlated with its anti-inflammatory components (e.g., cytokines, growth factors), lysozyme, IgG, prebiotic oligosaccharides and probiotics [29–32]. The activity of acetyl hydrolase (PAF-AH), an enzyme that degrades platelet-activating factor (PAF) is lower in neonates under 3 weeks of age than at any other time [33]. Considering the role of PAF in the pathogenesis of NEC the presence of PAF-AH activity may partly explain the protective effect of breast milk as formula milks don't contain it [34–38]. High use of breast milk is reported to lower the incidence of NEC, and result in a mild illness with more survivors [39]. Evidence indicates that freezing or thawing does not eliminate the benefits of breast milk in reducing the incidence and severity of NEC, possibly due to the increased anti-inflammatory cytokine IL-10 at the injury site [40]. A systematic review and meta analysis has reported that donor human milk feeding was associated with a significantly reduced risk of NEC [41]. The systematic review [42] of RCTs indicates that donor breast milk is associated with a lower risk of NEC and slower growth in the early postnatal period, but the quality of the evidence is limited. Sullivan et al. have recently evaluated the benefits of an exclusively human milk-based diet in extremely preterm neonates [43]. Neonates ($N = 207$) fed mothers' milk were randomized to 1 of the 3 study groups. Groups HM100 and HM40 received pasteurized donor human milk-based human milk fortifier when the enteral intake was 100 and 40 ml/kg/day, respectively, and both groups received pasteurized donor human milk if no mother's milk was available. Group BOV received bovine milk-based human milk fortifier when the enteral intake was 100 ml/kg/day and preterm formula if no mother's milk was available. All groups had comparable baseline demographics, duration of parenteral nutrition, rates of late-onset sepsis, and growth.

The groups receiving an exclusively human milk had significantly lower rates of NEC ($p = 0.02$) and NEC requiring surgery ($p = 0.007$) [43]. Meinzen-Derr et al. have reported that the likelihood of NEC or death after 14 days was decreased by a factor of 0.83 (95 % CI: 0.72, 0.96) for each 10 % increase in the proportion of total intake as human milk (HM) in ELBW neonates [44]. Each 100 ml/kg increase in HM intake during the first 14 days was associated with decreased risk of NEC or death (Hazard Ratio: 0.87 (95 % CI: 0.77, 0.97). There was a non-significant trend towards a decreased risk of NEC or death among infants who received 100 % HM as a proportion to total enteral intake (HM plus formula) [44]. Sisk et al. have reported that an intake of at least 50 % mother's milk was associated with fewer days to reach 100 and 150 ml/kg/day of enteral feeds in neonates who weighed $\leq 1{,}250$ g [45]. Sisk et al. have also reported that enteral feeding containing at least 50 % human milk in the first 14 days of life was associated with a sixfold decrease in the odds of NEC [46]. Breast milk alone has not eliminated NEC despite its advantages. Deficiency of immunologically beneficial components like IL-10, in the very preterm breast milk (< 30 weeks' gestation) may explain some of the susceptibility of extremely preterm neonates to NEC [47]. Emami et al. have recently reported the protective role of IL-10 in the pathogenesis of NEC in an animal model of the illness [48].

3 Hydrolysed Protein Formula (HPF)

HPF has been shown to accelerate the gastrointestinal transit of formula [49]. Results of a clinical trial by Mihatsch et al. suggest that HPF may have a role in accelerating feeding advancement in VLBW neonates [50]. The primary outcome was the time from initiation to reaching full enteral feeds (FEF: 150 ml/kg/day) in neonates who received < 10 % human milk (HM) to exclude HM as a confounder. Because the availability of HM was not predictable at enrolment, all eligible neonates ($n = 129$) were randomly assigned to receive HPF or standard preterm formula (SPF) if HM was unavailable; 87 neonates (HPF = 46, SPF = 41) received < 10 % HM. The baseline demographic characteristics were comparable. The time to reach FEF was significantly shorter with HPF feeds [10 (9–27) vs. 12 (9–28) days] [50]. Riezzo et al. have reported no significant difference in gastric electrical activity, gastric emptying time, and symptoms such as regurgitation and vomiting in preterm neonates fed standard compared with hydrolysed formula [51]. Maggio et al. have evaluated the urinary excretion of essential amino acids and weight gain in a small RCT in which preterm neonates ($n = 21$) were randomly allocated to feeding with either a HPF or an ordinary preterm formula [52]. Blood and amino acid concentrations were analysed before the start and after 14 and 28 days of feeding. Weight gain was slower in neonates fed the HPF. The renal excretion of essential amino acids was higher on day 14 days, indicating that the HPF did not have any nutritional advantage [52]. Routine use of HPF in preterm neonates can not be recommended considering the inadequate and conflciting evidence in this area.

4 Lactase Treated and Low Lactose Feeds

Lactase, the enzyme for digestion and absorption of lactose, is the last of the major intestinal disaccharidases to develop in preterm neonates. Lactase activity at 26–34 weeks gestation is only 30 % of that at term [53, 54]. Feed intolerance in preterm neonates may relate to their transient low functional lactase activity [55]. Prolonged periods of starvation could result in gut mucosal atrophy/damage that primarily involves injury to the microvillus tip, where lactase is produced [56, 57]. Lactase is therefore the first enzyme to be lost and the last to recover [58].

Early initiation of human milk feeds is important in preterm neonates as it is known to increase intestinal lactase activity [59]. Colonic fermentation may explain why lactose malabsorption manifests more commonly as large gastric residuals or abdominal distension rather than diarrhea in preterm neonates [60, 61]. Lactase has been used to hydrolyse lactose to minimize lactose malabsorption in preterm neonates. Erasmus et al. have studied whether lactase-treated feeds could enhance weight gain (primary outcome) and feed tolerance in preterm neonates [62]. A total of 130 preterm neonates of 26–34 weeks post-conceptual age (mean postnatal age at entry 11 days) were enrolled in this double blind RCT. Lactase treated feeds were initiated when enteral feeds provided > 75 % of daily intake. The RR for NEC was not statistically significant: 0.32 (95 % CI: 0.32 (0.01, 7.79); RD: −0.02 (95 % CI: −0.06, 0.03). Weight gain (mean ± SEM) of the treatment group was significantly greater (20.4 ± 1.8 g/day vs. 15.5 ± 1.6 g/day, $p < 0.05$) than that of the control group on day 10 but not at any other time points. There was no significant effect on feed intolerance. No adverse effects were noted [62]. The role of lactase treated feeds in enhancing weight gain without adversely affecting feed tolerance needs to be studied further in extremely preterm neonates. Griffin et al. have studied whether a low-lactose formula (LLF, < 5 % lactose) would improve feed intolerance in preterm neonates [63]. A total of 306 neonates (Gestation: < 36 weeks, Weight: < 1,800 g) were randomised to either lactose-containing formula (LCF) 24 kcal/oz or a specially prepared LLF, which was comparable to the LCF except for the replacement of lactose with maltose. Total 149 neonates were assigned to LCF (99 received only LCF); 152 were assigned to LLF (102 received only LLF). The remaining received LCF or LLF plus some quantity of human milk or human milk alone. Neonates receiving LLF had improved enteral caloric intake and weight gain, reached FEF faster, had less gastric residual, spent less time without oral intake, and had fewer feeds stopped than the LCF group. The incidence of NEC was too low to draw any conclusions [63].

5 Early Trophic Feeds (ETF)

Feeds are frequently stopped in extremely preterm neonates due to the signs of feed intolerance and the fear of NEC. Prolonged lack of enteral nutrients diminish gastrointestinal functional and structural integrity by diminishing hormonal activity,

growth of intestinal mucosa, lactase activity, or motor maturation [64]. ETF are therefore provided to facilitate gastrointestinal function and maturation to improve feed tolerance in extremely preterm neonates. In a systematic review, 9 RCTs ($N = 754$) of ETF were eligible for meta analysis [65]. ETF was generally started within the first 3 days of life and continued for various durations. Its volume varied from 12 to 24 cm^3/kg/day. Meta analysis ($I^2 = 74\,\%$) of 6 trials did not detect a significant effect on the time to FEF. (WMD = −0.95, 95 % CI: −2.47, 0.53 days) and NEC [RR: 1.07 (95 % CI: 0.67, 1.70), $I^2 = 0$]. It was concluded that the available data could not exclude important beneficial or harmful effects of ETF, and were insufficient to guide clinical practice [65].

6 Continuous Versus Intermittent Bolus Feeding

The benefits and risks of feeding preterm VLBW neonates intermittently, typically over 10–20 min every 1–3 h, or continuously, using an infusion pump, are not clear. A systematic review and meta analysis of 7 RCTs ($N = 511$) reports no significant differences between feeding methods in the time to reach FEF (WMD: 2 days; 95 % CI −0.3–3.9) [66]. In the subgroup analysis comparing continuous versus intermittent bolus nasogastric feeding the findings remained unchanged (WMD 2 days, 95 % CI −0.4–4.1). There was no significant difference in the incidence of NEC and somatic growth between feeding methods irrespective of tube placement. In a subgroup analysis, one trial suggested that neonates with birth weight < 1,000 g and between 1,000 and 1,250 g gained weight faster with continuous compared with intermittent nasogastric tube feeds (MD: 2.0 g/day; 95 % CI: 0.5–3.5; MD: 2.0 g/day; 95 % CI: 0.2–3.8, respectively). A trend toward earlier discharge was noted for ELBW neonates fed by the continuous versus intermittent nasogastric feeds (MD −11 days; 95 % CI −21.8–−0.2). Small sample sizes, methodologic limitations, inconsistencies in controlling possible confounders, and conflicting results of the trials made it difficult to make universal recommendations regarding the best tube feeding method for preterm VLBW neonates [66]. Grant et al. have reported higher rates of energy expenditure and diet-induced thermogenesis during intermittent versus continuous feeding in preterm neonates [67]. Energy expenditure was measured by open-circuit respiratory calorimetry, in 11 preterm neonates on 2 successive days for 5–7 h during and after intermittent or continuous feeding. Neonates were fed the same quantity of formula each day, either for 5 min or by continuous drip for 2–3 h, in a random sequence. There was no diet-induced thermogenesis in response to continuous feeding. However, a peak increase of 15 % over baseline was observed after intermittent feeding. Overall energy expenditure was significantly higher after intermittent feeding (2.18 ± 0.07 vs. 2.09 ± 0.05 kcal/kg/h; $p < 0.05$). Mean difference in energy expenditure between the two feeding modes was 4 % (range: up to 17 %) [67]. The clinical significance of increased energy efficiency of continuous feeding in ELBW neonates needs to be evaluated further.

7 Transpyloric Versus Gastric Tube Feeding

McGuire and Evans have conducted a systematic review of RCTs to assess whether transpyloric versus gastric tube feeding improves feed tolerance, growth and development without increasing adverse consequences in preterm neonates [68]. Meta analysis of 9 eligible trials did not indicate evidence of an effect on short term growth rates. Transpyloric feeding was associated with a greater incidence (RR: 1.45, 95 % CI: 1.05, 2.09) of gastrointestinal disturbance. There was some evidence that transpyloric feeding increased mortality (RR: 2.46, 95 % CI: 1.36, 4.46). The authors cautioned that the outcomes of the study that contributed most to this finding were likely to have been affected by selection bias. No significant differences were noted in the incidence of NEC and intestinal perforation. Considering the adverse effects, and no evidence of benefits, the authors concluded that transpyloric feeding cannot be recommended for preterm neonates [68].

8 Early Versus Delayed Initiation of Progressive Enteral Feeds

Delayed enteral feeding could affect the functional adaptation of the gastrointestinal tract resulting later in feed intolerance in preterm VLBW neonates. A systematic review by Morgan et al. has identified 5 RCTs ($N = 600$) dealing with this issue [69]. The trials defined delayed introduction as later than 5–7 days after birth and early introduction as within 4 days after birth. Two trials ($N = 488$) recruited only growth-restricted neonates with altered Doppler flows. Meta-analyses did not detect statistically significant effects on the risk of NEC (RR: 0.89, 95 % CI: 0.58–1.37) or all cause mortality (RR: 1.03, 95 % CI: 0.59–1.78). Neonates with delayed introduction of enteral feeds took significantly longer to establish FEF (MD: 3 days). Two trials reported no significant difference in feed intolerance. It was concluded that the data do not provide evidence that delayed introduction of progressive enteral feeds reduces the risk of NEC in VLBW neonates. Delaying the introduction of progressive enteral feeds results in several days delay in establishing FEF but the clinical importance of this effect is unclear [69].

9 Rapid versus Slow Advancement of Feeds

Morgan et al. have conducted a systematic review of RCTs evaluating the effect of slow compared with fast advancement of enteral feeds on NEC, mortality, and morbidity in VLBW neonates [70]. The review identified 4 RCTs with 496 participants. Few participants were ELBW or growth restricted. The trials defined slow advancement as daily increments of 15–20 ml/kg and faster advancement as 30–35 ml/kg. Meta-analyses did not detect statistically significant effects on the risk of NEC

(RR: 0.91, 95 % CI: 0.47–1.75) or all cause mortality (RR: 1.43, 95 % CI: 0.78–2.61). Neonates who had slow rates of feed volume advancement took significantly longer to regain birth weight (MD: 2–6 days) and to establish FEF (MD: 2–5 days). Data on feed intolerance was reported in 2 trials. There was no significant difference in the number of neonates who experienced feed intolerance resulting in interruption of enteral feeding: RR: 1.35 (95 % CI: 0.89–2.05); RD: 0.10 (95 % CI: −0.04–0.24). It was concluded that the data did not provide evidence that slow advancement of enteral feed volume reduces the risk of NEC in VLBW neonates. Advancing enteral feed volumes at slow rather than faster rates results in several days delay in regaining birth weight and establishing FEF but the long term clinical importance of these effects is unclear [70]. Further trials are needed to determine the ideal rate of advancement of enteral feeds in preterm, especially ELBW neonates.

10 Body Position and Gastric Emptying

Studies of the effect of body position upon gastric emptying/residuals have shown inconsistent results [71–75]. Cohen et al. reported that in a trial involving 31 healthy, stable, appropriate for gestational age preterm neonates tolerating FEF of 160 ml/kg/day, the right lateral decubitus position led to significantly less gastric residuals than the left lateral decubitus position and that prone position led to less residual than the left lateral decubitus [75]. The amount of gastric residuals 1 h after a meal appeared to be in the following decreasing order: left, supine, prone, right. After 3 h, there were no significant differences among the four positions in the amount of gastric residuals [75]. van Wijk et al. have assessed a body-positioning regimen that promotes gastric emptying and reduces gastroesophageal reflux (GER) by changing body position 1 h after feeding [76]. Ten healthy preterm neonates [Mean (range) postmenstrual age: 36 (33–38) weeks] were monitored with combined esophageal impedance-manometry. Neonates were positioned in the left (LLP) or right (RLP) lateral position and then gavage-fed. After 1 h, the position was changed to the opposite side. Subsequently, all were restudied with reversed order of positioning. The results indicated that a strategy of RLP for the first postprandial hour, and changing to LLP thereafter promotes gastric emptying and reduces liquid GER in the late postprandial period [76]. The applicability of these results to unstable/convalescing extremely preterm neonates with feed intolerance is questionable.

11 Enteral Feed Temperature

The effect of feed temperature on gastric emptying and feed tolerance is not clear [77–81]. Anderson et al. have reported no significant differences in the antral or duodenal motor responses in healthy preterm neonates after feeding with a 4,250 kJ/l formula at 6, 24, and 37 °C [77]. They concluded that thermoreceptors did not

appear to be functional, suggesting that the benefits of feeding warmed formula may have a limited physiological value in preterm neonates [77]. Gonzales et al. have reported that warming milk to body temperature may promote greater feed tolerance in preterm VLBW neonates [78]. Those fed warmer milk (body temperature) had significantly smaller (6 %) gastric residuals than those fed colder milk (Room temperature: 22 %, Control group: 18 %) [78]. Earlier Eckburg et al. studied the effect of formula temperature on the thermogenic response to gavage feeding in preterm neonates [79]. Feeding at room temperature was associated with drop in stomach temperature by 6.9 °C, in rectal temperature by 0.2 °C, and in mean skin temperature by 0.6 °C. Metabolic rate increased by 16 % in the first postprandial hour. After feeding formula at body temperature, mean skin temperature fell by 0.2 °C, but stomach and rectal temperatures did not change appreciably. The metabolic rate rose by 12 % in the first hour, which was not significantly less than the rise after room temperature feeding [79]. Despite the methodological and population differences in these studies it seems that warming the feeds to body temperature may be beneficial for preterm neonates.

12 Prokinetics

The manifestations of ileus of prematurity such as abdominal distension, and bile stained/large gastric residues constitute the common feeding difficulties in preterm neonates. Gastrointestinal hypomotility could permit bacterial overgrowth, which may initiate the cascade of events leading to NEC [82]. Ileus is a prominent feature of NEC. Manometry studies have reported that a single aspect of gastrointestinal motility, the frequency of contractions was similar in neonates who did and did not develop NEC [83]. Prokinetics were used fairly frequently in preterm neonates till recently to overcome their gastrointestinal hypomotility. However a national survey (2004) has reported that prokinetics such as cisapride and erythromycin were prescribed only infrequently by Australian neonatologists [84]. These results most probably reflect the increased awareness of adverse effects such as hypertrophic pyloric stenosis, and cardiac toxicity. A systematic review (2005) of RCTs has evaluated the efficacy and safety of erythromycin as a prokinetic agent in preterm neonates [85]. Meta-analysis could not be performed as there was significant heterogeneity between trials and specific data were either inadequate or not available. It was concluded that the conflicting results of the 7 trials probably related to the differences in dose, route, and mode (prophylaxis/rescue) of administration, and duration of treatment, and in gastrointestinal motor responses in the presence of different feeding conditions (e.g., fasting vs. fed, intermittent vs. continuous feeds). Influence of gestational and postnatal ages during erythromycin treatment was also considered important [85]. Lam and Ng [86] have recently evaluated the studies in this field to determine whether the use of prokinetics such as erythromycin is beneficial and justified in preterm neonates [86]. Overall, neither low-dose regimes nor prophylaxis seemed to be useful. High-dose regimes used as rescue therapy in neonates with established

gastrointestinal dysmotility consistently showed clinical benefits. Theoretical risks such as emergence of antibiotic resistance and abnormal intestinal flora were not fully evaluated. They concluded that judicious use of high-dose erythromycin as rescue therapy was probably justifiable in preterm neonates [86]. The need for large RCTs evaluating newer prokinetics with better safety profile, and the potential benefits of combining prokinetics with probiotics/prebiotics in preterm neonates has also been emphasised by Ng [87].

13 Additives and Medications

High osmolality of feeds, reflecting a high concentration of solute particles, has been implicated in the pathogenesis of NEC [88, 89]. High osmolality of enteral substrate may also slow gastric emptying. Evidence for direct intestinal mucosal injury due to hyperosmolar feeds is however limited. The currently recommended range of osmolarity of neonatal milk formulae is 246–320 mOsm/kg [90]. Osmolality of human breast milk is ~300 mOsm/kg, and that of fully fortified breast milk is just above 400 mOsm/kg. Addition of mineral and vitamin supplements to small volumes of milk can significantly increase osmolality. Radmacher et al. have measured the osmolality of common milk-medication combinations administered in neonatal units [91]. Only Elecare (30 kcal/oz) exceeded American Academy of Pediatrics recommendations for osmolality. Addition of multivitamins alone increased the osmolality above 400 mOsm/kg H_2O. Sometimes the addition of other medications raised it above 1,000 mOsm/kg H_2O [91].

Significant rise in breast milk osmolality following the addition of a fortifier may delay gastric emptying [92]. Studies of the effect of human milk fortifier (HMF) on gastric emptying have shown inconsistent results, possibly due to the small sample sizes, differences in study design and populations, type of fortifiers, and inter-observer variations [92–95]. Yigit et al. have recently studied the effect of a widely used fortifier on gastric emptying in VLBW neonates using a balanced crossover design [96]. Gastric emptying was determined in the same infant on the same day with each of the three test feedings (unfortified, half fortified and fully fortified breast milk) in random order. The antral cross-sectional area was measured before and immediately after feeding, and then at 10-min intervals until the pre-feeding value was reached. The average half-emptying time was 49 ± 23 min with breast milk, 54 ± 29 min with half-fortified breast milk, and 65 ± 36 min with fully fortified breast milk. The differences between groups were not significant. They concluded that fortification of breast milk does not play a clinically significant role in causing feed intolerance in preterm neonates when using the recommended concentration of the fortifier [96]. Results of a previous study by Moody et al. support these findings [95]. Thickened milk feeds are often used in preterm neonates with GER, and are associated with significantly increased microbial population, enterocolitis, and neonatal intestinal obstruction and gastric lactobezoar [97, 98]. Clarke et al. have reported temporal association of thickened feeds with NEC in two ELBW

neonates [99]. The Center for Food Safety and Applied Nutrition, USA has recently suggested a possible association between NEC and a commercial feed thickener in preterm neonates. Their review in 2011 of 22 cases with exposure revealed a distinct illness pattern [100]. Enteral theophylline has been shown to delay gastric emptying in preterm neonates [101]. Bonthala et al. have reported that current doses of mydriatics inhibit duodenal motor activity and delay gastric emptying, possibly explaining the feeding difficulties in preterm neonates after eye checks for retinopathy of prematurity [102]. Anecdotal evidence also suggests role of mydriatics and enteral theophylline in the pathogenesis of NEC [103, 104]. Soraisham et al. have reported that a single 10 mg/kg intravenous loading dose of caffeine does not cause a significant reduction in superior mesenteric artery (SMA) flow velocity and therefore does not place the preterm gut at increased risk of ischemic injury [105]. Considering their frequent use in neonatal nurseries, the impact of opioid analgesics like morphine on feed intolerance is probably underestimated [106]. Prostaglandin inhibitors like indomethacin may inhibit gastric emptying [107, 108]. Patent ductus arteriosus (PDA) and indomethacin can potentially compromise enteral function in preterm neonates. Bellander et al. report that tolerance to early human milk feeds is not compromised by indomethacin in preterm neonates with PDA [109]. Drugs such as indomethacin and dexamethasone are also associated with focal small bowel perforations (FSBP) [102–112]. Researchers have suggested that risk factors like gestational age, severity of illness, infection, and early indomethacin therapy may be synergistic with early postnatal dexamathasone therapy in causing FSBP [113, 114].

14 Manometry

Low-compliance, continuous-perfusion manometry has been used to monitor neonatal antroduodenal motility [115–118]. Manometric studies are useful in developing optimal feeding methods and predicting feed tolerance in preterm neonates [117, 118]. Koenig et al. have demonstrated that gastric and transpyloric feeds as small as 4 ml/kg are equally potent in eliciting an intestinal motor response in preterm and term neonates and that changes in the caloric density of formula affect the preterm intestinal motility significantly [116]. Diluted formula may therefore not be an optimal stimulant for the preterm gut. de Ville et al. have reported that when preterm neonates are fed by slow intragastric infusion over 120 min, their duodenal motor responses are more like those observed in adults and their gastric contents are emptied faster and more completely when compared with rapid bolus feeds [119]. Berseth et al. have studied motor activity during fasting and feeding in neonates with and without feed tolerance [120]. Among feed intolerant neonates, motor quiescence was less pronounced and clustered motor activity significantly more prominent compared with than in those who tolerated feeds. Neonates who were initially feed intolerant became feed tolerant coincident with the appearance of motor activity that was similar to that in neonates who were initially feed tolerant. The sensitivity and specificity of manometry to predict feed intolerance was

1.0 and 0.13 respectively [120]. Considering its invasive nature and need for a level of expertise manometry continues to remain a research tool despite its advantages [121–123].

15 Measurement of SMA Flow

Fang et al. have reported the utility of serial Doppler measurements of SMA flow velocity after the first enteral feed in predicting early feed tolerance in preterm neonates [124]. An increase in time averaged SMA mean velocity > 17 % at 60 min after the test feed of 0.5 ml had a sensitivity of 100 % and a specificity of 70 % for the prediction of early feed tolerance [124]. Pezzati et al. have reported that mean SMA flow velocity 30 min after the first feed can predict early feed tolerance in preterm neonates [125]. Bora et al. [126] have reported the utility of serial SMA flow measurements; especially the 60 min post feed measurement, in predicting feed intolerance in preterm neonates. Murdoch et al. [127] and Robel-Tillig et al. [128] have also reported the benefits of measuring SMA flow in monitoring the risk for feed intolerance and NEC.

16 PDA, Sepsis, and Phototherapy

Both, PDA requiring medical/surgical treatment and sepsis have been reported to independently influence the time to FEF [129–137]. Berseth et al. have determined the incidence and causes of delays in reaching FEF in 105 preterm (24–35 weeks' gestation) neonates [129]. Feed intolerance defined as failure to reach FEF of 140 ml/kg/day within 10 days of the initiation of feeds occurred in 28 and 49 % of neonates with gestation 30–35 weeks, and 24–29 weeks, respectively. Although several variables were associated with delay in reaching FEF, a diagnosis of PDA or PDA and late onset of sepsis was a significant risk factor. Effects of PDA, indomethacin and sepsis per se and their interplay on intestinal perfusion and mucosal integrity may explain these results [130]. In a clinical trial evaluating the effect of carboxymethylcellulose, a bulk laxative, the median (interquartile range) time to FEF was significantly longer [4 (3.3–6.0) vs. 7.5 (4.0–13.8) days, $p = 0.01$] in neonates with a PDA requiring medical/surgical treatment versus those without PDA [131]. The "start to FEF" interval has been reported to be the longest in preterm neonates ($\leq$ 28 weeks' gestation) with sepsis, followed by that in those with sepsis and PDA, and in those with PDA alone [132]. Personal belief about feeding in presence of PDA may affect decisions about ductal ligation in preterm neonates as noted in the results of a standardised questionnaire survey in the USA [133]. Manifestations of ileus are frequent in preterm neonates undergoing phototherapy, and could be the result of photorelaxation of the gastrointestinal smooth muscle [134, 135]. Further research in this area is needed considering the frequency of PDA, sepsis, and the use of phototherapy in preterm neonates.

17 Probiotics and Prebiotics

Probiotics are defined as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [136]. The most common types are Lactic acid bacteria and Bifidobacteria. Lactic acid bacteria produce lactic acid as the result of carbohydrate fermentation. Human milk oligosaccharides (HMO) which are highly abundant in and unique to human milk, were first discovered as prebiotic "bifidus factor" serving as metabolic substrate for desired intestinal microbiota, providing health benefits for the breast-fed neonate [137]. HMO act as soluble decoy receptors, prevent pathogen attachment to gut mucosa and lower the risk of infections. They can also modulate epithelial and immune cell responses, reduce excessive mucosal leukocyte infiltration and activation, and provide sialic acid as a potentially essential nutrient for brain development and cognition. A small percentage of HMO is believed to be absorbed intact in the small intestine and later excreted with the urine, opening speculations on possible systemic effects [138].

Prophylactic probiotic supplementation has been shown to significantly reduce the risk of death and NEC while facilitating feed tolerance in preterm VLBW neonates. Deshpande et al. [139] reported the first systematic review and meta analysis of RCTs evaluating the efficacy and safety of any probiotic supplementation (Started within first 10 days, duration: ≥ 7 days) in preventing $\geq$ Stage II NEC in preterm VLBW (gestation < 33 weeks; birth weight $< 1{,}500$ g) neonates. A total of 7/12 retrieved RCTs ($N = 1{,}393$) were eligible for inclusion in the analysis. Meta-analysis using a fixed effects model estimated a significantly lower risk of NEC [RR: 0·36(95 % CI: 0·20, 0·65)] and all cause mortality [RR: 0·47(95 % CI: 0·30, 0·73)] in the probiotic versus control group. The time to FEF was significantly shorter in the probiotic group [WMD $= -2{\cdot}74$ days (95 % CI: $-4{\cdot}98$, $-0{\cdot}51$]. The risk of blood culture positive sepsis did not differ significantly [RR: 0·94 (95 % CI: 0·74, 1·20)] [139]. These results were supported by their updated conclusive meta analysis, and systematic reviews by other investigators [140–143]. The beneficial effects of probiotics in the prevention of NEC are thought to be mediated via various pathways, including promoting colonisation by commensals and inhibiting pathogens**,** increasing gut mucous production, modulating gut mucosal permeability and immune responses, and protecting against free oxygen radical injury [144].

Indrio et al. have reported that specific probiotic strains can promote feed tolerance, improve bowel habits and facilitate gut motility in preterm neonates [145–148]. Thirty preterm neonates were enrolled in their double blind RCT [145]; 10 were exclusively breast-fed, and the remaining 20 were randomly assigned to either Lactobacillus reuteri ATCC 55730 (1×10^8 colony forming units/day) or placebo for 30 days [145]. Body weight gains/day was similar for all groups, and no adverse events were recorded. Neonates receiving probiotic had a significant decrease in regurgitation and mean daily crying time and a higher frequency of stools compared with those receiving placebo. Gastric emptying rate was significantly increased, and fasting antral area was significantly reduced in neonates receiving L. reuteri and breast-feeds compared with those receiving placebo [145]. Indrio et al. [146] have

also reported that prebiotic oligosaccharides can modulate the electrical activity and the gastric emptying and may improve the intestinal tolerance of enteral feeding in preterm neonates. In this double blind RCT the percentage of time in which propagation was detected in the electrogastrography (EGG) signal was twice in neonates receiving formula with prebiotics compared with formula with placebo, and the gastric half-emptying time was 30 % faster in the prebiotic group than the placebo group [146]. In another trial [147], cutaneous EGG and gastric emptying was studied in 49 preterm neonates. Seventeen were exclusively breast-fed; 32 were randomly assigned to prebiotic-added formula, a probiotic-added formula (L. reuteri: 1×10^8 colony forming units/day), or a formula with placebo for 30 days. After the intervention period, the prebiotic, probiotic, and breast milk groups showed a higher percentage of EGG slow wave propagation and faster gastric half emptying compared with the placebo group [147]. Indrio et al. [148] have also shown that in infants with functional gastroesophageal reflux, L. reuteri DSM 17938 reduces gastric distension, accelerates gastric emptying, and also reduces the frequency of regurgitation.

Oligosaccharides currently added to infant formula are structurally different from HMO and therefore likely not functionally equivalent [137, 138]. A systematic review has reported that prebiotic supplemented formula increased stool colony counts of bifidobacteria and lactobacilli in preterm neonates without adversely affecting weight gain [149].

Delayed evacuation of the thick viscous meconium is thought to play an important role in feed intolerence in preterm neonates. Westerbeek et al. [150] have studied the effect of neutral oligosaccharides [small-chain galacto-oligosaccharides/long-chain fructo-oligosaccharides (scGOS/lcFOS)] in combination with acidic oligosaccharides (pAOS) on stool viscosity, frequency and pH in preterm neonates. Neonates ($N = 113$, gestation < 32 weeks and/or birth weight $< 1{,}500$ g) were randomly allocated to either scGOS/lcFOS with pAOS or a placebo. Stool viscosity at day 30 was lower in the prebiotics (16.8N) (3.9–67.8) versus placebo group (26.3N) (1.3–148.0) ($p = 0.03$; 95 % CI: −0.80–0.03). There was a trend towards higher stool frequency in the prebiotics versus placebo group (3.1 ± 0.8 versus 2.8 ± 0.7; 95 % CI: −0.08–0.52; $p = 0.15$). Stool pH at day 30 was lower in the in the prebiotics versus placebo group (5.9 ± 0.6 versus 6.2 ± 0.3, 95 % CI: 0.08–0.53; $p = 0.009$) [150]. In another RCT, Westerbeek et al. [151] have reported that enteral supplementation with scGOS/lcFOS/AOS does not significantly reduce the risk of serious infectious morbidity in preterm (Gestation < 32 weeks) VLBW neonates. However, there was a trend toward a lower incidence of serious infectious morbidity, especially for infections with endogenous bacteria [151]. Mihatsch et al. [152] had earlier reported that formula supplementation with GosFos reduced stool viscosity and accelerated gastrointestinal transport in preterm neonates (gestation 27 (24–31) weeks) on FEF. No adverse effects were observed. Modi et al. have conducted a multicenter RCT comparing preterm formula containing 0.8 g/100 ml scGOS/lcFOS in a 9:1 ratio and an otherwise identical formula, using formula only to augment insufficient maternal milk supply [153]. Preterm (Gestation < 33 weeks), appropriately grown for gestational age neonates ($N = 160$) were randomized within 24 h of birth. There was no significant difference in the time to reach FEF of 150 ml/kg/day and the proportion of

time between birth and day 28/discharge that a total milk intake of ≥ 150 ml/kg/day was tolerated. There was also no significant difference in secondary outcomes including growth, fecal characteristics, gastrointestinal signs, NEC, and bloodstream infection. After covariate adjustment, a significant benefit in feed tolerance was noted from trial formula with increasing immaturity (2.9 % improved tolerance for a neonate born at 28-week gestation and 9.9 % at 26-week gestation; $p < 0.001$) but decreased or no benefit in neonates > 31-week gestation [153]. A pilot double blind RCT ($N = 28$) by Riskin et al. suggests the safety of low dose lactulose supplementation in preterm neonates [154]. Neonates on lactulose had more Lactobacilli-positive stool cultures that appeared earlier with larger number of colonies, less feed intolerance, fewer episodes of late-onset sepsis, lower NEC and be discharged home earlier. Their nutritional laboratory indices were better, especially calcium and total protein [154]. The role of probiotic and/or prebiotic supplementation in facilitating feed tolerance in preterm neonates needs to be evaluated further.

18 Discussion

Developing an optimal enteral feeding strategy for preterm neonates is difficult due to the lack of a clear understanding of the pathophysiology of NEC and feed intolerance due to ileus of prematurity. Most of the current feeding strategies for preterm neonates, especially those with gestation under 28 weeks, have either inadequate or no sound scientific basis [155]. Studies of feeding strategies have invariably selected soft-surrogate primary outcomes such as time to FEF, and volume of gastric residuals rather than NEC. High quality clinical research is needed to understand the significance of feed intolerance in the context of the developmental physiology of the gut and the immune system in preterm neonates. Only large, definitive pragmatic trials will help in developing an optimal method for enteral nutrition, and understanding whether the benefits of aggressive enteral nutrition can outweigh its risks such as NEC and altered programming in preterm neonates. Survival free of long-term NDI will be an ideal primary outcome for such trials. However, recruiting a large number of neonates within a realistic time will be difficult considering the relatively low incidence of important primary outcomes. Experts have suggested that Bayesian statistics may help in overcoming this issue [156]. Trials rigorously assessing the definition, benefits and risks of even simple strategies such as early trophic feeds will be difficult to conduct as the practice is now well routed. Systematic reviews have shown that apart from significantly reducing the risk of death and NEC, probiotics also facilitate enteral feeding in preterm neonates. It is not clear if improved feed tolerance relates to only selected probiotic strains with specific effects. Strain selection is an important issue if improvements in nutritional outcomes other than NEC are selected as primary outcomes. Whether benefits of probiotics can be further improved by combining them with prebiotic oligosaccharides should be assessed in future trials. The potential benefits of prebiotic oligosaccharides alone can be explored in larger RCTs with clinical outcomes. The potential of glycerine

Table 3.1 Strategies for optimising enteral feeding in preterm neonates

1.	Antenatal glucocorticoids
2.	Breast milk
3.	Hydrolysed protein formula
4.	Lactase treated and low lactose feeds
5.	Early trophic feeds
6.	Continuous versus intermittent bolus feeding
7.	Transpyloric versus gastric tube feeding
8.	Early versus delayed initiation of progressive enteral feeds
9.	Rapid versus slow advancement of feeds
10.	Body position and gastric emptying
11.	Enteral feed temperature
12.	Prokinetics
13.	Additives and medications
14.	Manometry
15.	Measurement of superior mesenteric artery flow
16.	Monitoring in presence of PDA, sepsis, and phototherapy
17.	Probiotics and prebiotics

suppositories or enemas in facilitating evacuation of meconium and improving feed tolerance could also be evaluated [157, 158].

As new frontiers continue to be explored the proven benefits of simple interventions like antenatal glucocorticoids and preferential use of breast milk must not be forgotten. The benefits and risks of fortified or supplemented donor breast milk in preterm neonates need to be assessed in the context of current clinical practices. Further research on aggressive parenteral nutrition is also essential for optimising nutrition in preterm neonates. The adverse effects of catch-up growth, especially in preterm neonates with intrauterine growth restriction also need to be monitored [159–163] (Table 3.1).

References

1. Clark RH, Thomas P, Peabody J (2003) Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics 111:986–990
2. Cooke RJ, Ainsworth SB, Fenton AC (2004) Postnatal growth retardation: a universal problem in preterm infants. Arch Dis Child Fetal Neonatal Ed 89:F428–F430
3. Embleton NE, Pang N, Cooke RJ (2001) Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 107:270–273
4. Cooke RJ, Ford A, Werkman S, Conner C, Watson D (1993) Postnatal growth in infants born between 700 and 1,500 g. J Pediatr Gastroenterol Nutr 16:130–135
5. Neu J, Walker WA Nenterocolitis (2011) N Engl J. Med 364:255–264
6. Berman L, Moss RL (2011) Necrotizing enterocolitis: an update. Semin Fetal Neonatal Med 16:145–150
7. Morgan JA, Young L, McGuire W (2011) Pathogenesis and prevention of necrotizing enterocolitis. Curr Opin Infect Dis 24:183–189
8. Lin PW, Stoll BJ (2006) Necrotising enterocolitis. Lancet 368:1271–1283

9. Blakely ML, Lally KP, McDonald S et al (2005) Postoperative outcomes of extremely low birth-weight infants with necrotizing enterocolitis or isolated intestinal perforation: a prospective cohort study by the NICHD Neonatal Research Network. Ann Surg 241:984–989; discussion 989–994
10. Rowe MI, Reblock KK, Kurkchubasche AG et al (1994) Necrotizing enterocolitis in the extremely low birth weight infant. J Pediatr Surg 29:987–990
11. Schulzke SM, Deshpande GC, Patole SK (2007) Neurodevelopmental outcome of very low birth weight infants with necrotizing enterocolitis—a systematic review of observational studies. Arch Pediatr Adolesc Med 16:583–590
12. Bisquera JA, Cooper TR, Berseth CL (2002) Impact of necrotizing enterocolitis on length of stay and hospital charges in very low birth weight infants. Pediatrics 109:423–428
13. Flidel-Rimon O, Branski D, Shinwell ES (2006) The fear of necrotizing enterocolitis versus achieving optimal growth in preterm infants—an opinion. Acta Paediatr 95:1341–1344
14. Celano P, Jumawan J, Horowitz C, Lau H, Koldovsky O (1977) Prenatal induction of sucrase activity in rat jejunum. Biochem J 162:469–472
15. Moog F (1962) Developmental adaptations of alkaline phophotases in the small intestine. Fed Proc 21:51–56
16. Neu J, Ozaki CK, Angelides KJ (1986) Glucocorticoid-mediated alteration of fluidity of brush border membrane in rat small intestine. Pediatr Res 20:79–82
17. Israel EJ, Schiffrin EJ, Carter EA, Freiberg E, Walker WA (1990) Prevention of necrotizing enterocolitis in the rat with prenatal cortisone. Gastroenterology 99:1333–1338
18. Shulman RJ, Schanler RJ, Lau C, Heitkemper M, Ou CN, Smith EO (1998) Early feeding, antenatal glucocorticoids, and human milk decrease intestinal permeability in preterm infants. Pediatr Res 44:519–523
19. Bousvaros A, Walker WA (1990) Development and function of the intestinal mucosal barrier. In: McDonald TT (ed) Ontogeny of the human system of the gut. CRC Press, Boca Raton, pp 2–22
20. Spencer T, McDonald TT (1990) The ontogeny of the immune system of the gut. CRC Press, Boca Raton, pp 23–50
21. Buchmiller TL, Shaw KS, Lam ML, Stokes R, Diamond JS, Fonkalsrud EW (1994) Effect of prenatal dexamethasone administration: fetal rabbit intestinal nutrient uptake and disaccharidase development. J Surg Res 57:274–279
22. Bauer CR, Morrison JC, Poole WK, Korones SB, Boehm JJ, Rigatto H, Zachman RD (1984) A decreased incidence of necrotizing enterocolitis after prenatal glucocorticoid therapy. Pediatrics 73:682–688
23. Crowley P, Chalmers I, Keirse MJ (1990) The effects of corticosteroid administration before preterm delivery: An overview of the evidence from controlled trials. Br J Obstet Gynaecol 97:11–25
24. Halac E, Halac J, Begue EF, Casanas JM et al (1990) Prenatal and postnatal corticosteroid therapy to prevent neonatal necrotizing enterocolitis: a controlled trial. J Pediatr 117:132–138
25. Roberts D, Dalziel S (2007) Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database of Syst Rev 4:CD004454. doi: 10.1002/14651858.CD004454.pub2
26. ACOG Committee on Obstetric P (2011) ACOG committee opinion no. 475: antenatal corticosteroid therapy for fetal maturation. Obstet Gynecol 117:422
27. Carlo WA, McDonald SA, Fanaroff AA et al (2011) Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Association of antenatal corticosteroids with mortality and neurodevelopmental outcomes among infants born at 22–25 weeks' gestation. JAMA 306:2348–2358
28. Lucas A, Cole TJ (1990) Breast milk and neonatal necrotising enterocolitis. Lancet 336: 1519–1523
29. Caplan MS, Amer M, Jilling T (2002) The role of human milk in necrotising enterocolitis. Adv Exp Med Biol 503:83–90

30. Hanson LA (1999) Human milk and host defence: immediate and long-term effects. Acta Paediatr 88:42–46
31. Schanler RJ (2001) The use of human milk for premature infants. Pediatr Clin North Am 48:207–219
32. Goldman AS (2000) Modulation of the gastrointestinal tract of infants by human milk. Interfaces and interactions. An evolutionary perspective J Nutr 130:426S–431S
33. Caplan M, Hsueh W, Kelly A, Donovan M (1990) Serum PAF acetylhydrolase increases during neonatal maturation. Prostaglandins 39:705–714
34. Rabinowitz SS, Dzakpasu P, Piecuch S, Leblanc P, Valencia G, Kornecki E (2001) Platelet-activating factor in infants at risk for necrotizing enterocolitis. J Pediatr 138:81–86
35. Caplan MS, Sun XM, Hseuh W, Hageman JR (1990) Role of platelet activating factor and tumor necrosis factor-alpha in neonatal necrotizing enterocolitis. J Pediatr 116:960–964
36. Amer MD, Hedlund E, Rochester J, Caplan MS (2004) Platelet activating factor concentration in the stool of human newborns: effects of enteral feeding and neonatal necrotizing enterocolitis. Biol Neonate 85:159–166
37. Furukawa M, Narahara H, Yasuda K, Johnston JM (1993) Presence of platelet-activating factor-acetylhydrolase in milk. J Lipid Res 34:1603–1609
38. Akisu M, Kultursay N, Ozkayin N, Coker I, Huseyinov A (1998) Platelet-activating factor levels in term and preterm human milk. Biol Neonate 74:289–293
39. Bunton GL, Durbin GM, McIntosh N et al (1977) Necrotizing enterocolitis. Controlled study of 3 years' experience in a neonatal intensive care unit. Arch Dis Child 52:772–777
40. Dvorak B, Halpern MD, Holubec H et al (2003) Maternal milk reduces severity of necrotizing enterocolitis and increases intestinal IL-10 in a neonatal rat model. Pediatr Res 53:426–433
41. McGuire W, Anthony MY (2003) Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. Arch Dis Child Fetal Neonatal Ed 88:F11–F14
42. Boyd CA, Quigley MA, Brocklehurst P (2007) Donor breast milk versus infant formula for preterm infants: systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed 92:F169–F175
43. Sullivan S, Schanler RJ, Kim JH, Patel AL, Trawöger R, Kiechl-Kohlendorfer U et al (2010) An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. J Pediatr 156:562–567.e1
44. Meinzen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B, Donovan EF (2009) Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. J Perinatol 29:57–62
45. Sisk PM, Lovelady CA, Dillard RG, Gruber KJ, O'Shea TM (2007) Early human milk feeding is associated with a lower risk of necrotizing enterocolitis in very low birth weight infants. J Perinatol 27:428–433
46. Sisk PM, Lovelady CA, Gruber KJ, Dillard RG, O'Shea TM (2008) Human milk consumption and full enteral feeding among infants who weigh $\leq$1,250 g. Pediatrics 121:e1528–e1533
47. Castellote C, Casillas R, Ramírez-Santana C et al (2011) Premature delivery influences the immunological composition of colostrum and transitional and mature human milk. J Nutr. 141(6):1181–1187
48. Emami CN, Chokshi N, Wang J et al (2012) Role of interleukin-10 in the pathogenesis of necrotizing enterocolitis. Am J Surg 203:428–435
49. Mihatsch WA, Ho·· gel J, Pohlandt F (2001) Hydrolyzed protein accelerates the gastrointestinal transport of formula in preterm infants. Acta Paediatr 90:196–198
50. Mihatsch WA, Franz AR, Hogel J, Pohlandt F (2002) Hydrolyzed protein accelerates feeding advancement in very low birth weight infants. Pediatrics 110:1199–1203
51. Riezzo G, Indrio F, Montagna O et al (2001) Gastric electrical activity and gastric emptying in preterm newborns fed standard and hydrolysed formulas. J Pediatr Gastroenterol Nutr 33:290–295

52. Maggio L, Zuppa AA, Sawatski G, Valsasina R, Schubert W, Tortorolo G (2005) Higher urinary excretion of essential amino acids in preterm infants fed protein hydrolysates. Acta Paediatr 94:75–84
53. Auricchio S, Rubino A, Muerset G (1965) Intestinal glycosidase activities in the human embryo, fetus, and newborn. Pediatrics 35:944–954
54. Antonowicz I, Chang SK, Grand RJ (1974) Development and distribution of lysosomal enzymes and disaccharidases in human fetal intestine. Gastroenterol 67:51–58
55. Raul F, Lacroix B, Aprahamian M (1986) Longitudinal distribution of brush border hydrolases and morphological maturation in the intestine of the preterm infant. Early Hum Dev 13:225–234
56. Levine GN, Deren JJ, Steiger E, Zinno R (1974) Role of oral intake in maintenance of gut mass and disaccharidase activity. Gastroenterol 67:975–982
57. Hughes CA, Dowling RH (1980) Speed of onset of adaptive mucosal hypoplasia and hypofunction in the intestine of parenterally fed rats. Clin Sci 59:317–327
58. Neu J, Koldovsky O (1996) Nutrient absorption in the preterm neonate. Clin Perinatol 23:229–243
59. Shulman RJ, Schanler RJ, Lau C et al (1998) Early feeding, feeding tolerance, and lactase activity in preterm infants. J Pediatr 133:645–649
60. Hamosh M (1996) Digestion in the newborn. In: Neu J (ed) Neonatal Gastroenterology. PA: WB Saunders, Philadelphia, pp 191–210
61. Kien CL (1996) Digestion, absorption, and fermentation of carbohydrates in the newborn. Clin Perinatol 23:211–228
62. Erasmus HD, Ludwig-Auser HM, Paterson PG, Sun D, Sankaran K (2002) Enhanced weight gain in preterm infants receiving lactase-treated feeds: a randomized, double-blind, controlled trial. J Pediatr 141:532–537
63. Griffin MP, Hansen JW (1999) Can the elimination of lactose from formula improve feeding tolerance in premature infants? J Pediatr 135:587–592
64. McClure RJ (2001) Trophic feeding of the preterm infant. Acta Paediatr Suppl 90:19–21
65. Bombell S, McGuire W (2009) Early trophic feeding for very low birth weight infants. Cochrane Database Syst Rev 3:CD000504
66. Premji SS, Chessell L (2011) Continuous nasogastric milk feeding versus intermittent bolus milk feeding for premature infants less than 1,500 g. Cochrane Database Syst Rev 11:CD001819
67. Grant J, Denne SC (1991) Effect of intermittent versus continuous enteral feeding on energy expenditure in premature infants. J Pediatr 118:928–932
68. McGuire W, McEwan P (2007) Transpyloric versus gastric tube feeding for preterm infants. Cochrane Database Syst Rev 3:CD003487
69. Morgan J, Young L, McGuire W (2011) Delayed introduction of progressive enteral feeds to prevent necrotising enterocolitis in very low birth weight infants. Cochrane Database Syst Rev 3:CD001970
70. Morgan J, Young L, McGuire W (2011) Slow advancement of enteral feed volumes to prevent necrotising enterocolitis in very low birth weight infants. Cochrane Database Syst Rev 3:CD001241
71. Victor YH (1975) Effect of body position on gastric emptying in the neonate. Arch Dis Child 50:500–504
72. Villanueva-Meyer J, Swischuk LE, Cesani F, Ali SA, Briscoe E (1996) Pediatric gastric emptying: Value of right lateral and upright positioning. J Nucl Med 37:1356–1358
73. Malhotra AK, Deorari AK, Paul VK, Bagga A, Singh M (1992) Gastric residuals in preterm babies. J Trop Pediatr 38:262–264
74. Blumenthal I, Pildes RS (1979) Effect of posture on the pattern of stomach emptying in the newborn. Pediatrics 63:532–536
75. Cohen S, Mandel D, Mimouni FB, Solovkin L, Dollberg S (2004) Gastric residual in growing preterm infants: effect of body position. Am J Perinatol 21:163–166

76. van Wijk MP, Benninga MA, Dent J et al (2007) Effect of body position changes on postprandial gastroesophageal reflux and gastric emptying in the healthy premature neonate. J Pediatr 151:585–590
77. Anderson CA, Berseth CL (1996) Neither motor responses nor gastric emptying vary in response to formula temperature in preterm infants. Biol Neonate 70:265–270
78. Gonzales I, Duryea EJ, Vasquez E, Geraghty N (1995) Effect of enteral feeding temperature on feeding tolerance in preterm infants. Neonatal Netw 14:39–43
79. Eckburg JJ, Bell EF, Rios GR, Wilmoth PK (1987) Effects of formula temperature on postprandial thermogenesis and body temperature of premature infants. J Pediatr 111:588–592
80. Costalos C, Ross I, Campbell AG, Sofi M (1979) Is it necessary to warm infants' feeds? Arch Dis Child 54:899–901
81. Blumenthal I, Lealman GT, Shoesmith DR (1980) Effect of feed temperature and phototherapy on gastric emptying in the neonate. Arch Dis Child 55:562–564
82. Vantrappen G, Janssens J, Hellemans J, Ghoos Y (1977) The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine. J Clin Invest 59:1158–1166
83. Morris FH Jr, Moore M, Gibson T, West MS (1990) Motility of the small intestine in preterm infants who later have necrotizing enterocolitis. J Pediatr 117:S20–S23
84. Patole SK, Muller R (2004) Enteral feeding of preterm neonates—a survey of Australian neonatologists. J Maternal Fetal Neonatal Med 16:309–314
85. Patole S, Rao S, Doherty D (2005) Erythromycin as a prokinetic agent in preterm neonates: a systematic review. Arch Dis Child Fetal Neonatal Ed 90:F301–F306
86. Lam HS, Ng PC (2011) Use of prokinetics in the preterm infant. Curr Opin Pediatr 23:156–160
87. Ng PC (2011) Erythromycin as a prokinetic agent in newborns—useful or doubtful? Neonatology 100:297–298
88. Laker MF, Menzies IS (1977) Increase in human intestinal permeability following ingestion of hypertonic solutions. J Physiol (Lond) 265:881–894
89. De Lemos RA, Rogers JH Jr, McLaughlin W (1974) Experimental production of necrotising enterocolitis in newborn goats. Pediatr Res 8:380A
90. Williams AF (1997) Role of feeding in necrotizing enterocolitis. Semin Neonatol 2:263–271
91. Radmacher PG, Adamkin MD, Lewis ST, Adamkin DH (2012) Milk as a vehicle for oral medications: hidden osmoles. J Perinatol 32:227–229
92. Agarwal R, Singal A, Aggarwal R, Deorari AK, Paul VK (2004) Effect of fortification with human milk fortifier (HMF) and other fortifying agents on the osmolality of preterm breast milk. Indian Pediatr 41:63–67
93. McClure RJ, Newell SJ (1996) Effect of fortifying breast milk on gastric emptying. Arch Dis Child Fetal Neonatal Ed 74:F60–F62
94. Ewer AK, Yu VY (1996) Gastric emptying in pre-term infants: the effect of breast milk fortifier. Acta Paediatr 85:1112–1115
95. Moody GJ, Schanler RJ, Lau C, Shulman RJ (2000) Feeding tolerance in premature infants fed fortified human milk. J Pediatr Gastroenterol Nutr 30:408–412
96. Yigit S, Akgoz A, Memisoglu A, Akata D, Ziegler EE (2008) Breast milk fortification: effect on gastric emptying. J Matern Fetal Neonatal Med 21:843–846
97. Mallett AK, Wise A, Rowland IR (1984) Hydrocolloid food additives and rat caecal microbial enzyme activities. Food Chem Toxicol 22:415–418
98. Mercier JC, Hartmann JF, Cohen R et al (1984) Intestinal occlusion and enterocolitis caused by Gelopectose. Arch Fr Pediatr 41:709–710
99. Clarke P, Robinson MJ (2004) Thickening milk feeds may cause necrotising enterocolitis. Arch Dis Child Fetal Neonatal Ed 89:F280
100. Beal J, Silverman B, Bellant J, Young TE, Klontz K (2012) Late onset necrotizing enterocolitis in infants following use of a xanthan gum-containing thickening agent. J Pediatr (Epub ahead of print)
101. Gounaris A, Kokori P, Varchalama L et al (2004) Theophylline and gastric emptying in very low birthweight neonates: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 89:F297–F299

102. Bonthala S, Sparks JW, Musgrove KH, Berseth CL (2000) Mydriatics slow gastric emptying in preterm infants. J Pediatr 137:327–330
103. Nair AK, Pai MG, da Costa DE, Khusaiby SM (2000) Necrotising enterocolitis following ophthalmological examination in preterm neonates. Indian Pediatr 37:417–421
104. Hufnal-Miller CA, Blackmon L, Baumgart S, Pereira GR (1993) Enteral theophylline and necrotizing enterocolitis in the low-birthweight infant. Clin Pediatr (Phila) 32:647–653
105. Soraisham AS, Elliott D, Amin H (2008) Effect of single loading dose of intravenous caffeine infusion on superior mesenteric artery blood flow velocities in preterm infants. J Paediatr Child Health 44:119–121
106. Murphy DB, Sutton JA, Prescott LF, Murphy MB (1997) Opioid-induced delay in gastric emptying: a peripheral mechanism in humans. Anesthesiology 87:765–770
107. Sanders KM (1984) Role of prostaglandins in regulating gastric motility. Am J Physiol 247:G117–G126
108. Corak A, Coskun T, Alican I, Kurtel H, Yegen BC (1997) The effect of nitric oxide synthase blockade and indomethacin on gastric emptying and gastric contractility. Pharmacology 54:298–304
109. Bellander M, Ley D, Polberger S, Hellstrom-Westas L (2003) Tolerance to early human milk feeding is not compromised by indomethacin in preterm infants with persistent ductus arteriosus. Acta Paediatr 92:1074–1078
110. Shorter NA, Liu JY, Mooney DP, Harmon BJ (1999) Indomethacin-associated bowel perforations: a study of possible risk factors. J Pediatr Surg 34:442–444
111. Fujii AM, Brown E, Mirochnick M, O'Brien S, Kaufman G (2002) Neonatal necrotizing enterocolitis with intestinal perforation in extremely premature infants receiving early indomethacin treatment for patent ductus arteriosus. J Perinatol 22:535–540
112. Gordon P, Rutledge J, Sawin R, Thomas S, Woodrum D (1999) Early postnatal dexamethasone increases the risk of focal small bowel perforation in extremely low birth weight infants. J Perinatol 19:573–577
113. Gordon PV, Marshall DD, Stiles AD, Price WA (2001) The clinical, morphologic, and molecular changes in the ileum associated with early postnatal dexamethasone administration: from the baby's bowel to the researcher's bench. Mol Genet Metab 72:91–103
114. Novack CM, Waffarn F, Sills JH, Pousti TJ, Warden MJ, Cunningham MD (1994) Focal intestinal perforation in the extremely-low-birth-weight infant. J Perinatol 14:450–453
115. Malagelada JR, Camilleri M, Stanghellini V (1986) Manometric diagnosis of gastrointestinal motility disorders. Thieme, New York
116. Koenig WJ, Amarnath RP, Hench V, Berseth CL (1995) Manometrics for preterm and term infants: a new tool for old questions. Pediatrics 95:203–206
117. Berseth CL (1999) Assessment in intestinal motility as a guide in the feeding management of the newborn. Clin Perinatol 26:1007–1015
118. Ittmann PI, Amarnath R, Berseth CL (1992) Maturation of antroduodenal motor activity in preterm and term infants. Dig Dis Sci 37:14–19
119. de Ville K, Knapp E, Al-Tawil Y, Berseth CL (1998) Slow infusion feedings enhance duodenal motor responses and gastric emptying in preterm infants. Am J Clin Nutr 68:103–108
120. Berseth CL, Ittmann PI (1992) Antral and duodenal motor responses to duodenal feeding in preterm and term infants. J Pediatr Gastroenterol Nutr 14:182–186
121. Berseth CL, Nordyke CK (1992) Manometry can predict feeding readiness in preterm infants. Gastroenterol 103:1523–1528
122. Berseth CL (1990) Neonatal small intestinal motility: motor responses to feeding in term and preterm infants. J Pediatr 117:777–782
123. Berseth CL (1989) Gestational evolution of small intestine motility in preterm and term infants. J Pediatr 115:646–651
124. Fang S, Kempley ST, Gamsu HR (2001) Prediction of early tolerance to enteral feeding in preterm infants by measurement of superior mesenteric artery blood flow velocity. Arch Dis Child Fetal Neonatal Ed 85:F42–F45

125. Pezzati M, Dani C, Tronchin M, Filippi L, Rossi S, Rubaltelli FF (2004) Prediction of early tolerance to enteral feeding by measurement of superior mesenteric artery blood flow velocity: appropriate- versus small-for-gestational-age preterm infants. Acta Paediatr 93:797–802
126. Bora R, Mukhopadhyay K, Saxena AK, Jain V, Narang A (2009) Prediction of feed intolerance and necrotizing enterocolitis in neonates with absent end diastolic flow in umbilical artery and the correlation of feed intolerance with postnatal superior mesenteric artery flow. J Matern Fetal Neonatal Med 22:1092–1096
127. Murdoch EM, Sinha AK, Shanmugalingam ST, Smith GC, Kempley ST (2006) Doppler flow velocimetry in the superior mesenteric artery on the first day of life in preterm infants and the risk of neonatal necrotizing enterocolitis. Pediatrics 118:1999–2003
128. Robel-Tillig E, Knüpfer M, Pulzer F, Vogtmann C (2004) Blood flow parameters of the superior mesenteric artery as an early predictor of intestinal dysmotility in preterm infants. Pediatr Radiol 34:958–962
129. Berseth CL (2003) Risk factors for delays in establishing full enteral feeding volume in preterm infants. Pediatr Res A2647
130. Patole S, McGlone L, Muller R (2003) Virtual elimination of necrotising enterocolitis for 5 years—reasons? Med Hypotheses 61:617–622
131. Patole SK, Muller R (2005) Does Carboxymethylcellulose have a role in reducing time to full enteral feeds in preterm neonates? Int J Clin Pract 59:544–548
132. Patole SK, Kumaran VS, Travadi JN, Brooks JM, Doherty DA (2007) Does patent ductus arteriosus affect feed tolerance in preterm neonates? Arch Dis Child Fetal Neonatal Ed 92:F53–F55
133. Jhaveri N, Soll RF, Clyman RI (2010) Feeding practices and patent ductus arteriosus ligation preferences-are they related? Am J Perinatol 27:667–674
134. Raghavan K, Thomas E, Patole SK, Muller R, Whitehall J (2001) Is phototherapy a risk factor for ileus in high-risk neonates? Pediatr Res A1855
135. Kadalraja R, Patole SK, Muller R, Whitehall JS (2004) Is mesenteric blood flow compromised during phototherapy in preterm neonates? Arch Dis Child Fetal Neonatal Ed 89:F564
136. Food and Agriculture Organization of the United Nations (FAO) (2001) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Amerian Córdoba Park Hotel, Córdoba, Argentina. http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf. Accessed 1-4 Oct 2001
137. Bode L (2012) Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology (Epub ahead of print)
138. Jantscher-Krenn E, Bode L (2012) Human milk oligosaccharides and their potential benefits for the breast-fed neonate. Minerva Pediatr 64:83–99
139. Deshpande G, Rao S, Patole S (2007) Probiotics for prevention of necrotising enterocolitis in preterm neonates. Lancet 369:1614–1620
140. Deshpande G, Rao S, Patole S, Bulsara M (2010) Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics 125:921–930
141. Guthmann F, Kluthe C, Bührer C (2010) Probiotics for prevention of necrotising enterocolitis: an updated meta-analysis. Klin Pediatr 222:284–290
142. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T (2011) Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 3:CD005496
143. Wang Q, Dong J, Zhu Y (2012) Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis. J Pediatr Surg 47:241–248
144. Pirie S, Patole S. (2012) Probiotics for the prevention of necrotising enterocolitis in preterm neonates. In: Ohls RK, Maheshwari A, (eds) Hematology, immunology and infectious disease. Neonatology questions and controversies. 2nd edn. Elsevier Saunders, New York, 237–252.
145. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R (2008) The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. J Pediatr 152:801–806

146. Indrio F, Riezzo G, Raimondi F, Francavilla R, Montagna O, Valenzano ML, Cavallo L, Boehm G (2009) Prebiotics improve gastric motility and gastric electrical activity in preterm newborns. J Pediatr Gastroenterol Nutr 49:258–261
147. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R (2009) Effects of probiotic and prebiotic on gastrointestinal motility in newborns. J Physiol Pharmacol 60:27–31
148. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Filannino A, Cavallo L, Francavilla R (2011) Lactobacillus reuteri accelerates gastric emptying and improves regurgitation in infants. Eur J Clin Invest 41:417–422
149. Srinivasjois R, Rao S, Patole S (2009) Prebiotic supplementation of formula in preterm neonates: a systematic review and meta-analysis of randomised controlled trials. Clin Nutr 28:237–242
150. Westerbeek EA, Hensgens RL, Mihatsch WA, Boehm G, Lafeber HN, van Elburg RM (2011) The effect of neutral and acidic oligosaccharides on stool viscosity, stool frequency and stool pH in preterm infants. Acta Paediatr 100:1426–1431
151. Westerbeek EA, van den Berg JP, Lafeber HN, Fetter WP, Boehm G, Twisk JW, van Elburg RM (2010) Neutral and acidic oligosaccharides in preterm infants: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 91:679–686
152. Mihatsch WA, Hoegel J, Pohlandt F (2006) Prebiotic oligosaccharides reduce stool viscosity and accelerate gastrointestinal transport in preterm infants. Acta Paediatr 95:843–848
153. Modi N, Uthaya S, Fell J, Kulinskaya E (2010) A randomized, double-blind, controlled trial of the effect of prebiotic oligosaccharides on enteral tolerance in preterm infants (ISRCTN77444690). Pediatr Res 68:440–445
154. Riskin A, Hochwald O, Bader D et al (2010) The effects of lactulose supplementation to enteral feedings in premature infants: a pilot study. J Pediatr 156:209–214
155. Patole S (2005) Strategies for prevention of feed intolerance in preterm neonates: a systematic review. J Matern Fetal Neonatal Med 18:67–76
156. Tyson JE, Kennedy KA, Lucke JF, Pedroza C (2007) Dilemmas initiating enteral feedings in high risk infants: how can they be resolved? Semin Perinatol 31:61–73
157. Khadr SN, Ibhanesebhor SE, Rennix C, Fisher HE, Manjunatha CM, Young D, Abara RC (2011) Randomized controlled trial: impact of glycerin suppositories on time to full feeds in preterm infants. Neonatol 100:169–176
158. Shim SY, Kim HS, Kim DH, Kim EK, Son DW, Kim BI, Choi JH (2007) Induction of early meconium evacuation promotes feeding tolerance in very low birth weight infants. Neonatol 92:67–72
159. Hales CN (1997) Metabolic consequences of intrauterine growth retardation. Acta Paediatr Suppl 423:184–187; discussion 188
160. Hack M, Schluchter M, Cartar L, Rahman M, Cuttler L, Borawski E (2003) Growth of very low birth weight infants to age 20 years. Pediatrics 112:e30–e38
161. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB (2000) Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 320:967–971 (Erratum in: BMJ 320:1244)
162. Hales CN, Ozanne SE (2003) The dangerous road of catch-up growth. J Physiol 15:5–10
163. Ozanne SE, Hales CN (2004) Lifespan: catch-up growth and obesity in male mice. Nature 427:411–412

Chapter 4
Prevention and Treatment of Necrotising Enterocolitis in Preterm Neonates

Sanjay Patole

Abstract Necrotising enterocolitis (NEC) is a potentially disastrous illness in preterm, especially extremely preterm (gestation under 28 weeks) neonates with significant mortality, and morbidity including long term neurodevelopmental impairment. With improved survival of neonates at the limits of viability, the size of the population at risk for NEC has increased in recent years. NEC is a common cause of death in preterm neonates who survive the first week of life. The estimated annual economic burden related to NEC is close a billion dollar in the USA. Prevention and treatment of NEC has thus become a priority. Recent research has improved the understanding of the role of innate immunity in the pathogenesis of NEC. As new frontiers like probiotics and lactoferrin continue to be explored, the impact of well-established simple strategies like antenatal glucocorticoid therapy, and early and preferential use of breast milk should not be forgotten for primary prevention of NEC. Clinical research is needed on feed intolerance, safety of minimal enteral feeds in terms of NEC, and benefits of standardised feeding regimens. Association of common clinical practices such as red cell transfusions, H2 receptor blockade, undue prolonged antibiotic treatment, and thickening of feeds with NEC also warrants attention. Evaluating potential strategies for secondary prophylaxis (e.g., pentoxifylline) is equally important considering the fact that almost the entire health burden of NEC is related to progression of the illness from Stage II to Stage III. A package of potentially better practices seems to be the most appropriate strategy for the prevention and treatment of NEC.

Key points

- The incidence (4–6 %) of necrotising enterocolitis (NEC) has not changed significantly in preterm very low birth weight (VLBW) neonates despite the advances in neonatal intensive care.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_4,

- Definite (≥ Stage II) NEC continues to have significant mortality (25 %) and morbidity (e.g., recurrent sepsis, dependence on parenteral nutrition, need for surgery, and survival with short bowel syndrome) in preterm VLBW neonates.
- Mortality is higher (40–45 %) and long-term neurodevelopmental impairment is a serious concern in extremely preterm neonates (gestation < 28 weeks) needing surgery for the illness.
- Antenatal glucocorticoid therapy, early preferential feeding with breast milk, standardised feeding protocol, and probiotic supplementation are beneficial for primary prevention of NEC. Exploring strategies for secondary prevention is important considering that the mortality and morbidity of NEC relates to progression of the illness from stage II to stage III.
- Improved understanding of the innate immune responses in the preterm neonate and the molecular and biochemical pathways involved in the illness will help in developing strategies for prevention, early detection, and treatment of NEC.

Necrotising enterocolitis (NEC) is a potentially disastrous illness in preterm neonates, characterised by inflammation of the gastrointestinal tract [1, 2]. NEC occurs in 6–8 % of preterm (gestation < 32 weeks) very low birth weight (VLBW) neonates with significant mortality (~25 %) and morbidity including need for surgery, and survival with short bowel syndrome (SBS) with protracted feed intolerance, complications of prolonged dependence on parenteral nutrition, recurrent infections, and prolonged hospital stay [1, 2]. The incidence (10–12 %), mortality (40–45 %), and morbidity of NEC including need for surgery, and long-term neurodevelopmental impairment (NDI) after surviving surgery for the illness is worse in extremely low birth weight (ELBW) neonates [3, 4]. NEC has become one of the common causes of death in preterm neonates surviving the first week of life. The economic burden of NEC is substantial considering the prolonged hospital stay due to many associated complications. The total cost of caring for NEC affected neonates in the USA is estimated to be between $ 500 million and $ 1 billion per year [1]. Bisquera et al. have reported that neonates with NEC were hospitalized 60 days longer than those without the illness if surgery was required and more than 20 days longer if surgery was not required [5]. The total mean cost of care over a 5-year period for a child with the SBS, the most serious complication of NEC, has been estimated to be nearly $ 1.5 million [5].

1 Pathogenesis of NEC

Despite decades of research, the pathogenesis of NEC remains poorly understood [6, 7]. However prematurity continues to be accepted as the single most important risk factor for the illness. Immaturity of the protective mucosal barrier of the gastrointestinal tract and the innate immune system of preterm neonates are considered as the key factors involved in development and progression of intestinal inflammation in NEC. The propagation of the inflammatory cascade in NEC is favoured by the fact that the cytokine balance for preterm neonates is skewed toward inflammation [8]. Nanthakumar et al. have recently proposed a hypothesis attributing the excessive inflammatory

response in NEC to immature expression of innate immune response genes [9]. It is hypothesized that the injury in NEC begins with a breach in the gut mucosal barrier leading to bacterial translocation across the epithelium, and exacerbation of the proinflammatory cascade, resulting in the clinical signs of NEC [10]. The breach in the gut mucosal barrier occurs because of the disruption in the tight junction proteins (e.g., zonula occludin proteins ZO-1, ZO-2, ZO-3) between the intestinal epithelial cells (IEC), or due to the destruction of these cells by an accelerated apoptosis through intrinsic and/or extrinsic pathways. The factors involved in the initial gut mucosal injury include hypoxia, ischemia-reperfusion- free radical generation, toxins from pathogens, and excess short chain fatty acid generation due to the inability to metabolise large amounts of substrate in the gut. It is currently believed that the complex interaction between these risk factors rather than one factor per se leads to the breakdown of the gut mucosal barrier in NEC in preterm neonates [10, 11].

Platelet activating factor (PAF) is an inflammatory mediator that plays an important role in the pathogenesis of NEC by inducing IEC apoptosis. PAF induces IEC apoptosis by causing loss of mitochondrial membrane potential and activation of caspase [12], inhibiting the phosphatidylinositol 3-kinase/protein kinase B Akt signaling pathway [13], and inducing intracellular acidosis [14]. The surge of proinflammatory agents such as PAF, IL-8, IL-6, TNF–α [15–17], and bacterial products triggers a cascade of inflammatory events including neutrophil activation, increase in vascular permeability, and release of free oxygen radicals that eventually lead to vasoconstriction followed by I-R injury. The consequent breakdown of the mucosal barrier leads to a self-perpetuating vicious cycle resulting in severe NEC, shock, sepsis and, sometimes, death [18]. IL-8 plays an important role in this process by stimulating the migration of neutrophils from intravascular to interstitial sites and direct activation of neutrophils and regulating the expression of neutrophil adhesion molecules [19–23]. Serum IL-8 levels have been reported to be significantly elevated in severe NEC [22], and IL-8 mRNA is upregulated throughout the serosa, muscularis, and intestinal epithelium in the resected specimens from neonates with NEC [14, 23]. Evidence from experimental studies indicates that IL-10 plays a protective role in the pathogenesis of NEC by attenuating the degree of intestinal inflammation [24]. IL-10 levels have been shown to be low in maternal breast milk of preterm neonates with NEC [25]. Considering the complex pathogenesis of this multifactorial illness it is not surprising that prevention and treatment of NEC is a difficult task.

2 Prevention of NEC

2.1 Probiotics

Probiotics are live microbial supplements which offer beneficial effects to the host when consumed in adequate amounts [26]. Probiotics have been used for prevention of NEC in preterm neonates considering the role of colonisation of the gut by aberrant, pathogenic flora in the illness [27]. The beneficial effects of probiotics in

the prevention of NEC are thought to be mediated via various pathways including promotion of colonisation by commensals and inhibition of pathogens, reduction of the intraluminal pH and production of antimicrobial peptides that inhibit growth of other bacteria [28, 29]. Probiotics also promote mucous production, modulate mucosal permeability and intestinal tight junction function, and increase secretory IgA production, which are an integral part of the gut's defence. Other beneficial functions of probiotics include decreasing adhesion of pathogens and their toxins by producing a biofilm or producing receptor analogues and competing with pathogens for binding sites, and displacing pathogens which are already attached to the intestinal surface. Immature intestinal cells show excessive inflammation when the toll like receptor (TLR) pathway is activated which has been proposed to be linked to the development of NEC [28, 29]. Probiotics act via TLRs such as TLR 2 and 4 to produce protective cytokines (e.g., IL-6) which mediate cell regeneration and inhibit cell apoptosis [30, 31]. Different probiotics appear to affect TLR expression in different ways [32]. Probitics manipulate immune function to improve anti-pathogen activity, but also mediate inflammatory responses to pathogens by increasing Th1 cytokine profiles resulting in increased anti-inflammatory cytokines and decreased inflammatory cytokines [33]. Probiotics can also upregulate TGF β1 signalling which has potent anti-inflammatory effects [34]. Free radical injury plays a role in the pathogenesis of NEC as reactive oxygen species are known to disrupt the tight junctions in the gut epithelium and affect the barrier function [35–38]. Studies have demonstrated that probiotics can protect against this [39]. Indrio et al. have reported that probiotics promote feeding tolerance, improve bowel habits and facilitate gastrointestinal motility in preterm neonates [40, 41]. Such effects of probiotics are expected to be beneficial in the prevention of NEC considering the role of gut immaturity and dysmotility in the pathogenesis of the illness.

Hoyas et al. [42] first reported benefits of probiotic supplementation in reducing the incidence of NEC in preterm neonates. All neonates ($N = 1{,}237$) admitted during the 12 months were given L. acidophilus and B. infantis (250 million each) every day until they were discharged; 1,282 neonates hospitalized during the previous year served as historical controls. The demographic and clinical characteristics were comparable between the groups. There was a significant reduction in the incidence (Probiotics vs. Control: 34 vs. 85 cases, $p < 0.0002$) and mortality (14/34 vs. 35/85, $p < 0.005$) related to NEC. There were no complications related to probiotic supplementation [42]. Based on these encouraging results many randomised controlled trials (RCT) were undertaken subsequently. Deshpande et al. [43] reported the first systematic review of RCTs in this field. Meta analysis of the data from 7 RCTs ($N = 1{,}393$) indicated that prophylactic probiotic supplementation reduces the risk of definite NEC and all cause mortality significantly in preterm VLBW neonates without adverse effects while facilitating enteral feeds. The risk of blood culture positive late onset sepsis (LOS) was not reduced significantly [43]. These results were later confirmed in their first conclusive updated meta analysis (11 RCTs with $N = 2{,}176$) with trial sequential analysis, and second updated meta analysis (17 RCTs with $N = 3{,}147$) [44, 45]. Their results are also supported by systematic reviews by other investigators [46–48].

Despite the overwhelming evidence, probiotic supplementation for preterm neonates is still not adopted by a wider scientific community considering the risk of antibiotic resistance, probiotic sepsis, altered immune responses in the long run, and lack of availability of high quality, safe and clinically effective products. Considering the sample sizes of the trials included in the meta analyses, many believe that there is still a need for large placebo controlled trials before adopting routine probiotic supplementation for preterm neonates [49–51]. Others, based on the current evidence, believe that probiotics, in general, do reduce the risk of NEC and death in preterm neonates, and issues such as the optimal strain, dose, and combinations, can be addressed without placebo controlled trials [52, 53]. It is important to note that adverse effects such as probiotic sepsis, which usually resolves with antibiotics, should be weighed against potential benefits such as reduced risk of death and a potentially disastrous illness like NEC. Experts argue that for bacteremias originating from endogenous flora (as in NEC), infection with lactobacilli is preferable over that from potential pathogens such as Klebsiella, Enterobacter, or yeast [54]. Results of follow up studies are reassuring in the context of long term NDI or altered immune responses [55–57]. Continued research, and cooperation between various stakeholders including the industry and regulatory authorities is necessary to overcome problems in adopting this unique intervention. Availability of a clinically effective inactivated/killed probiotic supplement may be helpful in avoiding the development and spread of antibiotic resistance, probiotic sepsis, and need for cold chain maintainence [58].

2.2 Prebiotic Oligosaccharides

Human Milk Oligosaccharides (HMO) are a family of structurally diverse unconjugated glycans that are highly abundant in and unique to human milk [59]. They were discovered as prebiotic "bifidus factor" that serves as metabolic substrate for promoting the beneficial gut flora in the breast-fed neonate. HMO serve as soluble decoy receptors, prevent pathogen attachment to mucosal surfaces and lower the risk for viral, bacterial and protozoan parasite infections. HMO may modulate epithelial and immune cell responses, reduce excessive mucosal leukocyte infiltration and activation, and lower the risk of NEC [59]. Results of in vitro studies indicate that acidic HMO reduce platelet-neutrophil complex formation leading to a decrease in neutrophil beta 2 integrin expression [60]. The neutral HMO fraction had no effect, supporting the hypothesis that acidic HMO may serve as anti-inflammatory components of human milk and contribute to the lower incidence of NEC in breast-fed infants [60]. HMO need to be fucosylated and sialylated to reduce selectin-mediated leukocyte rolling, adhesion, and activation, which may protect breast-fed infants from excessive immune responses. Evidence indicates that a single HMO that carries not 1 but 2 sialic acids protects neonatal rats from NEC [61]. Jantscher-Krenn et al. have reported that the HMO disialyllacto-N-tetraose (DSLNT) prevents NEC in neonatal rats. Rat pups were fed with formula without and with HMO and exposed to episodic hypoxia [62]. Ileum sections were scored blindly for signs of NEC. Compared to formula alone, pooled HMO significantly improved 96-h survival from 73.1 to 95.0 %

and reduced pathology scores from 1.98 ± 1.11–0.44 ± 0.30 ($p < 0.001$). Within the pooled HMO, a specific isomer of DSLNT was protective. Galacto-oligosaccharides, currently added to formula to mimic some of the effects of HMO, had no effect. It was concluded that, DSLNT could be used to prevent or treat NEC in formula-fed infants, and its concentration in the mother's milk could serve as a biomarker to identify breast-fed infants at risk of developing NEC [62]. Butel et al. have reported that oligofructose contributes to the protective role of bifidobacteria in experimental NEC [63]. Based on their results and the fact that oligosaccharides are a major component of breast milk, they concluded that the addition of oligofructose in formula milks may favour colonisation by a beneficial flora [63]. Srinivasjois et al. have reported a systematic review of RCTs assessing the efficacy and safety of prebiotic oligosaccharide supplemented formula in reducing the incidence of NEC and sepsis; in preterm neonates with gestation ≤ 37 weeks [64]. Only one trial reported that NEC did not occur in any of the enrolled neonates. Others did not report on NEC or sepsis. Two trials reported a statistically significant increase in bifidobacterial counts in the prebiotic supplemented group [64]. Based on the results of recent small/pilot RCTs, the role of prebiotic supplementation (either alone or in combination with probiotics) in promoting feed tolerance, reducing the risk of NEC and late onset infections in preterm neonates needs to be evaluated further in large definitive trials [65–69].

2.3 Lactoferrin

Lactoferrin, a mammalian milk glycoprotein involved in innate immune defences, has been shown to reduce the risk of LOS in preterm neonates [70, 71]. The benefits of lactoferrin were noted when it was administered either alone or in combination with the probiotic strain LGG. In a multicenter RCT, 472 preterm VLBW neonates were randomly assigned to oral bovine lactoferrin (BLF) 100 mg/day ($n = 153$), BLF plus LGG (6×109 cfu/day ($n = 151$), or placebo ($n = 168$) from birth until day 30 (day 45 for neonates $< 1{,}000$ g at birth) [70]. Incidence of LOS was significantly lower in BLF and BLF plus LGG versus placebo group. NEC $\geq$ Stage II occurred in 3/153 (1.9 %) of BLF group, 0/151 (0 %) of BLF plus LGG group, and 10/168 (6.0 %) of control group (BLF vs. Control: $p = 0.09$, BLF plus LGG vs. Control: $p = 0.002$). No adverse effects or intolerances to treatment occurred [70]. Considering the role of sepsis in the pathogenesis of NEC, and the fact that 30 % of the cases of NEC are associated with blood culture positive sepsis, the potential of Lactoferrin in prevention of the illness needs to be evaluated in further large definitive trials [72].

2.4 Standardised Feeding Protocols

Variations in clinical practice have been proposed to be the iatrogenic component of NEC [73–75]. In a study of biodemographic and clinical correlates of NEC, inter-centre differences in clinical practice were proposed to be the significant factors

linked to the prevalence of NEC in VLBW neonates [73]. The two centres with the highest prevalence of NEC had the shortest times required to regain birth weight. Similarly the centre with the lowest prevalence of NEC had the longest median time required to regain the birth weight. No other illnesses, including patent ductus arteriosus (PDA) and respiratory distress, were significantly different between centres, strongly suggesting that clinical practices determine the risk of NEC. The centres that practiced fluid restriction in the management of haemodynamically significant PDA had higher prevalence of suspected NEC whereas centres practising no fluid restriction for significant PDA had higher prevalence of definite NEC [73]. Variations in enteral feeding strategies for preterm neonates have also been reported by other authors [74, 75]. Considering the consistent reports of a significant and prolonged decline in the incidence of NEC following implementation of a SFR it is very much possible that variations in clinical practice contribute to the incidence of NEC [76–83]. A systematic review and meta analysis of observational studies indicates the significant potential of SFRs in reducing the risk of NEC [76]. This systematic review identified 6 eligible studies (1978–2003) reporting incidence of $\geq$ Stage II NEC in preterm, LBW neonates before and after implementation of a SFR. Meta analysis using a random effects model estimated a pooled risk ratio (RR) of 0.13 (95 % CI: 0.03–0.50). Although all studies showed a lowered risk of NEC after adopting a SFR, there was a significant heterogeneity ($p < 0.001$) between the studies indicating variations in the population characteristics and standards of neonatal care including feeding practices over a period of 25 years. Repeat analysis after excluding one of the studies that showed the strongest protective effect of SFR (RR: 0.03, 95 % CI: 0.004–0.227) indicated a more significant effect because the lack of heterogeneity resulted in a much lower variance. (RR: 0.71, 95 % CI: 0.52–0.97, $p = 0.03$, heterogeneity $p = 0.8$). Analysis using a fixed effects model showed almost identical results. Overall the results indicated that any SFR results in a 29 % (95 % CI: 3–48 %) decrease in the risk of NEC [76]. The benefits of SFR may relate to the process per se of developing and implementing a SFR that is associated with an increased awareness and early detection and treatment of NEC. Results of recent studies also support the importance of SFR as a simple tool for prevention of NEC by minimising variations in practice [84–86].

2.5 Antenatal Glucocorticoid Therapy

Antenatal exposure to glucocorticoids is known to significantly reduce the risk of NEC in preterm neonates. Prevention of prematurity, the single most important risk factor for NEC, has proved to be a difficult task. Maternal glucocorticoid treatment is thus the only tool available in the antenatal period to minimise the risk of NEC in the preterm neonate. The beneficial effects of antenatal glucocorticoids on gastrointestinal maturation and function include reduced uptake of macromolecules from the mucosa, reduced hepatic bacterial translocation, and increased activity of enzymes like lactase, maltase, and sucrase [87–94]. Nanthakumar et al. have used human intestinal xenografts to test whether a finite period of steroid responsiveness

exists as shown in animal models [95]. Developmental responsiveness was measured by lactase activity and inflammatory responsiveness by IL-8, IL-6, and monocyte chemotactic protein-1 (MCP-1) induction after an endogenous (IL-1) or exogenous (LPS) proinflammatory stimulus, respectively. Cortisone acetate accelerated the ontogeny of lactase at 20 week (immature) but the effect was lost by 30 week (mature) after transplant. Concomitant with accelerated maturation, the IL-8 response to IL-1 and LPS was significantly dampened by glucocorticoid pretreatment in the immature but not mature xenografts. The excessive activation of IL-8 in the immature gut was mediated by a prolonged activation of ERK and p38 kinases and nuclear translocation of NF-B due to low levels of IB. These results indicate the pathways by which antenatal glucocorticoids administered within the responsive period provide an effective preventive therapy for NEC by accelerating intestinal maturation [95]. A significant reduction in the incidence of NEC following antenatal glucocorticoid treatment was first reported by Bauer et al. [96]. The effect was more striking on NEC than on respiratory distress syndrome (RDS)—the desired primary outcome. Later a systematic review of RCTs by Crowley et al. and the RCT by Halac et al. confirmed this benefit of antenatal glucocorticoid treatment [97, 98]. Results of the updated Cochrane systematic review of 21 RCTs (3,885 women and 4,269 infants) show that antenatal corticosteroids reduce the risk of neonatal death, and morbidity including RDS, intraventricular haemorrhage (IVH), and NEC [99]. Current guidelines recommend antenatal corticosteroids for mothers in preterm labour from 24 to 34 weeks' gestation, but not before 24 weeks due to lack of data (ACOG) [100]. However, the results of the multicentre cohort study ($N = 10{,}541$) by Carlo et al. indicate that the neonatal benefits of antenatal glucocorticoid treatment for death and morbidity including NEC, likely occur even at 22 and 23 weeks [101].

2.6 Breast Milk

Human milk has been reported to reduce the incidence of NEC by up to seven fold compared with formula milk [102]. The protective effect of breast milk has been correlated with its anti-inflammatory components (e.g., cytokines, growth factors), lysozyme, IgG, prebiotic oligosaccharides and probiotics [103–106]. The activity of acetyl hydrolase (PAF-AH) that degrades PAF is lower in neonates under 3 weeks of age than at any other time [107]. Considering the role of PAF in the pathogenesis of NEC the presence of PAF-AH activity may partly explain the protective effect of breast milk [108–111]. High use of breast milk is reported to lower the incidence of NEC, and result in a mild illness with more survivors [112]. Evidence indicates that freezing or thawing does not eliminate the benefits of breast milk in reducing the incidence and severity of NEC [113]. Systematic reviews report that donor human milk feeding is associated with a lower risk of NEC [114].

Sullivan et al. have evaluated the benefits of an exclusively human milk-based diet in extremely preterm neonates [115]. Neonates ($N = 207$) fed mothers' milk were randomized to one of the three study groups. Groups HM100 and HM40 received pasteurized donor human milk-based human milk fortifier when the enteral intake

was 100 and 40 ml/kg/day, respectively. Both groups received pasteurized donor human milk if mother's milk was not available. Group BOV received bovine milk-based human milk fortifier after reaching enteral intake of 100 ml/kg/day and preterm formula if mother's milk was not available. All groups had comparable baseline demographics, duration of parenteral nutrition, rates of LOS, and growth. The groups receiving an exclusively human milk had significantly lower rates of NEC ($p = 0.02$) and NEC requiring surgery ($p = 0.007$) [115]. Ganapathy et al. have evaluated the cost-effectiveness of a 100 % human milk-based diet in extremely preterm neonates, based on the risks of overall NEC and surgical NEC in the RCT by Sullivan et al. [116]. The adjusted incremental costs of medical NEC and surgical NEC over and above the average costs incurred for extremely preterm neonates without NEC, in 2011 US$, were $ 74,004 (95 % CI: $ 47,051–100,957) and $ 198,040 (95 % CI: $ 159,261–236,819) per neonate, respectively. Extremely preterm neonates fed with 100 % human-milk based products had lower expected length of stay and total expected costs of hospitalization, resulting in net direct savings of 3.9 stay days and $ 8,167.17 (95 % CI: $ 4,405–11,930) per extremely preterm neonate ($p < 0.0001$). Costs savings from the donor HMF strategy were sensitive to price and quantity of donor HMF, percentage reduction in risk of overall NEC and surgical NEC achieved, and incremental costs of surgical NEC [116].

Meinzen-Derr et al. have reported that the likelihood of NEC or death after 14 days was decreased by a factor of 0.83 (95 % CI: 0.72, 0.96) for each 10 % increase in the proportion of total intake as human milk (HM) in ELBW neonates [117]. Each 100 ml/kg increase in HM intake during the first 14 days was associated with decreased risk of NEC or death (Hazard Ratio: 0.87; 95 % CI: 0.77, 0.97). There was a non-significant trend towards a decreased risk of NEC or death among neonates who received 100 % HM as a proportion to total enteral intake (HM plus formula) [117]. Sisk et al. have reported that an intake of at least 50 % mother's milk was associated with fewer days to reach 100 and 150 ml/kg/day of enteral feeds in neonates who weighed ≤ 1,250 g [118]. Sisk et al. have also reported that enteral feeding containing at least 50 % human milk in the first 14 days of life was associated with a 6 fold decrease in the odds of NEC [118]. It is important to note that despite its advantages preferential use of breast milk alone has not eliminated NEC. Deficiency of immunologically beneficial components like IL-10, in the very preterm breast milk (< 30 weeks' gestation) may explain this finding [24, 119].

2.7 Arginine Supplementation

Decreased concentration of nitric oxide (NO) is thought to play a role in the pathogenesis of NEC [2]. The rationale for arginine supplementation in prevention of NEC is based on the fact that it can act as a substrate for the production of NO in the tissues. Plasma arginine concentrations have been reported to be low in neonates who developed NEC [120, 121]. Amin et al. have evaluated the effect of L-arginine supplementation in reducing the incidence of all stages of NEC in preterm neonates [122]. In their double-blind, randomised, placebo-controlled trial, 152 preterm neonates

with birth weight $\leq$ 1,250 g and gestational age $\leq$ 32 weeks were randomly assigned to receive either supplemental L-arginine [1.5 mmol/kg/day; $n = 75$ (Intervention group A)] or placebo [$n = 77$ (Control group B)] with oral feeds/parenteral nutrition during the first 28 days of life. NEC developed in 5 neonates in group A compared with 21 in group B ($p < .001$). Arginine intake and plasma arginine concentrations were similar in both groups at study entry and increased in group A at days 14 and 28. Plasma arginine concentrations were lower in both groups at time of diagnosis of NEC. Maternal and neonatal demographics, nutrient intake, plasma ammonia and total and essential amino acid concentrations were not significantly different between the two groups [122]. Follow up of 132 (95 %) neonates at 36 months adjusted age showed no significant increase in neurodevelopmental disability in the intervention group [123]. In neonates given L-arginine, 5/61 (8.1 %) had major neurodevelopmental disabilities, defined as the presence of one or more of cerebral palsy, cognitive delay (cognitive index < 70), bilateral blindness or bilateral hearing loss requiring hearing aids compared with 9/71 (12.6 %) in the placebo group (RR: 0.64; 95 % CI: 0.22–1.82; $p = 0.40$) [123]. The safety and efficacy of arginine in prevention of NEC in preterm neonates needs to be evaluated in a definitive large trial before this intervention is adopted.

2.8 Restricted Fluid Intake

A systematic review has reported that restricted water intake significantly increases postnatal weight loss while significantly reducing the risk of death and morbidities such as NEC (RR: 0.43, 95 % CI: 0.21–0.87; RD: −0.05, 95 % CI: −0.09 to −0.01), Numbers needed to treat (NNT): [20; 95 % CI: 11–100] [124]. These findings are important considering that excess fluid intake has been implicated in the pathogenesis of NEC [124]. Results of a small case-control study by Morag et al. also indicate that excessive weight gain could be an early sign of NEC [125]. Preterm neonates ($n = 17$) with perforated NEC were matched with 17 neonates matched for birth weight and gestation. The postnatal age at diagnosis of NEC was identified, and weight changes as well as clinical and laboratory data were compared for 7 days before to 7 days after the diagnosis. A significant difference in weight gain was noticed between D1 and D0. The NEC and control groups gained 5.1 % and 1.2 %, respectively ($p = 0.002$). None of the sick infants lost weight on days −1 to D0 [125]. Careful restriction of water intake while avoiding dehydration and nutrient deprivation may decrease the risk of NEC without adverse consequences.

2.9 Acidification of Gastric Contents

Preterm neonates are often hypochlorhydric, and enteric, Gram-negative bacteria often colonize their stomachs, especially after gavage feeds [126, 127]. Acidifying the feeds to a pH low enough to inhibit gastric bacterial proliferation has been shown

to significantly lower the risk of NEC in preterm neonates [128]. In this double-blind RCT, supplementation with 0.01–0.02 ml of 1 N HCl/ml of milk was compared to that with a similar supplement of water. The median gastric pH of the acid supplemented group was lower (3.0) than the control group (4.0) throughout the study ($p < 0.001$). The gastric enteric bacterial colonisation rate and the quantitative bacterial counts were strongly correlated with gastric pH over 4 (p50.001). Somatic growth rates in the acid supplemented group were equal to, or higher than those in the control group. The incidence of NEC was significantly lower (1/34 vs. 8/34, $p = 0.02$) in the acid supplemented versus control group neonates [128]. The results of this trial and the association of H2-blocker therapy with higher incidence of NEC in VLBW neonates suggest that gastric acid is protective for NEC [129, 130].

3 Treatment of NEC

3.1 Bowel Rest and Broad Spectrum Antibiotics

Stopping milk feeds after the diagnosis of definite NEC allows the bowel to rest and recover from the inflammation. The duration of bowel rest is however arbitrary, generally around 1 week and 2–3 weeks for Stage II and advanced (stage III) NEC, respectively. Broad spectrum antibiotics such as penicillin and gentamycin are prescribed during this period considering the role of sepsis in the pathogenesis of NEC. Penicillin is often replaced by vancomycin given that coagulase negative staphylococci (e.g., staphylococcus epidermidis) are the commonest organisms responsible for LOS in preterm neonates. These organisms are also known to produce an enterotoxin that can cause an NEC like illness. Anaerobic cover with metronidazole is not warranted as a routine unless perforation is suspected (Figs. 4.1 and 4.2). Routine anaerobic cover with clindamycin does not provide any significant advantage in terms of prevention of intestinal gangrene/perforation and mortality [131]. Moreover its use in definite NEC has been associated with late onset strictures [131]. The choice of antibiotics should be based on the local microbial resistance patterns. It is important to know that pneumatosis intestinalis (Figs. 4.3 and 4.4), the characteristic finding in definite NEC disappears once milk feeds (the substrate for fermentation and formation of gas) are stopped and antibiotics are started (pathogens that ferment the substrate, are eliminated). Early recommencement of milk feeds and avoiding undue prolonged exposure to antibiotics is important after recovery from the illness considering the atrophy of the gut in absence of milk, and the elimination of beneficial gut flora, and increased colonisation by pathogens (including fungi) following the use of broad spectrum antibiotics. Brotschi et al. have compared the effect of fasting period duration on complication rates in neonates managed conservatively for Stage II NEC [132]. In a multicentre study, they retrospectively analyzed data collected by standardized questionnaire on all admissions for NEC over a period of 6 years. Complication rates were compared after dividing conservatively managed neonates with stage II NEC into two groups (those fasted for < 5 days and

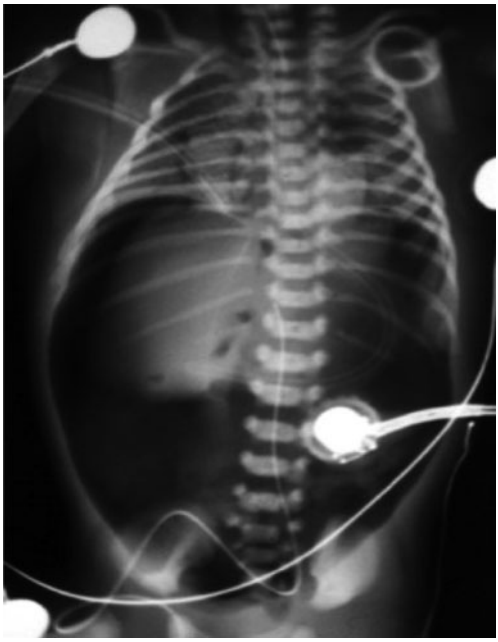

Fig. 4.1 Perforation with pneumoperitonium in necrotising enterocolitis. (Courtesy: Dr Vijay Shingde, Department of Neonatology, Nepean Hospital, Sydney, Australia)

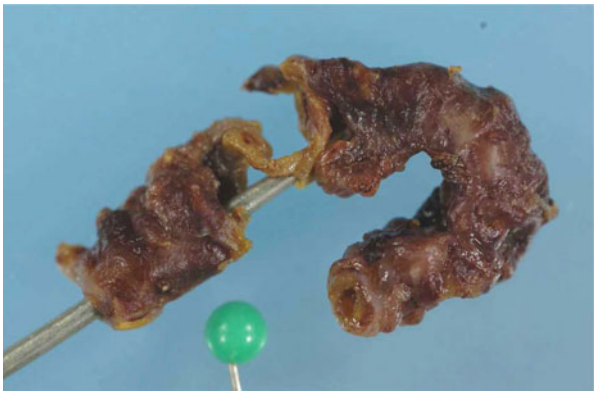

Fig. 4.2 Perforated sigmoid colon in a preterm neonate with necrotising enterocolitis. (Dimensions shown by comparing with a 3 mm size bobble on top of pin, Courtesy: Dr Adrian Charles, Department of Perinatal Pathology, KEM Hospital for Women, Perth, Western Australia)

those fasted for > 5 days). Of the 47 conservatively managed neonates, 30 (64 %) fasted for < 5 days (range 1–4 days) and 17 (36 %) for > 5 days (range 6–16 days). There were no significant differences for any of the patient characteristics analyzed. One (3 %) and four (24 %) neonates, respectively, developed post-NEC bowel stricture. One (3 %) and two neonates (12 %) suffered NEC relapse, and none and five (29 %) developed catheter-related sepsis. Overall, the results indicated that shorter fasting after NEC appeared to lower morbidity including catheter-related sepsis, after the acute phase of the illness [132]. There was no benefit in longer fasting neonates. Bohnhorst et al. have reported their experience with an early initiation of

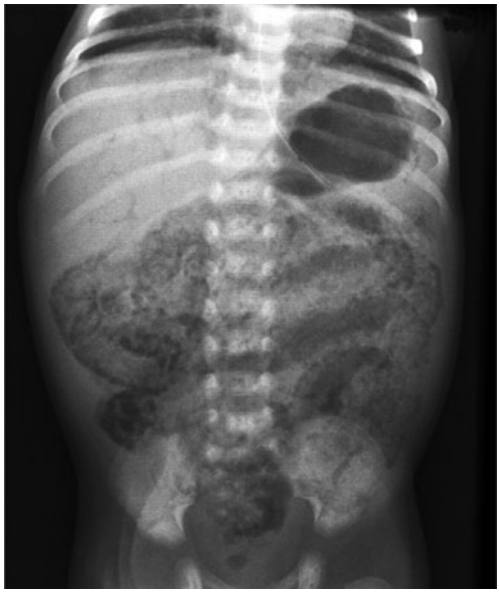

Fig. 4.3 Extensive pneumatosis intestinalis with portal venous gas in necrotising enterocolitis. (Courtesy: Dr Vijay Shingde, Department of Neonatology, Nepean Hospital, Sydney, Australia)

enteral feedings after NEC [133]. Over a 4-year period, all inborn neonates with Stage $\geq$ II NEC received enteral feed, increased by 20 ml/kg/day, once no portal vein gas had been detected on ultrasound for 3 consecutive days (group 1). These cases were compared with historic controls (group 2). NEC rates were 5 % (26/523) in the early feeding group and 4 % (18/436) in the comparison group. One early

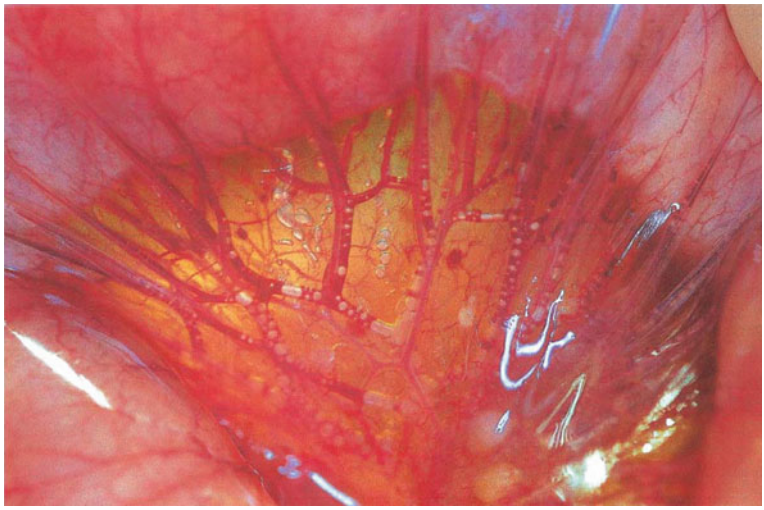

Fig. 4.4 Intra-operative view of extensive pneumatosis intestinalis. (J Pediatr 2003; 143:543 (Reproduced with permission))

feeding neonate and two comparison group neonates died of NEC, whereas 2 and 1, respectively, had recurrent NEC. Enteral feeds were restarted at a median (range) age of 4 (3–14) versus 10 (8–22) days after onset of NEC. Early feeding was associated with shorter median (range) time to reach full enteral feeds [10 (7–31) vs. 19 (9–76) days, $p < .001$), a reduced duration of central venous access [13.5 (8–24) vs. 26.0 (8–39) days, $p < .01$], less catheter-related septicemia (18 % vs. 29 %, $p < .01$), and a shorter duration of hospital stay [63 (28–133) vs. 69 (36–150) days], $p.05$). The limitations of the study design and the small numbers make it difficult to exclude a higher risk of recurrence of NEC following such a change in practice [133].

3.2 Abdominal Decompression

Abdominal distension is one of the characteristic signs of NEC. The significance of compartment syndrome is well known in pediatric and adult patients. However, it's potential to further compromise the perfusion of the affected bowel segment/s, and compromise the ventilation-perfusion by splinting the diaphragm, and impeding venous return, is probably underestimated in preterm neonates. Young age has been shown to be associated with a lower threshold for increased intraluminal pressure (ILP) leading to NEC in experimental studies [134]. Garstin et al. have showed development of histological changes consistent with NEC when the intestinal mucosa was exposed to both increased ILP and decreased pH [135]. Alterations in respiratory status are often the early signs of severe NEC. Dolgin et al. have reported unexplained changes in the respiratory status requiring increased respiratory support during the 24 h before clear evidence of severe NEC [136]. The early warning signs of NEC included decreased oxygenation, tachypnoea, and hypercarbia preceded by hypocarbia in some cases [136]. These changes could be due to either splinting of the diaphragm by the distended abdomen, or the inability of the immature respiratory system to meet the increased metabolic demand due to NEC. Dzakovic et al. have reported the beneficial effects of bedside PPD in preterm neonates ($n = 11$) with severe abdominal distension and increasing ventilatory requirements without free intraperitoneal air [137]. The mean airway pressure and oxygenation indices showed a significant improvement after PPD. The final outcome however did not indicate significant benefits [137].

3.3 Monitoring

Surveillance for sepsis and meningitis is important considering the association of sepsis with NEC in 30 % of the cases [72]. Fungal sepsis is a significant issue in surgical NEC. Pourcyrous et al. have reported that serum CRP correlates with Stage II and III NEC and a persistently elevated CRP may be a marker of complications such as a stricture or an abscess [138]. Hallstrom et al. have reported that persistent metabolic acidosis, decreasing platelet count, and increasing blood glucose level on

several successive days might predict a developing NEC [139]. Leukocyte counts $> 30 \times 10^9$/l, pH < 7.25, and a rise in blood glucose by ≥ 1.5 mmol/l within 24 h predicted NEC with intestinal perforation [139]. Severe thrombocytopenia within the first 3 days after a diagnosis of NEC suggests a higher likelihood of bowel gangrene, morbidity, and mortality [140]. It is important to note that platelet count cannot be used in isolation to predict extent of the illness or survival [141]. Monitoring circulating cytokines (e.g., IL-6) may help in judging the severity of NEC [142]. Srinivasjois et al. have reported that serial changes in CRP and plasma lactate level may predict progression of definite NEC to surgery or death in preterm neonates [143]. In their retrospective analysis of data over 6 years, serial changes in CRP, platelet count, plasma glucose and lactate, within 24 h before and over 72 h after the diagnosis of NEC, were correlated to progression to surgery or death in neonates with either medically (Group I) or surgically (Group II) managed NEC. CRP levels were significantly higher at 72 h in neonates in the surgical versus medical NEC group. Plasma glucose and lactate levels were significantly higher when compared with the baseline levels at all time points for both groups. Receiver operator curve analysis ($N = 30$) indicated that significant rise in CRP [baseline to 72 h (area under the curve, AUC: 0.933, $p = 0.001$)] and in lactate levels [baseline to 48 h (AUC: 0.818, $p = 0.047$)] had a strong potential as a predictor for progression to surgery or death [143]. Results of an earlier (2008) large multicentre prospective, observational study by Moss et al. indicate that clinical parameters do not adequately predict outcome in NEC [144]. Comprehensive data on mothers and their neonates with suspected or definite NEC was analysed. Of 455 neonates analyzed, 192 (42 %) progressed to severe disease, and 263 (58 %) advanced to full feeds without operation. The vast majority of the variables studied were not associated with progression to severe disease. A total of 12 independent predictors for progression were identified, including only 3 that were not described previously: having a teenaged mother (OR: 3.14; 95 % CI: 1.45–6.96), receiving cardiac compressions and/or drugs for resuscitation at birth (OR: 2.51; 95 % CI: 1.17–5.48), and having never received enteral feeds before the diagnosis (OR: 2.41; 95 % CI: 1.08–5.52). The investigators concluded that further analysis of clinical parameters alone will not be helpful, and future studies must focus on advanced biologic parameters in conjunction with clinical findings [144].

Thrombocytopenia is frequent in NEC. Kenton et al. have reported that platelet transfusions in preterm neonates with NEC may not lower mortality but may increase morbidity given that platelet transfusions contain a variety of bioactive factors including pro-inflammatory cytokines [145]. Neonates who developed SBS and/or cholestasis had been given a significantly higher number and volume of platelet transfusions compared with those who did not have such adverse outcomes [145]. Their observations do question the practice of routine platelet transfusions to maintain the platelet count above an arbitrary level. Red cell transfusions are often required for the correction of anemia in NEC. A recent systematic review and meta analysis of observational studies indicates an association of red cell transfusions for anemia with NEC in preterm neonates [146]. Transfusion associated gut injury (TRAGI) is probably similar to transfusion associated lung injury (TRALI) in adults. The benefits (improved oxygenation) versus potential risks (e.g., transfusion associated NEC) of such transfusions in preterm neonate are not clear [146]. Prevention and prompt

correction of metabolic derangements (MD) such as hyponatremia, hypotension, and metabolic acidosis is important. Tepas et al. have reported MD as the best predictor of mortality (OR: 4.76; 95 % CI: 1.41–16.13, $p = 012$) in NEC, which significantly increased with extending interval between diagnosis to surgery. Neonates with MD receiving peritoneal drain had a 4-fold increase in mortality (OR: 4.43; 95 % CI: 1.37–14.29; $p = .0126$) [147].

Abdominal ultrasound (AUS) may provide advantages over radiography for visualising portal venous gas (PVG), intra-abdominal echoic free fluid (EFF), bowel wall thickness and perfusion and be a useful adjuvant for timely surgical intervention [148]. AUS has been reported to be superior to plain radiography for early detection of perforation by demonstrating PVG and EFF [149]. Complex ascites with debris correlated well with gangrene or perforation and predicted mortality [150]. AUS can easily detect changes in bowel wall thickness and echogenicity, and abdominal aorta calibre, peak systolic and end-diastolic velocities, and resistive indices in superior mesenteric artery could facilitate early detection of gut injury in septic neonates with NEC [151].

3.4 Peritoneal Drainage versus Laparotomy

Peritoneal drainage (PD) is an alternative to laparotomy and resection of the necrotic/perforated segments as the standard surgical management of NEC or spontaneous intestinal perforation (SIP). A systematic review of randomised (RCT) or quasi-randomised controlled trials has evaluated the benefits and risks of these procedures as the initial surgical treatment for perforated NEC or SIP in preterm (< 37 weeks), low birth weight (< 2,500 g) neonates [152]. Only two RCTs met the eligibility criteria. Overall, no significant differences were seen between the PD and laparotomy groups regarding the incidence of mortality within 28 days of the primary procedure (28/90 versus 30/95; RR: 0.99, 95 % CI: 0.64–1.52); mortality by 90 days after the primary procedure (RR: 1.05, 95 % CI: 0.71–1.55) and the number of infants needing total parenteral nutrition for more than 90 days (RR: 1.18, 95 % CI: 0.72–1.95). Nearly 50 % of the neonates in the PD group could avoid the need for laparotomy during the study period (44/90 versus 95/96; RR: 0.49, 95 % CI: 0.39–0.61). One study found that the time to attain full enteral feeds in ELBW neonates was prolonged in the PD group (mean difference: 20.77, 95 % CI: 3.62–37.92). The reviewers concluded that the evidence from the two RCTs suggested no significant benefits or harms of PD over laparotomy. However, due to the very small sample size, clinically significant differences may have been easily missed. No firm recommendations could be made. Definitive large multicentre trials are needed to address this important issue [152].

3.5 Monitoring Long-Term Outcomes

Monitoring long-term neurodevelopmental outcomes is important. Results of a systematic review of observational studies indicate that survivors of Stage ≥ II NEC are at risk for long-term NDI, especially if they require surgery for the illness [4]. Eleven nonrandomized studies, including 5 with "matched controls," were included in this review. The risk of long-term NDI was significantly higher in the presence of at least stage II NEC vs. no NEC (OR: 1.82; 95 % CI: 1.46–2.27). Patients with NEC requiring surgery were at higher risk for NDI vs. those managed medically (OR: 1.99; 95 % CI: 1.26–3.14). Results of analyses based on study design, follow-up rate, and year of birth were not statistically significantly different from those of the overall analysis. Risk of cerebral palsy and cognitive and severe visual impairment was significantly higher in neonates with NEC [4]. The ORACLE Children Study has provided school age outcomes following neonatal NEC [153]. The outcomes of 119/157 (77 %) of children following proven or suspected NEC were compared with those of the remaining 6,496 in this study. NEC was associated with an increased risk of neonatal death (OR 14.6; 95 % CI: 10.4–20.6). At 7 years, NEC conferred an increased risk of all grades of impairment. Adjusting for confounders, risks persisted for any Health Utilities Index (HUI-3) defined functional impairment (adjusted OR: 1.55; 95 % CI: 1.05, 2.29), particularly mild impairment (adjusted OR: 1.61; 95 % CI: 1.03, 2.53) both in all NEC children and in those with proven NEC, which appeared to be independent. No behavioural or educational associations were confirmed. Following NEC, children were more likely to suffer bowel problems than non-NEC children (adjusted OR: 3.96; 95 % CI: 2.06, 7.61) [149]. Results of the systematic review by Schulzke et al. and of the follow up of Oracle Children study emphasise the importance of counselling the parents about the need for long-term follow up of neonates with Stage ≥ II NEC [4, 153].

3.6 Early Diagnosis and Secondary Prevention

The clinical utility of most of the markers/methods for early diagnosis of NEC is limited due to either cost, expertise, and accessibility issues or their poor properties as a diagnostic/screening test [154–157]. Recent evidence indicates that urinary I-FABP (fatty acid binding protein) and claudin-3 and fecal calprotectin are promising diagnostic markers for NEC, and urinary I-FABP may also be used to predict disease severity [158–160]. The potential of neutrophil CD64 expression, and C5a, a compliment activation product, as biomarkers for NEC needs to be studied further [161, 162]. Advances in laparoscopic techniques may help in early diagnosis of NEC [163]. The results from experimental studies indicate the potential role of immunomodulators such as pentoxifylline in secondary prevention of NEC [164]. The potential of endothelin receptor blockers (e.g., Bosentan) also needs to be evaluated considering the role of vasoconstriction in the pathogenesis and progression of NEC [165].

Table 4.1 Strategies for prevention and treatment of necrotising enterocolitis

Prevention	Treatment
Antenatal glucocorticoid therapy	Bowel rest and broad spectrum antibiotics (avoid unduly prolonged bowel rest)
Early preferential feeding with breast milk	Abdominal decompression and pain relief
Standardised feeding protocol	Surveillance for sepsis, meningitis, and complications (e.g., perforation, abscess, and stricture)
Probiotic supplementation	Prevention and prompt correction of metabolic derangements such as hyponatremia, hypotension, and metabolic acidosis
Restricted fluid intake without compromising nutrition and hydration	Ongoing consultations for timely surgical intervention
Aggressive surveillance and treatment for sepsis	Ongoing consultations for timely surgical intervention
Avoiding undue prolonged exposure to antibiotics, antacids, H_2 receptor blockers, and thickened feeds	
Lactoferrin, arginine supplementation (needs more research)	

In summary NEC continues to be a potentially disastrous illness in preterm neonates with significant mortality and morbidity. Recent research has improved the understanding of the role of innate immunity in the pathogenesis of NEC [166]. As new frontiers like probiotics and lactoferrin continue to be explored, the impact of well-established simple strategies like antenatal glucocorticoid therapy, and early preferential use of breast milk should not be forgotten for primary prevention of NEC. Evaluating strategies for secondary prophylaxis is equally important as almost the entire health burden of NEC is related to progression of the illness from Stage II to Stage III 9 perforation-peritonitis). A package of potentially better practices seems to be the most appropriate strategy for the prevention and treatment of NEC (Table 4.1).

References

1. Neu J, Walker WA (2011) Necrotizing enterocolitis. N Engl J Med 364:255–264
2. Berman L, Moss RL (2011) Necrotizing enterocolitis: an update. Semin Fetal Neonatal Med 16:145–150
3. Rowe MI, Reblock KK, Kurkchubasche AG, Healey PJ (1994) Necrotizing enterocolitis in the extremely low birth weight infant. J Pediatr Surg 29:987–990
4. Schulzke SM, Deshpande GC, Patole SK (2007) Neurodevelopmental outcomes of very low-birth-weight infants with necrotizing enterocolitis: a systematic review of observational studies. Arch Pediatr Adolesc Med 161:583–590
5. Bisquera JA, Cooper TR, Berseth CL (2002) Impact of necrotizing enterocolitis on length of stay and hospital charges in very low birth weight infants. Pediatrics 109:423–428
6. Schnabl KL, Van Aerde JE, Thomson AB, Clandinin MT (2008) Necrotizing enterocolitis: a multifactorial disease with no cure. World J Gastroenterol 14:2142–2161

7. Obladen M (2009) Necrotizing enterocolitis—150 years of fruitless search for the cause. Neonatol 96:203–210
8. Van Marter LJ, Dammann O, Allred EN, Leviton A, Pagano M, Moore M, Martin C (2002) Chorioamnionitis, mechanical ventilation, and postnatal sepsis as modulators of chronic lung disease in preterm infants. J Pediatr 140:171–176
9. Nanthakumar N, Meng D, Goldstein AM, Zhu W, Lu L, Uauy R, Llanos A, Claud EC, Walker WA (2011) The mechanism of excessive intestinal inflammation in necrotizing enterocolitis: an immature innate immune response. PLoS One 6(3):e17776
10. Claud EC, Lu L, Anton PM, Savidge T, Walker WA, Cherayil BJ (2004) Developmentally regulated IkappaB expression in intestinal epithelium and susceptibility to flagellin-induced inflammation. Proc Natl Acad Sci USA 101:7404–7408
11. Morgan JA, Young L, McGuire W (2011) Pathogenesis and prevention of necrotizing enterocolitis. Curr Opin Infect Dis 24:183–189
12. Lu J, Caplan MS, Saraf AP, Li D, Adler L, Liu X, Jilling T (2004) Platelet-activating factor-induced apoptosis is blocked by Bcl-2 in rat intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 286:G340–G350
13. Lu J, Caplan MS, Li D, Jilling T (2008) Polyunsaturated fatty acids block platelet-activating factor-induced phosphatidylinositol 3 kinase/Akt-mediated apoptosis in intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 294:G1181–G1190
14. Claud EC, Lu J, Wang XQ, Abe M, Petrof EO, Sun J, Nelson DJ, Marks J, Jilling T (2008) Platelet-activating factor-induced chloride channel activation is associated with intracellular acidosis and apoptosis of intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 294:G1191–G1200
15. Caplan MS, Sun XM, Hseuh W, Hageman JR (1990) Role of platelet activating factor and tumor necrosis factor-alpha in neonatal necrotizing enterocolitis. J Pediatr 116:960–964
16. Morecroft JA, Spitz L, Hamilton PA, Holmes SJ (1994) Plasma cytokine levels in necrotizing enterocolitis. Acta Paediatr Suppl 396:18–20
17. Morecroft JA, Spitz L, Hamilton PA, Holmes SJ (1994) Plasma interleukin-6 and tumour necrosis factor levels as predictors of disease severity and outcome in necrotizing enterocolitis. J Pediatr Surg 29:798–800
18. Fitzgibbons SC, Ching Y, Yu D, Carpenter J, Kenny M, Weldon C, Lillehei C, Valim C, Horbar JD, Jaksic T (2009) Mortality of necrotizing enterocolitis expressed by birth weight categories. J Pediatr Surg 44:1072–1075 (discussion 1075–1076)
19. Baggiolini M, Walz A, Kunkel SL (1989) Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 84:1045–1049
20. Djeu JY, Matsushima K, Oppenheim JJ, Shiotsuki K, Blanchard DK (1990) Functional activation of human neutrophils by recombinant monocyte-derived neutrophil chemotactic factor/IL-8. J Immunol 144:2205–2210
21. Huber AR, Kunkel SL, Todd RF 3rd, Weiss SJ (1991) Regulation of transendothelial neutrophil migration by endogenous interleukin-8. Science 254:99–102
22. Edelson MB, Bagwell CE, Rozycki HJ (1999) Circulating pro- and counterinflammatory cytokine levels and severity in necrotizing enterocolitis. Pediatrics 103:766–771
23. Nadler EP, Stanford A, Zhang XR, Schall LC, Alber SM, Watkins SC, Ford HR (2001) Intestinal cytokine gene expression in infants with acute necrotizing enterocolitis: interleukin-11 mRNA expression inversely correlates with extent of disease. J Pediatr Surg 36:1122–1129
24. Emami CN, Chokshi N, Wang J, Hunter C, Guner Y, Goth K, Wang L, Grishin A, Ford HR (2012) Role of interleukin-10 in the pathogenesis of necrotizing enterocolitis. Am J Surg 203:428–435
25. Fituch CC, Palkowetz KH, Goldman AS, Schanler RJ (2004) Concentrations of IL-10 in preterm human milk and in milk from mothers of infants with necrotizing enterocolitis. Acta Paediatr 93:1496–1500
26. Fuller R (1991) Probiotics in human medicine. Gut 32:439–442
27. Claud EC, Walker WA (2008) Bacterial colonization, probiotics, and necrotizing enterocolitis. J Clin Gastroenterol 42:S46–S52

28. Walker WA (2008) Mechanisms of action of probiotics. Clin Infect Dis 46:S87–S91 (discussion S144–S151)
29. Martin CR, Walker WA (2008) Probiotics: role in pathophysiology and prevention in necrotizing enterocolitis. Semin Perinatol 32:127–137
30. Boirivant M, Strober W (2007) The mechanism of action of probiotics. Curr Opin Gastroen 23:679–692
31. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F et al (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118:229–241
32. Ewaschuk JB, Blacker JL, Churchill TA et al (2007) Surface expression of Toll-like receptor 9 is upregulated on intestinal epithelial cells in response to pathogenic bacterial DNA. Infect Immun 75:2572–2579
33. Veckman V, Miettinen M, Pirhonen J et al (2004) Streptococcus pyogenes and Lactobacillus rhamnosus differentially induce maturation and production of TH1-type cytokines and chemokines in human monocyte-derived dendritic cells. J Leukoc Biol 75:764–771
34. Lavasani S, Dzhambazov B, Nouri M et al (2010) A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. PLoS One 5:e9009
35. O'Donovan DJ, Fernandes CJ (2004) Free radicals and diseases in premature infants. Antioxid Redox Signal 6:169–176
36. Baker RD, Baker SS, LaRosa K (1995) Polarized Caco-2 cells. Effect of reactive oxygen metabolites on enterocyte barrier function. Dig Dis Sci 40:510–518
37. Clark DA, Fornabaio DM, McNeill H, Mullane KM, Caravella SJ, Miller MJ (1988) Contribution of oxygen-derived free radicals to experimental necrotizing enterocolitis. Am J Pathol 130:537–542
38. Sheth PS, Basuroy S, Li C, Naren AP, Rao RK (2003) Role of phosphatidylinositol 3-kinase in oxidative stress-induced disruption of tight junctions. J Biol Chem 278:49239–49245
39. Rao RK, Polk DB, Seth A, Yan F (2009) Probiotics the good neighbour: guarding the gut mucosal barrier. Am J Infect Dis 5:188–192
40. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R (2009) Effect of probiotic and prebiotic on gastrointestinal motility in newborns. J Physiol Pharmacol 60:S27–S31
41. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R (2008) The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. J Pediatr 152:801–806
42. Hoyos AB (1999) Reduced incidence of necrotising enterocolitis associated with enteral administration of Lactobacillus acidophilus and Bifidobacterium infantis to neonates in an intensive care unit. Int J Infect Dis 3:197–202
43. Deshpande G, Rao S, Patole S (2007) Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. Lancet 369:1614–1620
44. Deshpande G, Rao S, Patole S, Bulsara M (2010) Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics 125:921–930
45. Patole S (2011) Safety and efficacy of probiotics in the neonatal period. Nestle symposium presentation at the 52nd annual meeting of the European society for pediatric research (ESPR), New Castle, UK
46. Guthmann F, Kluthe C, Bührer C (2010) Probiotics for prevention of necrotising enterocolitis: an updated meta-analysis. Klin Pediatr 222:284–290
47. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T (2011) Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev (3):CD005496
48. Wang Q, Dong J, Zhu Y (2012) Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis. J Pediatr Surg 47:241–248
49. Soll RF (2010) Probiotics: are we ready for routine use? Pediatrics 125:1071–1072

50. Garland SM, Jacobs SE, Tobin JM (2010) On behalf of the ProPrems study group. A cautionary note on instituting probiotics into routine clinical care for premature infants. Pediatrics 126:e741–2
51. Miller M, Wilks M, Fleming P, Costeloe K (2012) Should the use of probiotics in the preterm be routine? Arch Dis Child Fetal Neonatal Ed 97:F70–F74
52. Tarnow-Mordi WO, Wilkinson D, Trivedi A, Brok J (2010) Probiotics reduce all-cause mortality and necrotizing enterocolitis: it is time to change practice. Pediatrics 125:1068–1070
53. Barrington KJ (2011) Review: probiotics prevented necrotising enterocolitis and reduced mortality in preterm neonates. Arch Dis Child Education Ed 96:199. doi: 10.1136/adc.2011.214569
54. Kliegman RM, Willoughby RE (2005) Prevention of necrotizing enterocolitis with probiotics. Pediatrics 115:171–172
55. Chou IC, Kuo HT, Chang JS, Wu SF, Chiu HY, Su BH, Lin HC (2010) Lack of effects of oral probiotics on growth and neurodevelopmental outcomes in preterm very low birth weight infants. J Pediatr 156:393–396
56. Sari FN, Eras Z, Dizdar EA, Erdeve O, Oguz SS, Uras N, Dilmen U (2012) Do oral probiotics affect growth and neurodevelopmental outcomes in very low-birth-weight preterm infants? Am J Perinatol 29(8):579–586. doi: 10.1055/s-0032–1311981
57. Prescott SL, Wiltschut J, Taylor A, Westcott L, Jung W, Currie H, Dunstan JA (2008) Early markers of allergic disease in a primary prevention study using probiotics: 2.5-year follow-up phase. Allergy 63:1481–1490
58. Awad H, Mokhtar H, Imam SS, Gad GI, Hafez H, Aboushady N (2010) Comparison between killed and living probiotic usage versus placebo for the prevention of necrotizing enterocolitis and sepsis in neonates. Pak J Biol Sci 13:253–262
59. Bode L (2012) Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology 22(9):1147–1162. doi: 10.1093/glycob/cws074
60. Bode L, Rudloff S, Kunz C, Strobel S, Klein N (2004) Human milk oligosaccharides reduce platelet-neutrophil complex formation leading to a decrease in neutrophil beta 2 integrin expression. J Leukoc Biol 76:820–826
61. Bode L, Jantscher-Krenn E (2012) Structure-function relationships of human milk oligosaccharides. Adv Nutr 3(3):383S–391S. doi: 10.3945/an.111.001404
62. Jantscher-Krenn E, Zherebtsov M, Nissan C, Goth K, Guner YS, Naidu N, Choudhury B, Grishin AV, Ford HR, Bode L (2011) The human milk oligosaccharide disialyllacto-N-tetraose prevents necrotising enterocolitis in neonatal rats. Gut (Epub ahead of print)
63. Butel MJ, Waligora-Dupriet AJ, Szylit O (2002) Oligofructose and experimental model of neonatal necrotising enterocolitis. Br J Nutr 87:S213–S219
64. Srinivasjois R, Rao S, Patole S (2009) Prebiotic supplementation of formula in preterm neonates: a systematic review and meta-analysis of randomised controlled trials. Clin Nutr 28:237–242
65. Westerbeek EA, Hensgens RL, Mihatsch WA, Boehm G, Lafeber HN, van Elburg RM (2011) The effect of neutral and acidic oligosaccharides on stool viscosity, stool frequency and stool pH in preterm infants. Acta Paediatr 100:1426–1431
66. Westerbeek EA, van den Berg JP, Lafeber HN, Fetter WP, Boehm G, Twisk JW, van Elburg RM (2010) Neutral and acidic oligosaccharides in preterm infants: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 91:679–686
67. Mihatsch WA, Hoegel J, Pohlandt F (2006) Prebiotic oligosaccharides reduce stool viscosity and accelerate gastrointestinal transport in preterm infants. Acta Paediatr 95:843–848
68. Modi N, Uthaya S, Fell J, Kulinskaya E (2010) A randomized, double-blind, controlled trial of the effect of prebiotic oligosaccharides on enteral tolerance in preterm infants (ISRCTN77444690). Pediatr Res 68:440–445
69. Riskin A, Hochwald O, Bader D, Srugo I, Naftali G, Kugelman A, Cohen E, Mor F, Kaufman B, Shaoul R (2010) The effects of lactulose supplementation to enteral feedings in premature infants: a pilot study. J Pediatr 156:209–214

70. Manzoni P, Rinaldi M, Cattani S et al (2009) Italian task force for the study and prevention of neonatal fungal infections, Italian society of neonatology. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. JAMA 302:1421–1428
71. Manzoni P, Stolfi I, Messner H et al (2012) Italian task force for the study and prevention of neonatal fungal infections—the Italian society of neonatology. Bovine lactoferrin prevents invasive fungal infections in very low birth weight infants: a randomized controlled trial. Pediatrics 129:116–123
72. Candy DCA, Devane SP (1997) Role of micro-organisms in necrotising enterocolitis. Semin Neonatol 2:255–262
73. Uauy RD, Fanaroff AA, Korones SB, Phillips EA, Phillips JB, Wright LL (1991) Necrotizing enterocolitis in very low birth weight infants: biodemographic and clinical correlates. National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr 119:630–638
74. Patole S, Muller R (2004) Enteral feeding of preterm neonates—a survey of Australian neonatologists. J Matern Fetal Neonatal Med 16:309–314
75. Klingenberg C, Embleton ND, Jacobs SE, O'Connell LA, Kuschel CA (2012) Enteral feeding practices in very preterm infants: an international survey. Arch Dis Child Fetal Neonatal Ed 97:F56–F61
76. Patole SK, de Klerk N (2005) Impact of standardised feeding protocols on incidence of necrotising enterocolitis—a systematic review and meta analysis. Arch Dis Child Fetal Neonatal Ed 90:F147–F151
77. Patole S, McGlone L, Muller R (2003) Virtual elimination of necrotising enterocolitis for 5 years—reasons? Med Hypotheses 61:617–622
78. Kamitsuka MD, Horton MK, Williams MA (2000) The incidence of necrotizing enterocolitis after introducing standardized feeding schedules for infants between 1,250 and 2,500 g and less than 35 weeks of gestation. Pediatrics 105:379–384
79. Brown EG, Sweet AY (1978) Preventing necrotizing enterocolitis in neonates. JAMA 24:2452–2454
80. Spritzer R, Koolen AM, Baerts W, Fetter WP, Lafeber HN, Sauer PJ (1988) A prolonged decline in the incidence of necrotizing enterocolitis after the introduction of a cautious feeding regimen. Acta Paediatr Scand 77:909–911
81. Patole SK, Kadalraja R, Tuladhar R, Almonte R, Muller R, Whitehall JS (2000) Benefits of a standardised feeding regimen during a clinical trial in preterm neonates. Int J Clin Pract 54:429–431
82. Premji SS, Chessell L, Paes B, Pinelli J, Jacobson K (2002) A matched cohort study of feeding practice guidelines for infants weighing less than 1,500 g. Adv Neonatal Care 2:27–36
83. Kuzma-O'Reilly B, Duenas ML, Greecher C, Kimberlin L, Mujsce D, Miler D, Walker DJ (2003) Evaluation, development and implementation of potentially better practices in neonatal intensive care nutrition. Pediatrics 111:e461–e470
84. McCallie KR, Lee HC, Mayer O, Cohen RS, Hintz SR, Rhine WD (2011) Improved outcomes with a standardized feeding protocol for very low birth weight infants. J Perinatol 31:S61–S67
85. Street JL, Montgomery D, Alder SC, Lambert DK, Gerstmann DR, Christensen RD (2006) Implementing feeding guidelines for NICU patients $< 2{,}000$ g results in less variability in nutrition outcomes. JPEN J Parenter Enteral Nutr 30:515–518
86. Christensen RD, Gordon PV, Besner GE (2010) Can we cut the incidence of necrotizing enterocolitis in half-today? Fetal Pediatr Pathol 29:185–198
87. Celano P, Jumawan J, Horowitz C, Lau H, Koldovsky O (1977) Prenatal induction of sucrase activity in rat jejunum. Biochem J 162:469–472
88. Moog F (1962) Developmental adaptations of alkaline phosphatises in the small intestine. Fed Proc 21:51–56
89. Neu J, Ozaki CK, Angelides KJ (1986) Glucocorticoid-mediated alteration of fluidity of brush border membrane in rat small intestine. Pediatr Res 20:79–82

90. Israel EJ, Schiffrin EJ, Carter EA, Freiberg E, Walker WA (1990) Prevention of necrotizing enterocolitis in the rat with prenatal cortisone. Gastroenterol 99:1333–1338
91. Shulman RJ, Schanler RJ, Lau C, Heitkemper M, Ou CN, Smith EO (1998) Early feeding, antenatal glucocorticoids, and human milk decrease intestinal permeability in preterm infants. Pediatr Res 44:519–523
92. Bousvaros A, Walker WA (1990) Development and function of the intestinal mucosal barrier. In: McDonald TT (ed) Ontogeny of the human system of the gut. CRC Press, Boca Raton, pp 2–22
93. Spencer T, McDonald TT (1990) The ontogeny of the immune system of the gut. CRC Press, Boca Raton, pp 23–50
94. Buchmiller TL, Shaw KS, Lam ML, Stokes R, Diamond JS, Fonkalsrud EW (1994) Effect of prenatal dexamethasone administration: fetal rabbit intestinal nutrient uptake and disaccharidase development. J Surg Res 57:274–279
95. Nanthakumar NN, Young C, Ko JS, Meng D, Chen J, Buie T, Walker WA (2005) Glucocorticoid responsiveness in developing human intestine: possible role in prevention of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 288:G85–G92
96. Bauer CR, Morrison JC, Poole WK, Korones SB, Boehm JJ, Rigatto H, Zachman RD (1984) A decreased incidence of necrotizing enterocolitis after prenatal glucocorticoid therapy. Pediatrics 73:682–688
97. Crowley P, Chalmers I, Keirse MJ (1990) The effects of corticosteroid administration before preterm delivery: an overview of the evidence from controlled trials. Br J Obstet Gynaecol 97:11–25
98. Halac E, Halac J, Begue EF, Casanas JM et al (1990) Prenatal and postnatal corticosteroid therapy to prevent neonatal necrotizing enterocolitis: a controlled trial. J Pediatr 117:132–138
99. Roberts D, Dalziel S (2006) Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev (3):CD004454
100. ACOG Committee on Obstetric P (2011) ACOG committee opinion no 475: antenatal corticosteroid therapy for fetal maturation. Obstet Gynecol 117:422–424
101. Carlo WA, McDonald SA, Fanaroff AA et al (2011) Association of antenatal corticosteroids with mortality and neurodevelopmental outcomes among infants born at 22–25 weeks' gestation. JAMA 306:2348–2358
102. Lucas A, Cole TJ (1990) Breast milk and neonatal necrotising enterocolitis. Lancet 336:1519–1523
103. Caplan MS, Amer M, Jilling T (2002) The role of human milk in necrotising enterocolitis. Adv Exp Med Biol 503:83–90
104. Hanson LA (1999) Human milk and host defence: immediate and long-term effects. Acta Paediatr 88:42–46
105. Schanler RJ (2001) The use of human milk for premature infants. Pediatr Clin North Am 48:207–219
106. Goldman AS (2000) Modulation of the gastrointestinal tract of infants by human milk. Interfaces and interactions. An evolutionary perspective. J Nutr 130:426S–431S
107. Caplan M, Hsueh W, Kelly A, Donovan M (1990) Serum PAF acetylhydrolase increases during neonatal maturation. Prostaglandins 39:705–714
108. Rabinowitz SS, Dzakpasu P, Piecuch S, Leblanc P, Valencia G, Kornecki E (2001) Platelet-activating factor in infants at risk for necrotizing enterocolitis. J Pediatr 138:81–86
109. Caplan MS, Sun XM, Hseuh W, Hageman JR (1990) Role of platelet activating factor and tumor necrosis factor-alpha in neonatal necrotizing enterocolitis. J Pediatr 116:960–964
110. Amer MD, Hedlund E, Rochester J, Caplan MS (2004) Platelet activating factor concentration in the stool of human newborns: effects of enteral feeding and neonatal necrotizing enterocolitis. Biol Neonate 85:159–166
111. Furukawa M, Narahara H, Yasuda K, Johnston JM (1993) Presence of platelet-activating factor-acetylhydrolase in milk. J Lipid Res 34:1603–1609
112. Akisu M, Kultursay N, Ozkayin N, Coker I, Huseyinov A (1998) Platelet-activating factor levels in term and preterm human milk. Biol Neonate 74:289–293

113. Dvorak B, Halpern MD, Holubec H et al (2003) Maternal milk reduces severity of necrotizing enterocolitis and increases intestinal IL-10 in a neonatal rat model. Pediatr Res 53:426–433
114. McGuire W, Anthony MY (2003) Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. Arch Dis Child Fetal Neonatal Ed 88:F11–F14
115. Sullivan S, Schanler RJ, Kim JH, Patel AL et al (2010) An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. J Pediatr 156:562–567
116. Ganapathy V, Hay JW, Kim JH (2012) Costs of necrotizing enterocolitis and cost-effectiveness of exclusively human milk-based products in feeding extremely premature infants. Breastfeed Med 7:29–37
117. Meinzen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B, Donovan EF (2009) Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. J Perinatol 29:57–62
118. Sisk PM, Lovelady CA, Dillard RG, Gruber KJ, O'Shea TM (2007) Early human milk feeding is associated with a lower risk of necrotizing enterocolitis in very low birth weight infants. J Perinatol 27:428–433
119. Castellote C, Casillas R, Ramírez-Santana C, Pérez-Cano FJ, Castell M, Moretones MG, López-Sabater MC, Franch A (2011) Premature delivery influences the immunological composition of colostrum and transitional and mature human milk. J Nutr 141:1181–1187
120. Zamora SA, Amin HJ, McMillan DD et al (1998) Plasma L-arginine concentration oxygenation index, and systemic blood pressure in premature infants. Crit Care Med 26:1271–6
121. Becker RM, Wu G, Galanko JA et al (2000) Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. J Pediatr 137:785–793
122. Amin HJ, Zamora SA, McMillan DD, Fick GH (2002) Arginine supplementation prevents necrotizing enterocolitis in the premature infant. J Pediatr 140:425–441
123. Amin HJ, Soraisham AS, Sauve RS (2009) Neurodevelopmental outcomes of premature infants treated with l-arginine for prevention of necrotising enterocolitis. J Paediatr Child Health 45:219–223
124. Bell EF, Acarregui MJ (2008) Restricted versus liberal water intake for preventing morbidity and mortality in preterm infants. Cochrane Database Syst Rev (1):CD000503
125. Morag I, Goldman M, Kuint J, Heyman E (2007) Excessive weight gain as a possible predictor of necrotizing enterocolitis in premature infants. Isr Med Assoc J 9:24–27
126. Botsford KB, Weinstein RA, Boyer KM, Nathan C, Carman M, Paton JB (1986) Gram-negative bacilli in human milk feedings: quantitation and clinical consequences for premature infants. J Pediatr 109:707–710
127. Schreiner RL, Eitzen H, Gfell MA et al (1979) Environmental contamination of continuous drip feedings. Pediatrics 63:232–237
128. Carrion V, Egan EA (1990) Prevention of neonatal necrotizing enterocolitis. J Pediatr Gastroenterol Nutr 11:317–323
129. Guillet R, Stoll BJ, Cotton CM et al (2006) Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics 117:e137–e142
130. Terrin G, Passariello A, De Curtis M et al (2012) Ranitidine is associated with infections, necrotizing enterocolitis, and fatal outcome in newborns. Pediatrics 129:e40–e45
131. Faix RG, Polley TZ, Grasela TH (1988) A randomized, controlled trial of parenteral clindamycin in neonatal necrotizing enterocolitis. J Pediatr 112:271–277
132. Brotschi B, Baenziger O et al (2009) Early enteral feeding in conservatively managed stage II necrotizing enterocolitis is associated with a reduced risk of catheter-related sepsis. J Perinat Med 37:701–705
133. Bohnhorst B, Müller S, Dördelmann M, Peter CS, Petersen C, Poets CF (2003) Early feeding after necrotizing enterocolitis in preterm infants. J Pediatr 143:484–487
134. Chan KL, Ng SP, Chan KW, Wo YH, Tam PK (2003) Pathogenesis of neonatal necrotizing enterocolitis: a study of the role of intraluminal pressure, age and bacterial concentration. Pediatr Surg Int 19:573–577

135. Garstin WL, Kenny BD, McAneaney D, Patterson CC, Boston VV (1987) The role of intraluminal tension and pH in the development of necrotizing enterocolitis: an animal model. J Pediatr Surg 22:205–207
136. Dolgin SE et al (1998) Alterations in respiratory status: early signs of severe necrotizing enterocolitis. J Pediatr Surg 33:856–858
137. Dzakovic A, Notrica DM, Smith EO, Wesson DE, Jaksic T (2001) Primary peritoneal drainage for increasing ventilatory requirements in critically ill neonates with necrotizing enterocolitis. J Pediatric Surg 36:730–732
138. Pourcyrous M, Korones SB, Yang W, Boulden TF, Bada HS (2005) C-reactive protein in the diagnosis, management, and prognosis of neonatal necrotizing enterocolitis. Pediatrics 116:1064–1069
139. Hallstrom M, Koivisto AM, Janas M, Tammela O (2006) Laboratory parameters predictive of developing necrotizing enterocolitis in infants born before 33 weeks of gestation. J Pediatr Surg 41:792–798
140. Kenton AB, O'Donovan D, Cass DL, Helmrath MA, Smith EO, Fernandes CJ et al (2005) Severe thrombocytopenia predicts outcome in neonates with necrotizing enterocolitis. J Perinatol 25:14–20
141. Ververidis M, Kiely EM, Spitz L, Drake DP, Eaton S, Peirro A (2001) The clinical significance of thrombocytopenia in neonates with necrotizing enterocolitis. J Pediatr Surg 36:799–803
142. Harris MC, D'Angio CT, Gallagher PR, Kaufman D, Evans J, Kilpatrick L (2005) Cytokine elaboration in critically ill infants with bacterial sepsis, necrotizing enterocolitis, or sepsis syndrome: correlation with clinical parameters of inflammation and mortality. J Pediatr 147:462–468
143. Srinivasjois R, Nathan E, Doherty D, Patole S (2010) Prediction of progression of definite necrotising enterocolitis to need for surgery or death in preterm neonates. J Matern Fetal Neonat Med 23:695–700
144. Moss RL, Kalish LA, Duggan C et al (2008) Clinical parameters do not adequately predict outcome in necrotizing enterocolitis: a multi-institutional study. J Perinatol 28:665–674
145. Kenton AB, Hegemier S, Smith EO, O'Donovan DJ, Brandt ML, Cass DL et al (2005) Platelet transfusions in infants with necrotizing enterocolitis do not lower mortality but may increase morbidity. J Perinatol 25:173–177
146. Mohamed A, Shah PS (2012) Transfusion associated necrotizing enterocolitis: a meta-analysis of observational data. Pediatrics 129:529–540
147. Tepas JJ 3rd, Leaphart CL, Plumley D, Sharma R, Celso BG, Pieper P, Quilty J, Esquivia-Lee V (2010) Trajectory of metabolic derangement in infants with necrotizing enterocolitis should drive timing and technique of surgical intervention. J Am Coll Surgeons 210:847–852, 852–854
148. Bohnhorst B, Kuebler JF, Rau G, Gluer S, Ure B, Doerdelmann M (2011) Portal venous gas detected by ultrasound differentiates surgical NEC from other acquired neonatal intestinal diseases. Eur J Pediatr Surg 21:12–17
149. Dilli D, Suna Oguz S, Erol R, Ozkan-Ulu H, Dumanli H, Dilmen U (2011) Does abdominal sonography provide additional information over abdominal plain radiography for diagnosis of necrotizing enterocolitis in neonates? Pediatr Surg Int 27:321–327
150. McBride WJ, Roy S, Brudnicki A, Stringel G (2010) Correlation of complex ascites with intestinal gangrene and perforation in neonates with necrotizing enterocolitis. J Pediatr Surg 45:887–889
151. Kim HY, Kim IO, Kim WS, Kang GH (2011) Bowel sonography in sepsis with pathological correlation: an experimental study. Pediatr Radiol 41:237–243
152. Rao SC, Basani L, Simmer K, Samnakay N, Deshpande G (2011) Peritoneal drainage versus laparotomy as initial surgical treatment for perforated necrotizing enterocolitis or spontaneous intestinal perforation in preterm low birth weight infants. Cochrane Database Syst Rev (6):CD006182
153. Pike K, Brocklehurst P, Jones D, Kenyon S, Salt A, Taylor D, Marlow N (2012) Outcomes at 7 years for babies who developed neonatal necrotising enterocolitis: the ORACLE children study. Arch Dis Child Fetal Neonatal Ed (Epub ahead of print)

154. Patole S (2005) Prevention of necrotising enterocolitis—year 2004 and beyond. J Matern Fetal Neonat Med 17:69–80
155. Young C, Sharma R, Handfield M, Mai V, Neu J (2009) Biomarkers for infants at risk for necrotizing enterocolitis: clues to prevention? Pediatr Res 65:91R–97R
156. Evennett N, Alexander N, Petrov M, Pierro A, Eaton S (2009) A systematic review of serologic tests in the diagnosis of necrotizing enterocolitis. J Pediatr Surg 44:2192–2201
157. Thuijls G, Derikx JP, van Wijck K et al (2010) Non-invasive markers for early diagnosis and determination of the severity of necrotizing enterocolitis. Ann Surg 251:1174–1180
158. Evennett NJ, Hall NJ, Pierro A, Eaton S (2010) Urinary intestinal fatty acid-binding protein concentration predicts extent of disease in necrotizing enterocolitis. J Pediatr Surg 45:735–740
159. Aydemir O, Aydemir C, Sarikabadayi YU et al (2012) Fecal calprotectin levels are increased in infants with necrotizing enterocolitis. J Matern Fetal Neonat Med (Epub ahead of print)
160. Ayşe Selimoğlu M, Temel I, Yildirim C, Ozyalin F, Aktaş M, Karabiber H (2011) The role of fecal calprotectin and lactoferrin in the diagnosis of necrotizing enterocolitis. Pediatr Crit Care Med (Epub ahead of print)
161. Lam HS, Wong SP, Cheung HM, Chu WC, Wong RP, Chui KM, Liu FY, Li K, Fok TF, Ng PC (2011) Early diagnosis of intra-abdominal inflammation and sepsis by neutrophil CD64 expression in newborns. Neonatol 99:118–124
162. Tayman C, Tonbul A, Kahveci H, Uysal S, Koseoğlu B, Tatli MM, Dilmen U (2011) C5a, a complement activation product, is a useful marker in predicting the severity of necrotizing enterocolitis. Tohoku J Exp Med 224:143–150
163. Pierro A, Hall N, Ade-Ajayi A, Curry J, Kiely EM (2004) Laparoscopy assists surgical decision making in infants with necrotizing enterocolitis. J Pediatr Surg 39:902–906
164. Travadi J, Patole S, Charles A, Dvorak B, Doherty D, Simmer K (2006) Pentoxifylline reduces the incidence and severity of necrotizing enterocolitis in a neonatal rat model. Pediatr Res 60:185–189
165. Nowicki PT, Dunaway DJ, Nankervis CA et al (2005) Endothelin-1 in human intestine resected for necrotizing enterocolitis. J Pediatr 146:805–810
166. Athalye-Jape G, More K, Patole S (2012) Progress in the field of necrotising enterocolitis—year 2012. J Matern Fetal Neonat Med (Epub ahead of print)

Chapter 5
Aggressive Enteral Nutrition in Preterm Neonates

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Abstract Extrauterine growth restriction (EUGR) is a serious issue in extremely preterm (gestation under 28 weeks) neonates. Suboptimal nutrition and growth in the early postnatal life have been associated with impaired neurodevelopmental outcomes and growth in this population. Delay in commencing feeds, being too conservative whilst upgrading feeds, stopping feeds in presence of perceived risk factors for NEC, and failure to provide optimal balanced protein calorie intake during the critical early postnatal life, are the important contributors for EUGR. The concept of aggressive enteral nutrition has been widely accepted following the realisation of the frequency and consequences of EUGR in extremely preterm neonates. It is important to note that aggressive enteral nutrition is easier to advocate than practice considering the inherent limitations imposed on how much and how fast a preterm neonate can be fed without increasing the risk of NEC or long term adverse effects. This chapter provides a close scrutiny of the current evidence to assess if the safe upper limits of various aspects of aggressive enteral feeding of extremely preterm neonates are well defined and adequately assessed. The evidence for the management of manifestations of feed intolerance/ileus of prematurity (e.g., abdominal distension, large/bile stained gastric residuals) is also reviewed and guidelines for further research are provided.

Key points

- Suboptimal nutrition in the early postnatal life is associated with impaired long term neurodevelopment and growth in extremely preterm (gestation < 28 weeks) neonates.
- Early and aggressive enteral feeding is essential for optimising growth in extremely preterm neonates.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_5,

- Evidence for the benefits of early "aggressive" enteral nutrition in preterm neonates is mostly based on observational studies with risk of bias. They are also not powered adequately to assess the risk of NEC.
- Cochrane systematic review of randomised controlled trials has not ruled out the possibility that early trophic feeds may increase the risk of NEC. The definition and optimal duration of early trophic feeds is also not clear.
- Whilst it is accepted that early enteral feeding is essential, the safe upper limit of feed increment volume, and daily total milk intake is not clear for extremely preterm neonates, especially those with intrauterine growth restriction.
- Further research is necessary to understand the significance of the manifestations of ileus of prematurity and develop reliable biomarkers for early diagnosis of NEC, to avoid/minimise unnecessary withholding of enteral feeds due to the fear of the illness.

Extrauterine growth restriction (EUGR) has been reported as a serious issue in extremely preterm neonates [1]. The long-term consequences of EUGR can not be overemphasised given that early nutritional status may be related to later morbidity in neonates [1–3]. Nutritional exposures during critical perinatal period have been shown to influence the individual's risk of disease throughout life [4–7]. Birth weight and postnatal growth are known to influence the cognitive development and intelligence quotient [8–10]. Nutritional deficit and poor growth in very preterm neonates have been associated with impaired neurodevelopmental outcomes [11–13], short stature and metabolic disorders [14–16]. Postnatal growth impairment has been associated with neurological and sensory handicaps and poor school performance [1, 17, 18].

Clark et al. [1] have evaluated the incidence of EUGR in 24,371 preterm neonates from 124 American neonatal intensive care units at the time of discharge from the hospital. EUGR was defined as growth values (weight, length or head circumference) $\leq$ 10th percentile of intrauterine growth expectation based on estimated postmenstrual age in preterm (gestation: 23–34 weeks) neonates. The incidence of EUGR was 28 %, 34 %, and 16 % for weight, length, and head circumference, respectively. For each growth parameter, the incidence of EUGR increased with decreasing gestation and birth weight. Logistic regression analysis revealed that male gender, need for assisted ventilation on day 1, a history of necrotising enterocolitis (NEC), need for respiratory support at 28 days of age, and exposure to steroids during the hospital course were independently associated with EUGR [1]. Sweet et al. [19] have reported an almost universal pattern of impaired postnatal growth with extremely poor neurodevelopmental outcome at 2 years of age in preterm neonates. Of the 104 eligible neonates (birth weight $\leq$ 600 g) 24 survived to discharge, 2 died of chronic lung disease (CLD) after discharge, and 21 of the remaining 22 neonates returned for follow-up. Mean birth weight and gestation was 537 (430–600) g and 24 (22–27) weeks, respectively. At birth, 55 % were below the 10th percentile for birth weight. At hospital discharge and 2 years of age, 94 % were below the 10th percentile for weight, length, and head circumference. Nineteen of 21 (90 %) neonates were abnormal on neurodevelopmental follow-up [19]. Steward et al. [20] have reported

that ELBW neonates develop a growth deficit during the first few weeks of life that not only persists but also worsens during hospitalisation. The discharge weights of 35 ELBW appropriate for gestational age (AGA) neonates were compared with the median weight of a fetus of comparable gestational age based on an intrauterine growth reference. Growth velocity (grams/day) was determined. The weight-for-age z scores decreased significantly between birth and discharge. By discharge, 89 % of the neonates had discharge weights < 10th percentile. The mean discharge weight was significantly less than the median weight of a fetus of comparable gestational age. Days to regain birth weight also significantly affected growth outcomes [20]. Embleton et al. [21] have reported that postnatal malnutrition and growth retardation is an inevitable consequence of currently recommended dietary intakes (RDIs) in preterm neonates. Daily dietary intakes were prospectively collected in 105 preterm neonates (birth weight ≤ 1,750 g; gestation ≤ 34 weeks) admitted over a 6-month period. Actual energy and protein intake was subtracted from recommended energy (120 kcal/kg/day) and protein (3 g/kg/day) intakes and nutritional deficits calculated. Nutrient intakes meeting current RDIs were rarely achieved during early life. By the end of the first week, cumulative energy and protein deficits were 406 ± 92 and 335 ± 86 kcal/kg and 14 ± 3 and 12 ± 4 g/kg in neonates born ≤ 30 and those at ≥ 31 weeks. By the end of the fifth week, cumulative energy and protein deficits were 813 ± 542 and 382 ± 263 kcal/kg and 23 ± 12 and 13 ± 15 g/kg and the z scores were −1.14 ± 0.6 and −0.82 ± 0.5 for infants at ≤ 30 and ≥ 31 weeks. Variation in dietary intake accounted for 45 % of the variation in changes in z score [21]. In a large, prospective multicentre cohort study Ehrenkranz et al. [22] assessed growth in 1660 neonates (birth weight: 501–1,500 g) admitted by 24 h of age. Neonates were included if they survived > 7 days and were free of major congenital anomalies. The results indicated that once birth weight was regained, weight gain (14.4–16.1 g/kg/day) approximated intrauterine rates. However, at hospital discharge, most neonates born between 24 and 29 weeks' gestation had not achieved the median birth weight of the reference fetus at the same postmenstrual age. AGA neonates who survived to hospital discharge without developing CLD, severe intraventricular hemorrhage, NEC, or late onset-sepsis gained weight faster than comparable neonates with those morbidities. More rapid weight gain was associated factors such as an earlier age at the initiation of enteral feeds, and an earlier age at achievement of full enteral feedings [22]. Radmacher et al. [23] have evaluated the nutritional intake and subsequent growth and predictors of EUGR in a cohort of 221 ELBW neonates with gestation ≤ 29 weeks. Mean energy and protein intakes during hospitalisation did not reach recommendations of 120 and 3.0 g/kg/day and in utero growth rates could not be consistently reached or sustained. Birth weight percentile score, was highly predictive of EUGR ($p < 0.001$). When the independent effect of other predictors of EUGR was considered, only duration of total parenteral nutrition support ($p < 0.001$) and head circumference percentile at regaining of birth weight ($p < 0.001$) contributed significantly to the prediction of EUGR, once the effect of birth weight was taken into account [23]. A multicentre study (Cooke et al. [24]) from a single region in UK has reported that EUGR was universal in preterm neonates who survived to discharge. The extent of EUGR however varied among units and

could not be explained by differences in patient characteristics including the severity of illness. The findings indicate the importance of variations in nutritional practices and their impact on neonatal nutrition.

Senterre and Rigo have recently reported that the first week of life is a critical period to promote growth in preterm neonates and that early nutrition from the first day of life is essential [25, 26]. Cumulative protein deficit during the first week of life was the major determinant of the postnatal growth during the first 6 weeks of life [25]. Their results indicate that the cumulative nutritional deficit may be drastically reduced in both extremely preterm (gestation < 28 weeks) and very preterm (gestation: 28–30 weeks) neonates after optimizing nutritional policy during the first weeks of life, and the postnatal growth restriction could even be prevented [26]. Hanson et al. [27] have recently reported that changes in nutrition practices including early aggressive parenteral nutrition, early enteral feedings, trophic feedings, continuous feeds, protein fortification of 24-cal/oz mother's own breast milk, and development of a "feed intolerance" algorithm, improve growth outcomes significantly without causing adverse effects in VLBW neonates. Implementing the nutrition practice changes decreased EUGR from 57 % in the pre-implementation group to 28 % in the post-implementation group ($p = 0.01$). Weight percentile ranking at gestation 36 weeks' increased significantly in neonates 1,001–1,500 g, from the 13th to the 27th percentile ($p = 0.004$ and $p = 0.01$, respectively). CLD decreased significantly ($p = 0.02$). There was no increase in NEC (6 % pre vs 3 % post) or in blood urea nitrogen. Days of parenteral nutrition and central line use were decreased ($p = 0.02$ and $p = 0.07$, respectively) [27].

Considering the overall health burden of EUGR investigators have encouraged neonatologists to improve their nutritional practice while continuing further research considering the positive impact of nutrition on growth in extremely preterm neonates [28–30]. Over the last decade "aggressive" parenteral and enteral nutrition has been adopted as a strategy to optimise nutrition and reduce the incidence of EUGR in extremely preterm neonates [31–33]. An aggressive approach to parenteral nutrition alone may not bridge the gap unless its safety in terms of long-term neurodevelopmental outcomes is documented. Focussing only on parenteral nutrition is also not appropriate considering the risk of gut atrophy in absence of direct tropic effects of milk on the gut [34]. The policy of aggressive enteral nutrition is however difficult to practice given our inability to interpret common signs of feed intolerance from a potentially devastating illness like NEC [35]. Moreover despite the plausible rationale and suggested benefits even the definition and the benefit/risk ratio of trophic feeds in extremely preterm neonates is not clear [34]. It is therefore important to review the evidence indicating the scope of aggressive enteral nutrition in extremely preterm neonates.

1 Early Trophic Feeds

Evidence indicates that enteral fasting diminishes the functional adaptation of the immature gut and prolongs the need for parenteral nutrition. Administration of very small volumes of milk during the first week of life ("early trophic feeds"), may

promote intestinal maturation, and decrease time to reach full enteral feeds by facilitating feed tolerance. A systematic review of randomised or quasi-randomised trials has determined the effect of early trophic feeds versus enteral fasting on feed tolerance, growth, and the risk of NEC, mortality and other morbidities in VLBW neonates [34]. Early trophic feeds were defined as milk volumes up to 24 ml/kg/day introduced before 96 h postnatal age and continued for at least one week after birth. The nine trials ($N = 754$) included in the analysis, did not provide any evidence that early trophic feeds affected feed tolerance or growth rates in VLBW neonates. Meta-analysis did not detect a statistically significant effect on the incidence of NEC: RR: 1.07 (95 % CI: 0.67, 1.70). It was concluded that the available data cannot exclude important beneficial or harmful (increased risk of NEC) effects and are insufficient to guide clinical practice [34]. Considering that the practice of early trophic is well established, conducting large pragmatic trials to address these issues will be difficult. Physiologic benefits of early trophic feeds may occur at volumes as low as < 1 ml/kg/day [36]. Experts suggest that early trophic feeds can be defined as milk volumes under 10 ml/kg/day, and have pointed out that their optimal duration is not clear [37]. Given these issues and the significant variations in practice (volume and type of milk, age at start, and duration) the true effects of early trophic feeds will probably remain unclear.

2 Early and Aggressive Nutritional Feeds

Irrespective of significant overlap in the range and duration of milk volumes used for early trophic versus nutritional feeds, it is agreed that early and aggressive enteral feeding is essential for optimising growth and avoiding the adverse effects of parenteral nutrition. Evidence indicates that small volume feeds can be tolerated even by critically ill neonates. Current approach favours starting enteral feeds as soon as possible after birth (hour zero) rather than within the first 24–48 h [38]. Such a policy is easy to adopt when colostrum, and mother's/donor milk are available. However the benefits and risks of early feeding with formula milk when mother's/donor milk is not available need to be studied. Early aggressive enteral feeding has been shown to decrease the risk of late onset sepsis and improve nutritional outcomes without increasing adverse effects in extremely preterm neonates [39, 40].

3 Intrauterine Growth Restriction

Feed intolerance is a common problem in preterm neonates with intrauterine growth restriction (IUGR) and antenatal altered umbilical Doppler flows (AUDF). A systematic review of observational studies ($N = 14$) has shown that the risk of all stages of NEC is higher in these neonates (AUDF: 85/659 vs Control: 66/1178, OR: 2.13; 95 % CI: 1.49–3.03) [41]. The risk of confirmed NEC (6 studies) was also significantly

higher (OR: 6.9; 95 % CI: 2.3–20) [41]. Adverse effects of chronic hypoxia on an immature gut and the increased oxygen demand imposed by enteral feeds relate to the feeding difficulties and increased risk of NEC in these neonates [41]. Experimental studies show that hypoxia reduces intestinal blood flow and oxygen delivery through adrenergic vasoconstriction [42]. Increased oxygen extraction can compensate for a 30 % reduction in gut blood flow, but enteral feeding reduces the ability of oxygen extraction to compensate for the effects of hypoxia [43, 44]. Metabolic demands of enteral feeds increase oxygen consumption by the intestine [45]. Investigators have reported that the superior mesenteric artery (SMA) and Coeliac axis flow is significantly reduced on day one and the recovery in the baseline gut flow is slow during the first week of life in IUGR neonates. Despite the recovery in baseline SMA and Coeliac axis blood flow, the dynamic response to the first feed is still impaired in IUGR neonates [46–48]. It is important to note that even when Doppler variables are taken into consideration, birthweight remains a predominant risk factor for NEC [49, 50]. Based on Santulli's theory for the pathogenesis of NEC (triad of ischaemia, bacteria, and substrate), and the possibility that prolonging small feeding volumes early in life may decrease the incidence of NEC, delay in starting and increasing enteral feeds for the first 5–7 days of life of preterm VLBW neonates became a common practice [51, 52]. However Mihatsch et al. [53] reported that VLBW neonates with IUGR, increased antenatal umbilical arterial (UA) resistance, and brain sparing tolerated enteral feeds as well as appropriate for gestational age VLBW neonates. A total of 124 inborn VLBW neonates were enrolled in their prospective trial evaluating early enteral nutrition using a standardized feeding protocol with daily feed increments of 16 ml/kg. Full enteral feeds (FEF: 150 ml/kg/day) were achieved at 15 days (12–21 days) of age for all neonates. IUGR [FEF: 14 (12–21) days], increased UA resistance [FEF: 14 (11–16) days], and brain sparing [FEF: 15 (14–20) days] were not associated with early feed intolerance [53]. Later Karagianni et al. [50] assessed the effect of early (≤ 5 days) Vs delayed (≥ 6 days) minimal enteral feeds (MEF) on the incidence of NEC and feed intolerance in preterm IUGR neonates with abnormal Dopplers. This was a randomized, non-blinded pilot trial comparing "early" versus "delayed" MEF in addition to PN within 48 h. A total of 40 neonates received early [2 (1–5) days] and 41 received delayed [7 (6–14) days] MEF. There was no significant difference in NEC ($p = 0.353$) and feeding intolerance ($p = 0.533$) after early MEF [50]. Leaf et al. [54] have recently reported the first multicentre randomised controlled trial evaluating the effects of early versus delayed enteral feeds in preterm IUGR neonates. Neonates with gestation < 35 weeks, birth weight $<$ 10th centile and abnormal antenatal UA Doppler waveforms were randomly allocated to early (day 2) or "late" (day 6) enteral feeds after birth. Feeds were gradually increased by a feeding protocol with equal rate of increase for both groups. The primary outcomes included the time to achieve FEF sustained for 72 h and NEC. A total of 404 neonates (median gestation 31 weeks) were enrolled from 54 hospitals in UK and Ireland. The median age at FEF was 18 vs 21 days in the early versus late group neonates (HR: 1.36; 95 % CI: 1.11–1.67). All Stage NEC occurred in 18 % versus 15 % in the early versus late groups (RR: 1.2; 95 % CI: 0.77–1.87). Incidence of Stage II/III NEC was 8 % in both groups. Early feeds resulted in shorter duration of

TPN and high-dependency care, decreased cholestatic jaundice, and increases SD score for weight at discharge. It was concluded that early introduction of feeds in preterm IUGR neonates resulted in earlier achievement of FEF without increasing the risk of NEC [54]. The applicability of these results to extremely preterm neonates (gestation < 28 weeks) with IUGR is debatable considering the mean gestation (31 weeks) of the enrolled neonates, and the small number of neonates (44 vs 42) with gestation under 29 weeks at birth. The trial was also not adequately powdered to detect a minimum significant change in incidence of NEC, the real concern in this high-risk cohort.

Gupta et al. [55] have studied feeding tolerance in the 82/404 growth restricted preterm infants with gestation < 29 weeks from the ADEPT trial as a subgroup analysis. Gestation and birth weight were comparable between the early versus late feeding groups. Both groups started TPN at a median age of 2 days and had central lines in place for an average of 18 days. Median number of days of feed intolerance was 7 days in both groups. The early feeding group had significantly more frequent episodes of intolerance compared with the late feeding group. Birth weight under 600 g, late passage of meconium (> 72 h) and cholestasis were significantly associated with days of feeding intolerance. The median volume of feeds on first day of feeding intolerance was similar (9 ml/kg/day) in both groups. The median volume of feeds tolerated by infants in the first 10 days of life was much lower than the target volume in the trial. This feed intolerance in early days of life was present in both early and late feeding group. It was concluded that growth restricted infants of < 29 weeks' gestation with abnormal antenatal Dopplers failed to tolerate even the careful graded feeding regime used in the ADEPT trial. They suggested that this cohort of infants may require an increased duration of minimal enteral feeds and slower increments to decrease intolerance and establish full feeds [55]. Overall the current evidence indicates that delaying enteral feeds is not justified in preterm IUGR neonates however the optimal safe (with respect to the risk of NEC) feeding protocol for this population is not clear.

4 Rapid Advancement of Feeds

At start a volume of 20–25 ml/kg/day of nutritional feeds is generally considered safe for infants < 32 weeks' gestation [52]. Incremental volumes in various studies have been between 10–35 ml/kg/day in neonates with birth weight ≤ 750–1,500 g and gestation up to 33 weeks [56]. None includes significant number of neonates with gestation under 28 weeks with or without IUGR [56]. A systematic review has assessed the effect of slow rates (up to 24 ml/kg/day) of feed advancement on the incidence of NEC, mortality and other morbidities in VLBW neonates [56]. The review identified 4 RCTs ($N = 496$) with few ELBW and IUGR neonates. The trials defined slow advancement as daily increments of 15–20 ml/kg and faster advancement as 30–35 ml/kg. Meta-analyses did not detect statistically significant effects on the risk of NEC (RR: 0.91, 95 % CI: 0.47–1.75) or all cause mortality (RR: 1.43,

95 % CI: 0.78–2.61). neonates who had slow rates of feed volume advancement took significantly longer to regain birth weight [Median difference 2–6 days] and to establish full enteral feeding [reported median difference 2–5 days]. It was concluded that the current data do not provide evidence that slow advancement of enteral feed volumes reduces the risk of NEC in VLBW neonates [56]. Pending large pragmatic trials, the safe upper limit of daily increment volume, especially for ELBW and/or IUGR neonates remains unclear.

5 Higher Daily Total Volume of Enteral Feeds

Kuschel et al. [57] have reported the results of their RCT assessing the effects of a higher daily milk intake. Preterm neonates (Gestation < 30 weeks, $N = 44$), who reached FEF were randomized to remain on 150 ml/kg/day (A Group) or increase to 200 ml/kg/day (B Group). Primary outcome was growth at 35 weeks corrected gestational age (CGA). The groups were comparable at baseline but there was a trend for A group neonates to be lighter (895 vs 1,020 g, $p = 0.27$). There was a significant cross over: Group A: 43 % vs Group B: 54 %. B group had better weight gain/day (16.7 vs 15.2 g/kg/day, $p = 0.047$), and better weight (2,020 vs 1,885 g, $p = 0.014$), and greater arm fat area (282 vs 218 mm^2, $p = 0.009$) at 35 weeks CGA**.** There was no effect on length and head circumference. There was no significant difference in any growth parameters at 1 year. Morbidity was not different between the groups [57]. The significant crossover rates and the possibility that the weight gain related significantly to fat deposition are worrisome findings in this trial. Thomas et al. [58] have recently reported a RCT comparing two enteral feeding volumes in VLBW neonates in a resource poor set up where poor post-natal growth of preterm neonates is common and fortification is not available. A total of 64 VLBW neonates, once they reached full feeds, were randomised to either continue feeds at 200 ml/kg/day (standard volume) or increase to 300 ml/kg/day (high volume) of expressed breast milk. There was a significantly higher daily weight gain in the high-volume group as compared to the standard volume group (24.9 vs. 18.7 g/kg/day, $p < 0.0001$) without increasing any adverse effects such as feed intolerance, NEC, patent ductus arteriosus (PDA), or sepsis [58]. The small sample size and the gestation of enrolled neonates limits the applicability of these results to extremely preterm neonates in other set ups. Pending further research attention to provision of optimal protein calorie ratio rather than excess calories alone is important.

6 Feeding in Presence of PDA, Sepsis, and Umbilical Catheters

Both, hemodynamically significant PDA and its treatment with indomethacin are associated with NEC in preterm neonates [59, 60]. Berseth et al. [61] have analysed feeding outcomes in 105 preterm neonates (24–35 weeks' gestation) to determine the

incidence and causes of delays in reaching full enteral feeds (FEF: 140 ml/kg/day). Feed intolerance, defined as failure to reach FEF within 10 days of starting feeds, occurred in 13/46 (28 %) neonates with 30–35 weeks' gestation and in 29/59 (49 %) of those with 24–29 weeks' gestation. Although several factors were associated with delays in reaching FEF, a diagnosis of PDA, or PDA and late onset sepsis (after day 3), was a major risk factor [61]. Patole et al. [62] have reported the results of a clinical trial of prophylactic carboxymethylcellulose in reducing the time to FEF in neonates with < 32 weeks' gestation. The only variable showing any independent influence on the time to FEF, irrespective of the allocation to cellulose or placebo, was the presence of a significant PDA. The median (interquartile range) time to FEF was significantly longer [7.5 (4.0–13.8) vs. 4 (3.3–6.0) days, $p = 0.01$] in neonates with significant PDA than in neonates with no PDA [62]. Patole et al. [63] have also reported PDA, especially PDA with sepsis, as a risk factor for feed intolerance in preterm neonates. In their study, the start to full feeds interval was found to be longest in preterm neonates (≤ 28 weeks' gestation) with sepsis, followed by that in preterm neonates with sepsis and PDA, and in those with PDA alone [63]. A systematic review of observational studies has also raised the possibility that enteral feeding in the presence of significant PDA alone or PDA and sepsis may be related to NEC [64]. Adverse effects of PDA, cyclooxygenase inhibitors (e.g., indometacin, ibuprofen), and sepsis per se on intestinal perfusion and mucosal integrity as well as their interplay may explain such results. Considering the inability to differentiate between feed intolerance and early NEC, reluctance to feed during treatment of hemodynamically significant PDA and/or sepsis is common. In a national survey of Australian neonatologists, 56.6 % agreed that a significant PDA should be closed before initiating feeds [65]. Bellander et al. [66] have reported that tolerance to early human milk is not compromised by indometacin in preterm neonates with PDA. However, it is difficult to draw clear conclusions from their study, especially in terms of NEC, given the limitations of their study design and small sample size. Feeds are often not commenced, upgraded in presence of umbilical vascular catheters due to the risk of impaired gut perfusion. Davey et al. [67] have studied the frequency of feeding problems and NEC between a group of preterm neonates who received early enteral feeds while low umbilical artery catheters (LUACs) were in place, and a late group who were not fed until 24 h after removal of LUACs. Twenty-nine stable preterm neonates (Gestation: 28.5 ± 3.0 SD weeks) received early enteral feed at a median of 2 days while a LUAC was in place; 31 (Gestation: 28.6 ± 2.7 SD weeks) received late enteral feed at a median of 5 days of age, 24 h after the removal of the LUAC. There were no significant differences in the baseline characteristics, and the incidence of gastric residuals and abdominal distension between the two groups. The early feeding group had significantly fewer percutaneous central venous catheters, evaluations for sepsis, and episodes of receiving nothing by mouth. The early-fed group neonates received parenteral nutrition for a median of 13 days versus 30 days for the late-fed group ($p = 0.0028$). The incidence of NEC was 2 vs 4 cases in the early versus late group [67]. Pending large pragmatic trials with NEC as the primary outcome, balancing the benefits versus risks of an aggressive approach to feeding in presence of PDA, sepsis, and unmbilical catheters is difficult.

7 Facilitating Meconium Evacuation

Apart from poor gastroduodenal coordination and excessive quiescence in motor activity it is likely that delayed and slow colonic motility also plays a role in feed intolerance in preterm neonates [68]. Mihatsch et al. [69] have evaluated the correlation between the timings of the first and the last stool and feed intolerance in ELBW neonates. Forty one ELBW neonates were fed following a standardised protocol (day 3–14). Bolus gavage feeds were started at 48 h of age (12 mg/kg/day increments, 12 feeds a day). Gastric residuals up to 2 ml or up to 3 ml were tolerated for neonates with birth weight ≤ 750 g and > 750 g, respectively. No enemas or laxatives were given during the study. The impact of the time until the passage of the first (M-1) and the last (M-last) meconium on the feeding volume on day 14 (V14) was assessed by linear regression analysis. The median (range) V14, M-1, and M-last were as follows: V14: 99 (0–156) ml/kg, M-1: 31(0.5–77) h, M-last: 6 (1.4–22) days. There was a significant correlation between feed tolerance and the time for the last and not the first evacuation of meconium (i.e., V14 increased with decreasing M-last, $p < 0.001$). These findings were interpreted as the passage of the first meconium only indicates terminal large bowel function and total evacuation of the meconium is a far better parameter of feed intolerance. The authors suggested that passage of meconium should be considered when decisions on feeding ELBW neonates are made, and hypothesised if V14 could be advanced by accelerating passage of meconium [69]. Their hypothesis indicates a potential role of therapeutic agents accelerating passage of meconium in facilitating feed tolerance in preterm neonates. Use of glycerine suppositories and small volume enemas, for evacuating meconium, meconium plugs is not uncommon in preterm neonates [65]. It is however important to note that total evacuation of meconium and feed tolerance requires normal function of both, the upper as well as lower gastrointestinal tract, and glycerin suppositories do not have any effect on the right colon and the small bowel. Shim et al. [70] have recently reported a small observational study ($n = 58$) in preterm VLBW neonates to assess the benefits of glycerin enema (1 ml/kg every 12–24 h) starting within 24 h after birth and continued till meconium passage was complete. Compared to the control group, the study group neonates achieved FEF faster (HR: 2.9; 95 % CI: 1.8–4.8). This effect was more defined in ELBW neonates (HR = 4.6; 95 % CI = 1.9–11.1). Study group neonates also passed first meconium earlier than control group (1.4 v/s 3.7 days; $p < 0.001$), and had significantly less culture positive sepsis (7.7 v/s 27.8 %, $p = 0.02$) [70]. Khadr et al. [71] have assessed the benefits of regular glycerin suppositories in preterm neonates in a small ($n = 54$) open RCT. The intervention was started from 24 h of age, and continued for 10 days. The median time to FEF was 1.6 days shorter in study group, but not statistically significant: [7.4 (4.6–30.9) vs 9.0 (4.4–13.3) days, $p = 0.780$; 95 % CI: −1.917, 2.166]. Passage of first meconium occurred earlier in study group than controls (day 2 vs. day 4, $p = 0.016$) [71]. Small sample sizes and limitations of the design make it difficult to draw conclusions from these studies.

8 Feeding in Presence of Gastric Residuals

The significance of the volume and colour of gastric residuals in preterm neonates is not clear. Gastric aspirate volume above 30 or 50 % of feeds over previous 4 h is generally considered as abnormal [65]. Rising aspirate volume is reported to correlate with development of NEC in high-risk neonates [72, 73]. Bile stained (various shades of green or yellow) gastric residuals are considered as indicators of feed intolerance or early NEC. Considering the fear of NEC feeds are often not commenced or stopped in presence of abnormal gastric residuals. It is important to note that the frequency and need for monitoring gastric aspirates is not evidence based and that the aspirate volume and colour is affected by many variables. Bile stained gastric residuals are very common in the first few weeks of life in extremely preterm neonates, and should not be a contraindication to feeds if clinical examination is otherwise normal [74]. Persistence with at least small volume of milk feeds is expected to overcome the ileus of prematurity under such situation. Mihatsch et al. [74] have reported an observational study in 99 LBW neonates fed following a standardised feeding regime (day 3–14). Feeds were started at 48 h of age (12 ml/kg/day increments, 2 h feeds). Gastric residuals (GR) were checked before each feeding, and a GR volumes (GRV) up to 2 ml/3 ml in infants ≤ 750 g/ > 750 g was tolerated. In cases of increased GRV, feeds were reduced or withheld. The colour of GR was assessed as clear, milky, green-clear, green-cloudy, blood-stained, or hemorrhagic. Multiple regression analysis was used to study the effect of the mean GRV and the colour of GR on the feed volume on D14 (V14). The median V14 was 103 ml/kg/day (0–166). V14 increased with increasing percentage of milky GR, whereas the mean GRV and the green colour did not have a significant effect. It was concluded that early feeds could be established in ELBW neonates. The critical GRV seemed to be > 2 ml/3 ml as there was no significant negative correlation between the mean GRV and V14. Green GRs were not negatively correlated with V14 and should not slow down feed volumes in absence of other signs and symptoms [74]. Overall the current evidence indicates giving undue importance to abnormal gastric residuals is probably not justified if an aggressive approach to enteral nutrition is to be adopted provided the clinical examination is normal. Large pragmatic trials are needed to address this important issue.

9 Probiotics and Prebiotics

Indrio et al. [75–77] have reported that specific probiotic strains can promote feed tolerance, improve bowel habits and facilitate gut motility in preterm neonates. Thirty preterm neonates were enrolled in their double blind RCT; 10 were exclusively breast-fed, and the remaining 20 were randomly assigned to either Lactobacillus reuteri ATCC 55730 (1×10^8 colony forming units/day) or placebo for 30 days [75]. Body weight gains/day was similar for all groups, and no adverse events were recorded. Neonates receiving probiotic had a significant decrease in regurgitation and mean

daily crying time and a higher frequency of stools compared with those receiving placebo. Gastric emptying rate was significantly increased, and fasting antral area was significantly reduced in neonates receiving L. reuteri and breast-feeds compared with those receiving placebo [75]. Indrio et al. [76] have also reported that prebiotic oligosaccharides can modulate the electrical activity and the gastric emptying and may improve the intestinal tolerance of enteral feeding in preterm neonates. In this double blind RCT the percentage of time in which propagation was detected in the electrogastrography (EGG) signal was twice in neonates receiving formula with prebiotics compared with formula with placebo, and the gastric half-emptying time was 30 % faster in the prebiotic group than the placebo group [76]. In another trial (Indrio et al. [77]), cutaneous EGG and gastric emptying was studied in 49 preterm neonates. A total of 17 were exclusively breast-fed; 32 were randomly assigned to prebiotic-added formula, a probiotic-added formula (L. reuteri: 1×10^8 colony forming units/day), or a formula with placebo for 30 days. After the intervention period, the prebiotic, probiotic, and breast milk groups showed a higher percentage of EGG slow wave propagation and faster gastric half emptying compared with the placebo group [77].

Apart from hypomotility of the gut, high viscosity of meconium is thought to contribute to delayed passage of meconium and feed intolerence in preterm neonates. Westerbeek et al. [78] have studied the effect of neutral oligosaccharides [small-chain galacto-oligosaccharides/long-chain fructo-oligosaccharides (scGOS/lcFOS)] in combination with acidic oligosaccharides (pAOS) on stool viscosity, frequency and pH in preterm neonates. Neonates ($N = 113$, gestation < 32 weeks and/or birth weight $< 1{,}500$ g) were randomly allocated to either scGOS/lcFOS with pAOS or a placebo. Stool viscosity at day 30 was lower in the prebiotics (16.8N) (3.9–67.8) versus placebo group (26.3N) (1.3–148.0) ($p = 0.03$; 95 % CI: -0.80–0.03). There was a trend towards higher stool frequency in the prebiotics versus placebo group (3.1 ± 0.8 versus 2.8 ± 0.7; 95 % CI: -0.08–0.52; $p = 0.15$). Stool pH at day 30 was lower in the in the prebiotics versus placebo group (5.9 ± 0.6 versus 6.2 ± 0.3, 95 % CI: 0.08–0.53; $p = 0.009$) [78]. Mihatsch et al. [79] have also reported that formula supplementation with GosFos reduced stool viscosity and accelerated gastrointestinal transport in preterm neonates (gestation 27 (24–31) weeks) on FEF. No adverse effects were observed. Modi et al. [80] have conducted a multicenter RCT comparing preterm formula containing 0.8 g/100 ml scGOS/lcFOS in a 9:1 ratio and an otherwise identical formula, using formula only to augment insufficient maternal milk supply. Preterm (Gestation < 33 weeks), appropriately grown for gestational age neonates ($N = 160$) were randomized within 24 h of birth. There was no significant difference in the time to reach FEF of 150 ml/kg/day and the proportion of time between birth and day 28/discharge that a total milk intake of ≥ 150 ml/kg/day was tolerated. There was also no significant difference in secondary outcomes including growth, NEC, and bloodstream infections. A significant benefit in feed tolerance was noted from trial formula with increasing immaturity (2.9 % improved tolerance for a neonate born at 28-week gestation and 9.9 % at 26-week gestation; $p < 0.001$) but decreased or no benefit in neonates > 31 week gestation [80]. A pilot double blind RCT ($N = 28$) by Riskin et al. [81] suggests the safety of low dose lactulose supplementation in preterm

neonates. Neonates on lactulose had more Lactobacilli-positive stool cultures that appeared earlier with larger number of colonies, less feed intolerance, fewer episodes of late-onset sepsis, lower NEC and be discharged home earlier. Their nutritional laboratory indices were better; especially calcium and total protein [81]. The role of probiotic and/or prebiotics in facilitating feed tolerance in preterm neonates needs to be evaluated further.

10 Discussion

The concept of aggressive enteral nutrition has been widely accepted following the realisation of the frequency and consequences of EUGR in preterm neonates. Delay in commencing feeds, being too conservative whilst upgrading feeds, stopping feeds in presence of perceived risk factors for NEC, and failure to provide optimal balanced protein calorie intake during the critical early postnatal life, are the important contributors for EUGR. It is important to note that aggressive enteral nutrition is easier to advocate than practice considering the inherent limitations imposed on how much and how fast a preterm neonate can be fed without increasing the risk of NEC or long term adverse effects. A close scrutiny of the current evidence indicates that the safe upper limits of various aspects of aggressive enteral feeding of extremely preterm neonates are either poorly defined or inadequately assessed. Even the definition and safety of early trophic feeds in terms of an increased risk of NEC is not clear. Not many studies include enough number of extremely preterm neonates who are at the highest risk of NEC. Most of the recent studies reporting benefits of early aggressive enteral nutrition are observational in nature with high probability of bias [82–84]. Results of the large retrospective cohort study showed that earlier initiation of enteral nutrition was associated with lower risk of late-onset bacteremia only in most mature VLBW infants (i.e., ≥ 28 and < 32 weeks) [82]. Generating evidence from high quality definitive RCTs in extremely preterm neonates to address current gaps in knowledge is difficult considering the low incidence of NEC that necessitates large sample sizes. The long list of gaps in the knowledge also means conducting a definitive pragmatic trial to address each one of the issues is not practical. Factorial trials, cluster randomised trials, or the use of Bayesian statistics may help in overcoming this difficulty [85]. The need for follow up studies to assess the long term consequences of neonatal feeding strategies can not be overemphasised [86]. Pending further research what can be realistically achieved in terms of aggressive enteral nutrition for extremely preterm neonates is rather limited in the context of current evidence. However it is time to question an unduly conservative approach to enteral feeding that is not evidence based either.

References

1. Clark RH, Thomas P, Peabody J (2003) Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics 111:986–990
2. Barker DJ, Forsen T, Eriksson JG, Osmond C (2002) Growth and living conditions in childhood and hypertension in adult life: a longitudinal study. J Hypertens 20:1951–1956
3. Eriksson JG, Forsen T, Tuomilehto J, Jaddoe VW, Osmond C, Barker DJ (2002) Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. Diabetologia 45:342–348
4. Lucas A (1998) Programming by early nutrition: an experimental approach. J Nutr 128:401s–406s
5. Singhal A, Farooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A (2002) Early nutrition and leptin concentrations in later life. Am J Clin Nutr 75:993–999
6. Singhal A, Cole TJ, Lucas A (2001) Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. Lancet 357:413–419
7. Fewtrell MS, Cole TJ, Bishop NJ, Lucas A. (2000) Neonatal factors predicting childhood height in preterm infants: evidence for a persisting effect of early metabolic bone disease? J Pediatr 137:668–673
8. Jefferis BJ, Power C, Hertzman C (2002) Birth weight, childhood socioeconomic environment, and cognitive development in the 1958 British birth cohort study. BMJ 325:305
9. Richards M, Hardy R, Kuh D, Wadsworth ME (2001) Birth weight and cognitive function in the British 1946 birth cohort: longitudinal population based study. BMJ 322:199–203
10. Richards M, Hardy R, Kuh D, Wadsworth ME (2002) Birthweight, postnatal growth and cognitive function in a national UK birth cohort. Int J Epidemiol 31:342–348
11. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK (2006) Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 117:1253–1261
12. Franz AR, Pohlandt F, Bode H, Mihatsch WA, Sander S, Kron M et al (2009) Intrauterine, early neonatal, and postdischarge growth and neurodevelopmental outcome at 5.4 years in extremely preterm infants after intensive neonatal nutritional support. Pediatrics 123:e101–e109
13. Stephens BE, Walden RV, Gargus RA, Tucker R, McKinley L, Mance M et al (2009) First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123:1337–1343
14. Odberg MD, Sommerfelt K, Markestad T, Elgen IB (2010) Growth and somatic health until adulthood of low birthweight children. Arch Dis Child Fetal Neonatal Ed 95:F201–F205
15. Singhal A, Fewtrell M, Cole TJ, Lucas A (2003) Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 361:1089–1097
16. Vohr BR, Allan W, Katz KH, Schneider KC, Ment LR (2010) Early predictors of hypertension in prematurely born adolescents. Acta Paediatr 99:1812–1818
17. Hack M, Breslau N, Weissman B, Aram D, Klein N, Borawski E (1991) Effect of very low birth weight and subnormal head size on cognitive abilities at school age. N Engl J Med 325:231–237
18. Hack M, Taylor HG, Klein N, Eiben R, Schatschneider C, Mercuri-Minich N (1994) School-age outcomes in children with birth weights under 750 g. N Engl J Med 331:753–759
19. Sweet MP, Hodgman JE, Pena I, Barton L, Pavlova Z, Ramanathan R (2003) Two-year outcome of infants weighing 600 g or less at birth and born 1994 through 1998. Obstet Gynecol 101:18–23
20. Steward DK, Pridham KF (2002) Growth patterns of extremely low-birth-weight hospitalized preterm infants. J Obstet Gynecol Neonatal Nurs 31:57–65
21. Embleton NE, Pang N, Cooke RJ (2001) Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 107:270–273
22. Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL, Katsikiotis V et al (1999) Longitudinal growth of hospitalized very low birth weight infants. Pediatrics 104:280–289

23. Radmacher PG, Looney SW, Rafail ST, Adamkin DH (2003) Prediction of extrauterine growth retardation (EUGR) in VVLBW infants. J Perinatol 23:392–295
24. Cooke RJ, Ainsworth SB, Fenton AC (2004) Postnatal growth retardation: a universal problem in preterm infants. Arch Dis Child Fetal Neonatal Ed 89:F428–F430
25. Senterre T, Rigo J (2012) Reduction in postnatal cumulative nutritional deficit and improvement of growth in extremely preterm infants. Acta Paediatr 101:e64–e70
26. Senterre T, Rigo J (2011) Optimizing early nutritional support based on recent recommendations in VLBW infants and postnatal growth restriction. J Pediatr Gastroenterol Nutr 53:536–542
27. Hanson C, Sundermeier J, Dugick L, Lyden E, Anderson-Berry AL (2011) Implementation, process, and outcomes of nutrition best practices for infants < 1500. Nutr Clin Pract 26:614–624
28. Clark RH, Wagner CL, Merritt RJ, Bloom BT, Neu J, Young TE et al (2003) Nutrition in the neonatal intensive care unit: how do we reduce the incidence of extrauterine growth restriction? J. Perinatol 23:337–344
29. Bloom BT, Mulligan J, Arnold C, Ellis S, Moffitt S, Rivera A et al (2003) Improving growth of very low birth weight infants in the first 28 days. Pediatrics 112:8–14
30. Dusick AM, Poindexter BB, Ehrenkranz RA, Lemons JA (2003) Growth failure in the preterm infant: can we catch up? Semin Perinatol 27:302–310
31. Ziegler EE, Thureen PJ, Carlson SJ (2002) Aggressive nutrition of the very low birthweight infant. Clin Perinatol 29:225–244
32. Thureen PJ, Hay WW Jr (2001) Early aggressive nutrition in preterm infants. Semin Neonatol 6:403–415
33. Wilson DC, Cairns P, Halliday HL, Reid M, McClure G, Dodge JA (1997) Randomised controlled trial of an aggressive nutritional regimen in sick very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 77:F4–F11
34. Bombell S, McGuire W (2009) Early trophic feeding for very low birth weight infants. Cochrane Database Syst Rev 3:CD000504–C
35. Jadcherla SR, Kliegman RM (2002) Studies of feeding intolerance in very low birth weight infants: definition and significance. Pediatrics 109:516–517
36. Thureen PJ (1999) Early aggressive nutrition in the neonate. NeoReviews 22:e45–e55
37. Ziegler EE (2011) Meeting the nutritional needs of the low-birth-weight infant Ann Nutr. Metab 58:8–18
38. Taylor SN, Kiger J, Finch C, Bizal D (2010) Fluid, electrolytes, and nutrition: minutes matter. Adv Neonatal Care 10:248–255
39. Rønnestad A, Abrahamson TG, Medbø S et al (2005) Septicemia in the first week of life in a Norwegian national cohort of extremely premature infants. Pediatrics 115:e262
40. Härtel C, Haase B, Browning-Carmo K et al (2009) Does early feeding advancement affect short-term outcomes in very low birth weight infants? J Pediatr Gastroenterol Nutr 48:464–470
41. Dorling J, Kempley S, Leaf A (2005) Feeding growth restricted preterm infants with abnormal antenatal Doppler results. Arch Dis Child Fetal Neonatal Ed 90:F359–F363
42. Nowicki P, Caniano DA, Szaniszlo K (1988) Effect of intestinal denervation on intestinal vascular response to severe arterial hypoxia in newborn swine. Am J Physiol 254:G189–G193
43. Bulkley GB, Kvietys PR, Parks DA*etal* (1985) Relationship of blood flow and oxygen consumption to ischemic injury in the canine small intestine. Gastroenterology 89:852–857
44. Szabo JS, Mayfield SR, Oh W (1987) Postprandial gastrointestinal blood flow and oxygen consumption: effects of hypoxemia in neonatal piglets. Pediatr Res 21:93–98
45. Nowicki PT, Stonestreet BS, Hansen NB et al (1983) Gastrointestinal blood flow and oxygen consumption in awake newborn piglets: effect of feeding. Am J Physiol 245:G697–G702
46. Gamsu HR, Kempley ST (1997) Enteral hypoxia/ischaemia and necrotising enterocolitis. Semin Neonatal 2:245–254
47. Maruyama K, Koizumi T (2001) Superior mesenteric artery blood flow velocity in small for gestational age infants of very low birth weight during the early neonatal period. J Perinat Med 19:64–70

48. Murdoch EM, Sinha AK, Kempley ST (2002) Impaired splanchnic haemodynamic responses to enteral feeding in preterm growth restricted infants. Abstract Presented at Summer Meeting of the UK Neonatal Society, 28–29th June 2002. Faculté de Médecine de Tours, France
49. Manogura AC, Turan O, Kush ML, Berg C, Bhide A, Turan S et al (2008) Predictors of necrotizing enterocolitis in preterm growth-restricted neonates. Am J Obstet Gynecol 198:638.e1–e5
50. Karagianni P, Briana DD, Mitsiakos G (2010) Early versus delayed minimal enteral feeding and risk for necrotizing enterocolitis in preterm growth-restricted. Am J Perinatol 27:367–373
51. Santulli TV, Schullinger JN, Heird WC et al (1975) Acute necrotising enterocolitis in infancy: a review of 64 cases. Pediatrics 55:376–387
52. Berseth CL, Bisquera JA, Paje VU (2003) Prolonging small feeding volumes early in life decreases the incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics 111:529–534
53. Mihatsch WA, Pohlandt F, Franz AR, Flock F (2002) Early feeding advancement in very low-birth-weight infants with intrauterine growth retardation and increased umbilical artery resistance. J Pediatr Gastroenterol Nutr 35:144–148
54. Leaf A, Dorling J, Kempley S, McCormick K, Mannix P, Linsell L, Juszczak E, Brocklehurst P, Abnormal Doppler Enteral Prescription Trial Collaborative Group (2012) Early or delayed enteral feeding for preterm growth-restricted infants: a randomized trial. Pediatrics 129:e1260–e1268
55. Gupta N, Kempley S on behalf of ADEPT study group (2010) Analysis of feeding intolerance in growth restricted < 29 weeks infants. Presented at the Neonatal society London, UK, 2010, Autumn Meeting. http://www.neonatalsociety.ac.uk/abstracts/guptan_2010_feedintolerance29weeks.shtml
56. Morgan J, Young L, McGuire W (2011) Slow advancement of enteral feed volumes to prevent necrotising enterocolitis in very low birth weight infants. Cochrane Database Syst Rev 3:CD001241–C
57. Kuschel CA, Evans N, Askie L, Bredemeyer S, Nash J, Polverino J (2000) A randomized trial of enteral feeding volumes in infants born before 30 weeks' gestation. J Paediatr Child Health 36:581–586
58. Thomas N, Cherian A, Santhanam S, Jana AK (2012) A randomized control trial comparing two enteral feeding volumes in very low birth weight babies. J Trop Pediatr 58:55–58
59. Capozzi G, Santoro G (2011). Patent ductus arteriosus: patho-physiology, hemodynamic effects and clinical complications. J Matern Fetal Neonatal Med 24:15–16
60. Dollberg S, Lusky A, Reichman B (2005) Patent ductus arteriosus, indomethacin and necrotizing enterocolitis in very low birth weight infants: a population-based study. J Pediatr Gastroenterol Nutr 40:184–188
61. Berseth CL (2003) Risk factors for delays in establishing full enteral feeding volume in preterm infants. Pediatr Res 2003:A2647–A
62. Patole SK, Muller R (2005) Does Carboxymethylcellulose have a role in reducing time to full enteral feeds in preterm neonates? Int J Clin Pract 59:544–548
63. Patole SK, Kumaran VS, Travadi JN, Brooks JM, Doherty DA (2007) Does patent ductus arteriosus affect feed tolerance in preterm neonates? Arch Dis Child Fetal Neonatal Ed 92:F53–F55
64. Patole SK, de Klerk N (2005) Impact of standardised feeding regimens on incidence of neonatal necrotising enterocolitis-a systematic review and meta analysis of observational studies. Arch Dis Child Fetal Neonatal Ed 90:F147–F151
65. Patole SK, Muller R (2004) Enteral feeding of preterm neonates—a survey of Australian neonatologists. J Maternal Fetal Neonatal Med 16:309–314
66. Bellander M, Ley D, Polberger S, Hellstrom-Westas L (2003) Tolerance to early human milk feeding is not compromised by indomethacin in preterm infants with persistent ductus arteriosus. Acta Paediatr 92:1074–1078
67. Davey AM, Wagner CL, Cox C, Kendig JW (1994) Feeding premature infants while low umbilical artery catheters are in place: a prospective, randomized trial. J Pediatr 124:795–799

68. Jadcherla SR, Kliegman RM (2002) Studies of feeding intolerance in very low birth weight infants: definition and significance. Pediatrics 109:516–517
69. Mihatsch WA, Franz AR, Lindner W, Pohlandt F (2001) Meconium passage in extremely low birthweight infants and its relation to very early enteral nutrition. Acta Paediatr 90:409–411
70. Shim SY, Kim HS, Kim DH, Kim EK, Son DW, Kim BI, Choi JH (2007) Induction of early meconium evacuation promotes feeding tolerance in very low birth weight infants. Neonatology 92:67–72
71. Khadr SN, Ibhanesebhor SE, Rennix C et al (2001) Randomized controlled trial: impact of glycerin suppositories on time to full feeds in preterm infants. Neonatology 100:169–176
72. Cobb BA, Carlo WA, Ambalavanan N (2004) Gastric residuals and their relationship to necrotizing enterocolitis in very low birth weight infants. Pediatrics 113:50–53
73. Bertino E, Giuliani F, Prandi G, Coscia A, Martano C, Fabris C (2009) Necrotizing enterocolitis: risk factor analysis and role of gastric residuals in very low birth weight infants. J Pediatr Gastroenterol Nutr 48:437–442
74. Mihatsch WA, von Schoenaich P, Fahnenstich Het al (2002) The significance of gastric residuals in the early enteral feeding advancement of extremely low birth weight infants. Pediatrics 109:457–459
75. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R (2008) The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. J Pediatr 152:801–806
76. Indrio F, Riezzo G, Raimondi F et al (2009) Prebiotics improve gastric motility and gastric electrical activity in preterm newborns. J Pediatr Gastroenterol Nutr 49:258–261
77. Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R (2009) Effects of probiotic and prebiotic on gastrointestinal motility in newborns. J Physiol Pharmacol 60:27–31
78. Westerbeek EA, Hensgens RL, Mihatsch WA, Boehm G, Lafeber HN, van Elburg RM (2011) The effect of neutral and acidic oligosaccharides on stool viscosity, stool frequency and stool pH in preterm infants. Acta Paediatr 100:1426–1431
79. Mihatsch WA, Hoegel J, Pohlandt F (2006) Prebiotic oligosaccharides reduce stool viscosity and accelerate gastrointestinal transport in preterm infants. Acta Paediatr 95:843–848
80. Modi N, Uthaya S, Fell J, Kulinskaya E (2010) A randomized, double-blind, controlled trial of the effect of prebiotic oligosaccharides on enteral tolerance in preterm infants (ISRCTN77444690). Pediatr Res 68:440–445
81. Riskin A, Hochwald O, Bader D et al (2010) The effects of lactulose supplementation in premature infants—a pilot study. J Pediatr 156:209–214
82. Lavoie PM (2009) Earlier initiation of enteral nutrition is associated with lower risk of late-onset bacteremia only in the most mature VLBW infants. J Perinatol 29:448–454
83. Terrin G, Passariello A, Canani RB, Manguso F, Paludetto R, Cascioli C (2009) Minimal enteral feeding reduces the risk of sepsis in feed-intolerant very low birth weight newborns. Acta Paediatr 98:31–35
84. Cakmak CF, Aygun C, Cetinoglu E (2009) Does early enteral feeding of very low birth weight infants increase the risk of necrotizing enterocolitis? Eur J Clin Nutr 63:580–584
85. Tyson JE, Kennedy KA, Lucke JF, Pedroza C (2007) Dilemmas initiating enteral feedings in high risk infants: how can they be resolved? Semin Perinatol 31:61–73
86. Desai M, Beall M, Ross MG (2013) Developmental origins of obesity: programmed adipogenesis. Curr Diab Rep 13(1):27–33

Chapter 6
Metabolic Bone Disease of Prematurity

Suresh Birajdar, Mary Sharp and Sanjay Patole

Abstract Metabolic bone disease (MBD) is a significant complication that commonly affects extremely preterm (Gestation under 28 weeks) or very low birth weight (Birth weight under 1,500 g) infants. Reduced bone mineralization is the pathognomonic feature of MBD. Many hormonal and nutritional factors contribute to its pathogenesis. Conditions such as bronchopulmonary dysplasia, necrotising enterocolitis, and medications such as glucocorticoids and loop diuretics increase the risk. MBD is often asymptomatic and can present with failure to wean off the ventilator and/or long bone and rib fractures. Serum phosphate and alkaline phosphatase levels can help in early diagnosis but lack sensitivity and specificity. Plain X-rays of bones have low sensitivity because significant demineralization needs to occur before the diagnostic changes become visible. Dual Energy X ray absorptiometry (DEXA) is a more specific and sensitive diagnostic tool but it is not portable and involves increased radiation exposure to the infant. Early enteral feeding, fortification of preterm human milk with calcium, phosphorus and supplementation of vitamin D have an important role in the prevention of MBD. Current evidence suggests that MBD can reduce bone mineral contents and height in childhood but may not affect eventual adult height and bone mineralization. However there are uncertainties about its role in development of osteoporosis in adulthood. Future research is required to evaluate the long term outcomes such as the risk of fractures and skeletal deformities following MBD, and the role of systematic physical activity programs and maternal vitamin D deficiency in the condition.

Key points

- Metabolic bone disease (MBD) of prematurity is a morbid condition affecting mainly extremely preterm or very low birth weight neonates.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_6,

- Reduced bone mineralization, the pathognomonic feature of MBD, occurs due to insufficient dietary intake of bone minerals.
- MBD is often asymptomatic and there is no gold standard investigation for its diagnosis.
- Optimisation of nutritional intake especially proteins and minerals can help in prevention of MBD.

1 Introduction

The third trimester of pregnancy is the period for the greatest fetal mineral accretion [1]. A fetus born at 24 weeks of gestation has total body calcium content only 10–15 % of its value at term. Preterm birth thus denies the opportunity to the infant for mineral accretion. The nutritional needs of preterm infants in the early postnatal period are mostly provided via parenteral route considering their intolerance to significant volume of milk feeds and supplements. However this route cannot match the significant transplacental supply of essential nutrients and bone minerals (e.g., calcium and phosphorus) that the fetus receives [2]. Providing the required intake of calcium and phosphorus is especially difficult given the complex bioavailability and pharmacological properties of these bone minerals. The resultant inadequate bone mineralization has been labelled as osteopenia of prematurity or more recently, metabolic bone disease of prematurity (MBD) [1, 3]. Considering its insidious course MBD is often diagnosed late when it manifests with complications such as fracture of the bone. The survival of extremely preterm (gestation under 28 weeks) infants has improved following the recent advances in neonatal intensive care, increasing the absolute number of those who are at high risk for MBD. A clear understanding of the epidemiology and pathogenesis of MBD in preterm infants is therefore necessary for its prevention and optimal management.

2 Epidemiology and Health Burden

The incidence of MBD has been reported to vary from 3.4 % to 30 % in the studies from 1990s [4] whereas earlier studies have reported it to be high as up to 55 % in extremely low birth weight (ELBW) infants [5]. The few recent reports indicate that the incidence of MBD in preterm infants is declining following the advances in nutritional care [6, 7]. MBD predominantly affects preterm (gestation < 32 weeks) very low birth weight (birth weight $< 1{,}500$ g) infants [8, 9]. Those exposed to prolonged parenteral nutrition (PN), sepsis, prolonged immobility and medications such as steroid and diuretics are at a higher risk. The morbidity of MBD is significant and includes reduced bone mineralization, abnormal bone remodeling and fractures. Rib fractures can prolong mechanical ventilation and make successful extubation difficult [9, 10]. The long term potential adverse effects include fractures in infancy

and reduced childhood height [11]. However no effect on adult height has been demonstrated [12].

3 Pathophysiology

3.1 Intrauterine Bone Formation and Mineral Metabolism

A detailed discussion on the development of fetal and neonatal bones is beyond the scope of this chapter. It is described in great detail by Rigo et al. [13]. The process of bone formation and mineralization is influenced by multiple factors. An adequate supply of protein and energy is required for collagen matrix synthesis whereas an adequate calcium and phosphorus supply is necessary for bone mineralization [14]. Calciotropic hormones such as parathyroid hormone (PTH), parathyroid hormone related protein (PTHrP), 1,25 dihydroxyvitamin D (1,25 $(OH)_2D$) and calcitonin maintain calcium, phosphorus and magnesium homeostasis by modulating their physiological effects on each other and on target organs such as kidney, intestine and bone [15]. Other factors such as growth hormone, insulin-like growth factor 1, cortisol and tumor necrosis factor (TNF) can also affect secretion or function of calciotropic hormones and eventually mineral homeostasis [13, 16, 17].

The fetus receives most of the essential nutrients through placenta. Calcium is transported across placenta by high affinity calcium pump. PTHrP and PTH facilitate the transplacental transport of calcium and magnesium to the fetus [18, 19]. PTH stimulates 25 (OH) D to bind to placental calcium receptor to promote calcium transport to the fetus. PTH also increases the phosphorus flux. PTHrP regulates foetal bone growth by stimulating foetal growth plate chondrocytes and cartilage specific proteoglycans that modulate bone mineralization [20]. The net effect of the placental active transport and hormonal influences makes the fetus relatively hypercalcemic in comparison with its mother [18]. This relative hypercalcemia results in high fetal calcitonin levels and consequently reduces the biological activity of osteoclasts. Thus bone remodeling in the fetus favors bone formation [19].

3.2 Vitamin D Metabolism and its Role in Bone Development

The generic term "Vitamin D" denotes the precursors and metabolites of the vitamin including ergocalciferol (vitamin D2), cholecalciferol (vitamin D3), 25-Hydroxyvitamin D (25 (OH) D)—the most abundant metabolite in the circulation and an useful index of vitamin D reserve, and the active hormone 1,25 $(OH)_2$ D (calcitriol) formed after further hydroxylation of 25 (OH) D in the kidneys [17, 21]. The fetus receives vitamin D from the placenta. 25-Hydroxyvitamin D [25 (OH) D] crosses placenta and gets hydroxylated to 1,25 $(OH)_2$ D in the fetal kidney [19]. The process of renal hydroxylation of vitamin D develops by 26 weeks of gestation [22].

Calcitriol is active in bone remodeling process and calcium homeostasis. It remains unclear whether fetal or maternal vitamin D directly influences neonatal or childhood bone mass. Animal studies have reported that offspring of vitamin D deficient rats or 1-hydroxylase null pigs have normal skeletal length, morphology and bone mineral contents [23–25]. Additionally, placental transfer of calcium has been shown to be adequate in absence of vitamin D [26, 27]. Collectively these studies indicate that vitamin D or its receptor are not essential for fetal calcium homeostasis, skeletal development, and mineralization [20]. However an observational study has reported that maternal serum vitamin D levels under 27.5 mmol/L was associated with lower bone mineral content in offspring at 9 years of age [28]. Confounding factors such as maternal obesity, poor diet and poor antenatal care that may influence childhood bone mineral content were unaccounted for in this study. Other investigators have also hypothesised that apart from low birth weight and length, maternal low vitamin D levels modulate intrauterine bone mineral acquisition and program childhood peak bone mass and the risk of fractures in osteoporotic bones in adult life [29]. This hypothesis has led to the recommendation of vitamin D supplementation in mothers with serum 25(OH) D level under 25 nmol/L [21, 29, 30]. It is important to note that there is no high quality evidence to support this recommendation. Supplementation of vitamin D in excess amounts is not without adverse effect, as it is a fat-soluble vitamin that can be stored in body for a long time [31].

3.3 Postnatal Bone Mineralization in Preterm Infants

Fetal skeletal mineralization occurs late in pregnancy. Between 24 weeks of gestation and term, the fetus accrues approximately 80 % of calcium, phosphorus and magnesium [1]. Preterm infants therefore miss in part or completely the period of greatest mineral accretion. They are also susceptible to hypocalcaemia because of abrupt stoppage of transplacental mineral supply after umbilical cord clamping. The relatively high plasma calcitonin levels and an immature response to increase in plasma PTH concentration also increases the risk of hypocalcaemia [32]. Together these factors put newborn infants in a state favoring bone resorption rather than deposition [33]. The situation is further compounded by the difficulties in ensuring an adequate mineral intake during the neonatal period particularly in the smallest, and the sickest infants [34].

Postnatal bone mineralization needs adequate levels of minerals such as calcium and phosphorus and vitamin D. Dietary calcium is absorbed in the small intestine by both active and passive transports. Vitamin D is essential for active absorption of calcium [21]. Other factors affecting calcium absorption in preterm infants include the source of calcium (human milk or preterm formula), type of calcium salt, and the amount and type of fat in the diet. Infants fed on exclusive human milk absorb 60 % of the total dietary calcium [35]. Up to 80–90 % of the dietary phosphorus is absorbed through the intestines and the process of absorption is independent of vitamin D. The absolute amount of dietary phosphorus and relative concentrations of calcium

and phosphorus influence the net phosphorus absorption. An excess concentration of either one reduces the absorption of the other [35].

Gastrointestinal conditions such as necrotising enterocolitis (NEC) and short bowel syndrome can cause restricted enteral feeding, prolonged PN and reduced absorption of minerals, predisposing the preterm infant to MBD [21].

Renal immaturity of preterm neonates plays an important role in the pathogenesis of MBD. In adults and children almost all-urinary Ca is reabsorbed through the renal tubule. However this reabsorption does not occur in preterm infants who have a tendency to excrete high amount of calcium through urine [35]. Preterm infants also have low urinary threshold for phosphate excretion, even in presence of low serum levels. The hypercalciuria is increased by concomitant hypophosphatemia [36].

Associated comorbidities such as bronchopulmonary dysplasia (BPD) can increase the risk of MBD in preterm infants. Infants with BPD are often fluid restricted and have higher energy expenditure. As a result they have an inadequate supply of nutrients required for bone growth. Infants treated with loop diuretics (e.g., Furosemide) and caffeine have increased urinary calcium losses [37]. The use of corticosteroids in the treatment of BPD has been associated with increased MBD. In vitro studies have shown that corticosteroids can suppress human osteoblastic cell function and DNA synthesis [38]. Infants treated with dexamethasone are reported to have low bone mineral content as measured by Dual Energy X ray Absorptiometry (DEXA) [39]. Dexamethasone treatment for BPD has also been associated with reduced markers for bone formation (Bone specific alkaline phosphatase and osteocalcin) and increased markers for bone resorption (urinary deoxypyridinoline) [40] (Table 6.1, 6.2) (Fig. 6.1, 6.2).

4 Mechanical Factors Contributing to MBD

Mechanical factors play an important role in fetal mineral accretion. The regular fetal kicks against the uterine wall represent an intrauterine form of resistance training that increases osteoblast activity and helps in bone growth and remodeling [41]. Several studies have demonstrated that physical activity increases bone density in children, adolescents and adults [42–44]. Lack of activity is known to result in bone resorption and decreased bone mineral density [45, 46]. The prolonged period of hospitalization of preterm infants without physical stimulation may contribute to bone demineralization. The preterm infant is usually hypotonic and its spontaneous movements do not meet any resistance as in utero against uterine wall. This leads to less bone loading and less new bone formation [41]. Congenital neuromuscular disorders, sepsis and use of paralytic agents are often associated with prolonged periods of immobility resulting in bone resorption, hypercalciuria, and demineralization [47].

Table 6.1 Risk factors for MBD of prematurity

Gestational age under 28 weeks
Very low birth weight (birth weight under 1,500 g)
Feeding practices
Delayed feeding/restricted feeds
Unfortified human milk
Inadequate dietary supplements
Medications
Steroids
Diuretics
Methylxanthines
Lack of mechanical stimulation
Sedation/paralysis
Sepsis
Syndromic causes
Spina bifida
Arthrogryposis
Gastrointestinal/surgical conditions requiring prolonged parenteral nutrition
Necrotizing enterocolitis
Short bowel syndrome
Congenital gastrointestinal tract anomalies
Maternal and placental pathologies
Intrauterine growth restriction
Chorioamnionitis
Preeclampsia
Magnesium sulphate therapy
Cholestasis
Bronchopulmonary dysplasia
Vitamin D deficiency

5 Clinical Features and Diagnosis

MBD is usually an asymptomatic disease. Clinical manifestations are often non-specific and appear between the 6th and 12th postnatal week [48]. Undiagnosed MBD can present with failure to wean off the ventilator and bone fractures [8, 49]. Sometimes it is incidentally detected on routine chest or abdominal radiographs.

There are no definitive diagnostic criteria for MBD in preterm infants. Osteopenia conventionally implies that there is reduced bone mass. Historically plain radiographs of long bones have been used to define the extent of MBD (Table 6.3) [50]. The interpretation of reduced bone mass on plain radiographs is very subjective [3, 51]. In 1994 the World Health Organisation established the criteria for diagnosis of osteopenia as reduced bone mineral density (BMD) below 1 standard deviation from normal [52]. Since the term osteopenia now has a specific quantitative definition, its general use in the context of MBD in preterm infants is best avoided. The diagnosis of MBD involves clinical signs, radiological findings, biochemical markers and measurement of bone mineral content (BMC).

There are no screening tests that have high sensitivity and specificity. Nevertheless serial screening of at risk infants is suggested by some authors. Screening starting as

Table 6.2 Biochemical markers for MBD in preterm infants

Biochemical Parameter	Changes in MBD	Advantages	Limitations
Serum ALP Bone ALP	Increased	Easy availability	Not specific/sensitive Poor correlation with mineralization
Serum Calcium	Normal, increased or decreased	Easy availability	Poor sensitivity and specificity
Serum Phosphorus	Decreased		
Urinary Ca and Ph	Decreased or Normal	Non invasive, easy to perform	Difficult to interpret
Urinary pyridinoline and deoxypyridinoline	Increased		Not widely available Marker of bone turnover than resorption
Procollagen type I C-terminal prepetide (PICP)	-	Indicates osteoblastic activity	Not widely available

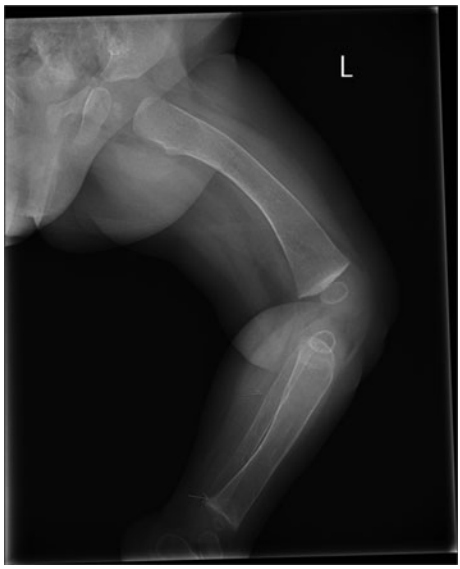

Fig. 6.1 X-ray of the lower limb at 12 weeks after birth in a preterm (gestation 28 weeks) infant with gastroschisis and chronic lung disease. Note the severe demineralization and cortical thinning of long bones. The infant received long term parenteral nutrition for short bowel syndrome, and required prolonged ventilation, and treatment with furosemide and dexamethasone

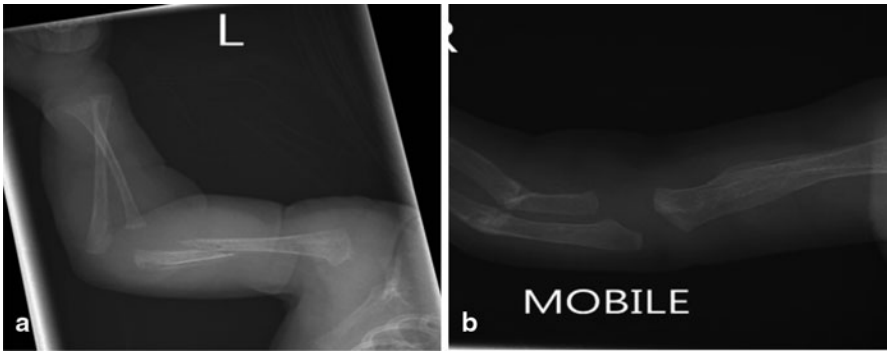

Fig. 6.2 (**a**) X-ray of the forearm bone in a preterm (gestation 26 weeks) infant with intestinal atresia requiring prolonged parenteral nutrition and chronic lung disease requiring treatment with furosemide and dexamethasone. Note demineralization of bone matrix and thin cortex along with fracture of humerus. (**b**) X-ray showing healing of the fracture and improved mineralization after 2 weeks of increasing oral intake and supplementation with calcium and phosphate

Table 6.3 Radiological grading of metabolic bone disease (MBD) in preterm infants [50]

Grade of MBD	X-ray changes
Normal (Grade 0)	Normal density of bone cortex
Grade 1	Thinning of cortex Increased submetaphyseal lucency
Grade 2	Irregularity and fraying of metaphyses Splaying and cupping: signs of rickets
Grade 3	Changes of rickets with bone fractures

early as 3 weeks of postnatal age in extremely preterm and low birth weight infants can help in the diagnosis of MBD in asymptomatic infants [53].

5.1 Biochemical Markers

There is no gold standard biochemical marker to diagnose MBD in preterm infants. Various serum and urinary parameters are used as indicators of MBD (Table 6.2). The typical findings include normal serum calcium; low serum phosphate and high serum alkaline phosphatase (ALP) concentrations. Increasing serum ALP levels have been associated with increased risk of MBD. Serum ALP value above 700 IU/L at 3 weeks postnatal age in ex preterm infants has been proposed as an indicator of MBD (sensitivity 73 % and specificity 74 %, positive likelihood ratio 2.79, negative likelihood ratio 0.369) [53]. The sensitivity and specificity of detection of MBD increased to 100 % and 97 %, respectively when high serum ALP values (above 900 IU/L) were combined with low serum inorganic phosphorus (under 1.8 mmol/mL) [54]. Alkaline phosphatase can come into serum from different sources like bone, liver and placenta. BALP, an isoenzyme of ALP originating from bones has been studied

as a more specific marker of bone turnover and demineralization [55]. However recent studies have showed that total ALP had a highly positive relation with BALP from 3 weeks to 17 weeks post-natal age and BALP provided no additional benefit in early detection of MBD [54]. Biochemical markers have been shown to have poor correlation with more specific radiological markers such as Dual-Energy X-ray absorptiometry (DEXA), Quantitative ultrasound and X ray of bones [54, 56–59].

Transient rise in serum parathyroid hormone (PTH) has been reported in response to hypocalcemia early in the course of MBD of prematurity [60]. It may be secondary to associated vitamin D deficiency and inadequate dietary intake of calcium. More studies are needed to know the exact incidence of secondary hyperparathyroidism in MBD of prematurity.

Urinary excretion of calcium and phosphate has been suggested as an indicator of bone mineral storage. A high urinary concentration of calcium and phosphorus (1–2 mmol/L of each) can indicate surplus of both minerals that may be enough for adequate bone mineralization [61]. However urinary calcium and phosphorus concentrations depend on a complex interaction between intake, absorption, retention, losses via faeces and sweat, as well as renal function and water balance. The low renal phosphate threshold in preterm infants results in urinary excretion of phosphate despite a low serum phosphate concentration [62]. These factors make interpretation of urinary phosphate excretion pattern even more difficult.

Urinary excretion of markers of bone turnover may be used to assess changes in the skeletal system. Degradation products of type-1 collagen, pyridinoline and deoxypyridinoline (DPD) can be excreted in excess quantity during periods of bone turnover [63]. However interpretation of these findings is difficult as they reflect either bone formation or bone resorption. There is no significant correlation between collagen cross-link excretion and bone mineral density [64]. The serum levels of C-terminal propeptide of type I collagen (PICP), which indicates osteoblast activity, have been proposed to be good surrogate markers for bone mineralization in preterm infants [38]. However there is lack of normative data in preterm infants.

5.2 Radiological Features

Early radiological appearance of MBD is characterized by reduced bone density of the metaphyses of long bones. With more severe disease, the changes become more pronounced, showing demineralization or frank osteomalacia and rickets with loss of zone of provisional calcification and fraying of metaphyses [50]. The evidence of osteopenia is best seen in areas of rapid bone growth such as the wrist and knee. However these areas are seldom available routinely for evaluation. It is therefore important to carefully inspect the proximal humerus and the hip while assessing chest and abdominal X-rays [65]. It is important to note that a significant amount of reduction (20–50 %) in bone mineral density is required to detect these changes on conventional X-rays [66, 67]. Dual Energy X-ray absorptiometry (DEXA) is a more specific and sensitive tool to evaluate bone mineralization in infants and young children [68, 69]. However it is not portable, involves increased radiation exposure

to the infant, and interpretation of the results are influenced by movement artifact [68–70]. Quantitative ultrasound (QS) has been used as a tool to assess bone heath in adults as well as in infants, by measuring the cortical thickness, elasticity and bone architecture. This modality has the advantage of being portable, low cost and not involving radiation exposure [57, 71]. QS can identify the changes in bone mineral accretion after preterm birth and subsequent increase in cortical porosity and bone, but has no correlation with markers of bone resorption [72]. In absence of data supporting its ability to predict changes of MBD or fracture in preterm infants, QS continues to remains a research tool [58, 71].

6 Prevention of MBD of Prematurity

6.1 Supplementation of Minerals and Vitamins

Postnatal supplementation of minerals and vitamins has an important role in prevention of MBD of prematurity. Current recommendations for daily requirements are as follows: (1) **Calcium** (mg/kg/day): Enteral: 120–140, Parenteral: 60–90 (2) **Phosphate** (mg/kg/day): Enteral: 60–90, Parenteral: 40–70 (3) **Vitamin D** (IU/day): Enteral: 800–1,000, Parenteral: 400 [73, 74]

6.1.1 Supplementation of Minerals Through PN

Many extremely preterm VLBW infants are solely dependent on PN for mineral and energy supply during the first few days after birth, as oral feeds are not tolerated well. Due to their high requirements of minerals [74], neither the standard PN solutions nor the enteral intake can safely deliver the amounts of calcium and phosphorus necessary to match intrauterine accretion in extremely preterm infants [75, 76]. Increasing concentrations of calcium and phosphate in PN can result in calcium phosphate precipitation [77]. The presence of large calcium phosphate crystals may occlude pulmonary arterioles leading to life threatening consequences such as acute respiratory distress, pulmonary embolus, interstitial pneumonitis, thrombosis and catheter occlusion [77–79]. The solubility of calcium and phosphorus is affected by many factors including the concentration of calcium and phosphorus itself, glucose; and amino acid concentration and temperature and pH of the PN solution [75, 80]. The higher the concentration of glucose and amino acids in the PN, the greater the amount of calcium and phosphorus that can be mixed in the solutions without causing precipitation [81, 82]. Addition of L-cysteine hydrochloride can reduce pH of the PN solution, which in turn can promote compatibility of calcium and phosphorus [81]. Formulations of PN solution containing glycerophoshate and monobasic phosphate allow delivery of greater amounts of calcium and phosphorus without precipitation [76, 80, 83]. Addition of calcium and phosphorus in a ratio of 1.7:1 in parenteral solutions has been suggested for balanced retention of both minerals [84, 85].

6.1.2 Supplementation of Minerals Through Enteral Feeding

Supplementation of minerals through PN cannot achieve optimal calcium and phosphorus retention. Supplementation of minerals through enteral feeding is therefore of paramount importance. Once oral feeds are started, these infants can get the nutrients from either mother's milk or pasteurized donor human milk or from preterm formula. Historically these mineral supplements have been provided by direct addition to breast milk, or via preterm formula or fortified breast milk.

Unfortified human milk is known to have low nutritional value for extremely preterm infant, which includes low calcium and phosphorus levels [2, 86–88]. The exclusive feeding of unfortified human milk in extremely preterm infants has been associated with poor rates of growth and nutritional deficiencies [89]. Compared to preterm formula, infants fed with unfortified breast milk have increased incidence of MBD [88, 90]. Human milk fortification is therefore very commonly undertaken to provide extra supply of the much-needed nutrients to this high-risk population of neonates. Studies have shown that fortification of preterm human milk with calcium, phosphorus and protein improves the bone mineralization and growth comparable to preterm formula in low birth weight infants. However insufficient data is available for evaluating long term neurodevelopment and growth [91, 92] or for assessing complications of MBD such as fractures.

Even though human milk contains less calcium and phosphorus than formula, it is postulated to have higher bioavailability [93]. In preterm infants fed human milk, 60–70 % of calcium is absorbed compared to 35–60 % for preterm formulas [35]. Still a significant positive association was found between the neonatal intake of human milk in preterm infants and whole-body skeletal size and bone mineral content at 5 years of age. This apparent beneficial effect of human milk is hypothesized to be due to nonnutritive factors in human milk [12].

The dietary absorption of calcium can be variable depending on the availability of vitamin D and dietary sources. Daily oral vitamin D supplementation (800–1,000 IU/day) [73] is recommended in all preterm infants, as they are deficient of it. Furthermore, breast milk is a poor source of vitamin D [94], which plays an important role in maintaining bone mineral-hormonal milieu. The type of salt used in calcium supplements also affects its absorption (e.g., calcium glycerophosphate used in human milk fortifier has high calcium retention rate up to 90 mg/kg/day) [14].

Direct addition of calcium and phosphorus to milk is not preferable as they can precipitate and may result in decreased absorption. Preterm formulas containing high amount of calcium can result in increased faecal calcium excretion, increased stool hardness and decreased gastrointestinal transit time resulting in increased risk of NEC [35].

Minimal enteral feedings (MEF), i.e., feeds between 12 and 24 mL/kg/day, started as early as day 1 of life, have been shown to improve calcium retention, lower alkaline phosphatase levels and increase bone mineral content, compared to infants who were not fed or received only PN [95–97]. Even though MEF increases bone mineral content in preterm infants, they remain osteopenic compared to term infants. Further

research is needed to establish the safety of aggressive MEF in critically sick preterm infants and its benefits with respect to long term bone growth and mineralization [96].

6.2 Physical Activity Programs

Minimal handling facilitates stability and minimizes stress in hospitalized preterm infants. However the resultant inactivity has been proposed to contribute to suboptimal stimulation of bone metabolism [34]. Physical activity programs (PAP) have been shown to reduce bone loss and risk of osteoporotic fractures in adults and children [44, 98, 99]. Based on these benefits PAP have been adopted to improve bone mineral content in preterm infants. Systematic PAP consists of extension and flexion, range-of-motion exercises of both the infant's upper and lower limbs, administered for several minutes at a time several times a week for at least two weeks [68, 100–103]. A systematic review of randomised and quasi-randomised trials has assessed whether PAP improves bone mineralization and growth and reduce the risk of fractures in preterm infants [104]. Six small and single centre trials enrolling 169 preterm infants (gestation: 26–34 weeks) were included in this review. All evaluated daily physical activity for 3.5–4 weeks during initial hospitalization. The methodological quality and reporting of all trials was poor. Two trials ($N = 55$) reported moderate short-term benefits on bone mineralization at completion of the PAP. The only trial ($N = 20$) assessing long-term effects showed no effect of PAP during initial hospitalization on bone mineralization at 12 months corrected age. Meta-analysis from three trials ($N = 78$) demonstrated an effect of PAP on daily weight gain (WMD 2.77 g/kg/day, 95 % CI 1.62, 3.92). Two trials reported no effect on linear growth or head growth during the PAP. The authors concluded that the **c**urrent evidence does not support the routine PAP in preterm infants, and further trials recruiting infants with a high baseline risk of osteopenia and assessing adverse events (e.g., fractures, skeletal deformities), and the effects of nutrient intakes are necessary [104].

7 Treatment of MBD in Preterm Infants

Optimisation of nutritional intake through PN and/or enteral feeds is paramount in the treatment of established MBD in preterm infants. If mineral intake is inadequate in spite of fortification, an additional oral supplementation of calcium and phosphate salts is recommended. Monitoring serum levels of phosphorus, calcium and ALP is necessary to guide replacement of calcium and phosphorus. Review of medications that predispose to MBD (steroids, diuretics and caffeine) is warranted so that their doses can be altered. Ongoing surveillance for early detection of fractures and their standardized management in the form of analgesia, splinting and serial X-rays should be considered in all cases of MBD with fractures.

8 Fractures in MBD

Fractures have been reported to occur at variable frequency (1.2–30 %) in preterm infants. The incidence of fractures is reported to be higher in VLBW infants [8, 49]. It is important to note that such reports are based on retrospective reviews of X rays done for clinical suspicion of a fracture or following incidental detection. The true incidence of fractures in preterm infants thus remains unknown. Rib fractures are commonest amongst all non-traumatic neonatal fractures, most likely due to the higher frequency of chest X rays as opposed to other radiographs [49, 105]. Rib fractures have been most commonly associated with infants with MBD who were extremely sick, septic, ventilated and required high percentage of oxygen [105]. Fractures associated with MBD are usually asymptomatic and except for simple splinting, specific orthopaedic interventions are not required. This is also partly related to the presence of satisfactory callus formation at the time of diagnosis and the increased risk of bone resorption with immobilisation.

Prospective long-term follow up of fractures in VLBW infants with MBD has shown complete resolution after 6 months with no residual skeletal deformities. However fractures sustained post discharge from the neonatal intensive care unit pose a difficult diagnostic dilemma as to whether they are related to MBD or secondary to non-accidental injury (NAI). Fractures involving multiple sites and ribs, especially posterior ribs, are classically viewed as highly suspicious of Non Accidental Injury (NAI) [106–108]. Posterior rib fractures from NAI have been thought to be associated with a shaking injury where infants are squeezed [106, 109, 110]. They have been reported to have positive predictive value as high as 95 % to indicate NAI [107]. However in preterm babies with MBD, posterior ribs are the commonest sites for bone fractures. In babies with MBD, rib and long bone fractures can be noted in first six months of infantile life and can occur post discharge from Neonatal Intensive Care Units [105]. Ignorance of this knowledge can have medico legal repercussions as attribution the rib fractures to NAI without consideration of pathologies like congenital rickets, MBD and maternal vitamin D deficiency has recently led to wrongful persecution of parents for child abuse [111]. The legal fraternity has called for further research into different aspects of fractures, vitamin D deficiency in mother and babies under 6 months of age [112].

Like calcium and phosphorus, Copper deficiency can present as metabolic bone disease in VLBW infants especially those requiring long-term parenteral nutrition. The radiographic appearances of long bones in Copper deficient infants can mimic those seen in infants with NAI [113].

9 Nutritional Support for Infants with MBD After Hospital Discharge

The ideal duration of mineral supplementation in preterm infants is currently unknown. Considering the underlying pathophysiology of MBD, the supplementation of minerals till term gestation or weight comparable to normal term infant is logical

[35]. The supportive data for this strategy is based on the facts that (1) maximum intrauterine mineral accretion occurs from the third trimester till term [1] (2) complications associated with MBD including fractures usually occur at 2–4 months in VLBW infants [8, 9] (3) mineral supplementation for shorter duration (6–8 weeks) has not shown to prevent MBD and fractures in VLBW infants [8]. There are no published studies assessing the benefits of longer periods of supplemental nutrition in the preterm infant after discharge from the hospital. Kurl et al. have reported that extremely preterm infants exclusively breast fed after discharge from hospital had low bone mineral content, but they had normal catch up growth and weight gain [114]. Apart from being observational, this study has other limitations including the fact that the milk intake was not quantitated, and postnatal use of steroid was very prevalent. Currently there is no sufficient evidence to continue supplementation of minerals in ex preterm infants that are exclusively breastfed, after hospital discharge.

10 Long-Term Complications

10.1 Adult Height

Preterm infants are known to have lesser than normal height as well as reduced bone mineral density in their adulthood compared to population references. Suboptimal early nutrition and energy intake has been considered as preventable cause for this finding. However dietary interventions in the form of early enteral feeding in preterm infants have shown to have no significant effect on their peak bone mass or bone turnover as adults [115]. It is likely that prematurity may influence final height and bone mass by another unknown mechanism.

10.2 Osteoporosis in Adulthood

There is increasing focus on optimizing the accretion of bone mass during infancy and childhood to maximize the peak bone mass attained at skeletal maturity [116]. This has been proposed as a strategy for reducing later osteoporosis in adulthood. Preterm infants have been followed up to test this hypothesis as they are at the risk of MBD and have suboptimal bone growth in infancy and early childhood. However studies suggest that preterm infants may eventually attain a level of bone mineralization in proportion to their body size [114, 117].

11 Summary and Future Research

Considering that the clinical manifestations of MBD in preterm infants appear very late and can cause significant morbidity, it is important to identify it early by regular screening and having a high index of suspicion. Early detection and treatment

Table 6.4 Guidelines for managing metabolic bone disease (MBD) in preterm infants

Screening
Born at less than 28 weeks or with birth weight less than 1,500 g
Infants requiring parenteral nutrition for more than 4 weeks
Infants with prolonged courses of diuretics or steroids
When
Start at 4 weeks of age and repeat monthly (if normal results) or fortnightly if results are abnormal until discharge
Measure
Alkaline phosphatase, serum phosphate and serum calcium
Consider diagnosis of MBD
Serum ALP greater than 900 IU/L and serum phosphate less than 1.8 mmol/L
Treatment
Optimize calcium and phosphate intake by fortification of feeds and/or additional phosphate, calcium and vitamin D supplements
Monitoring
Measure serum ALP, Calcium and phosphate levels weekly till normal levels achieved
Urinary calcium and phosphate measures can be helpful to ensure adequate supplements are prescribed

of MBD is especially more important in extremely preterm infants born at gestational age of 23–26 weeks, who are at the highest risk for the condition (Table 6.4). MBD appears to be a self-resolving disease in preterm infants, but a period of demineralization associated with the condition is not acceptable. Although the potential long-term consequences of MBD on attainment of peak bone mass are not clear at present, there is definite merit in preventing its short-term consequences to avoid fractures and improve linear growth.

The role of maternal Vitamin D in bone development and mineralization during fetal and neonatal life and beyond childhood needs to be explored further. The hypothesis suggesting its influence on intrauterine programming of childhood bone mass also needs to be studied. Evidence indicates that systematic physical activity programs may be beneficial in prevention of MBD. Definitive large randomised controlled trials are necessary to evaluate their safety and efficacy and long-term outcomes such as fractures and skeletal deformities.

References

1. Ryan S et al (1988) Mineral accretion in the human fetus. Arch Dis Child 63(7):799–808
2. Ryan S (1996) Nutritional aspects of metabolic bone disease in the newborn. Arch Dis Child Fetal Neonatal Ed 74(2):F145–F148
3. Griscom NT, Jaramillo D (2000) "Osteoporosis," "osteomalacia," and "osteopenia": proper terminology in childhood. AJR Am J Roentgenol 175(1):268–269
4. Backstrom MC, Kuusela AL, Maki R (1996) Metabolic bone disease of prematurity. Ann Med 28(4):275–282
5. McIntosh N, Livesey A, Brooke OG (1982) Plasma 25-hydroxyvitamin D and rickets in infants of extremely low birthweight. Arch Dis Child 57(11):848–850

6. Caksen H et al (2002) Reports of osteopenia/rickets of prematurity are on the increase because of improved survival rates of low birthweight infants. J Emerg Med 23(3):305–306
7. Thattakkat K, Matthews YY (2009) A clinical study of spectrum of metabolic bone disease in preterm infants admitted to the special care baby unit over a 14 year period. In: Paediatric research society autumn meeting, Churchill Livingstone, Newcastle upon Tyne, United Kingdom, p 253–254
8. Koo WW et al (1989) Fractures and rickets in very low birth weight infants: conservative management and outcome. J Pediatr Orthop 9(3):326–330
9. Lyon AJ et al (1987) Radiological rickets in extremely low birthweight infants. Pediatr Radiol 17(1):56–58
10. Glasgow JF, Thomas PS (1977) Rachitic respiratory distress in small preterm infants. Arch Dis Child 52(4):268–273
11. Fewtrell MS et al (2000) Neonatal factors predicting childhood height in preterm infants: evidence for a persisting effect of early metabolic bone disease? J Pediatr 137(5):668–673
12. Fewtrell MS (2011) Does early nutrition program later bone health in preterm infants? Am J Clin Nutr 94(6 Suppl):1870S–1873S
13. Rigo J et al (2000) Bone mineral metabolism in the micropremie. Clin Perinatol 27(1):147–170
14. Rigo J, Senterre J (2006) Nutritional needs of premature infants: current issues. J Pediatr 149:S80–S88
15. Simmonds CS, Kovacs CS (2010) Role of parathyroid hormone (PTH) and PTH-related protein (PTHrP) in regulating mineral homeostasis during fetal development. Crit Rev Eukaryot Gene Expr 20(3):235–273
16. Rigo J, De Curtis M (2006) Disorders of calcium, phosphorus, and magnesium metabolism. In: Martin RJ, Fanaroff AA, Walsh MC (eds) Fanaroff and Martin's neonatal-perinatal medicine: diseases of the fetus and infant. Mosby Elsevier, Philadelphia, PA, p 1491–1453
17. Kovacs CS, Kronenberg HM (1997) Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. Endocr Rev 18(6):832–872
18. Mitchell DM, Juppner H (2010) Regulation of calcium homeostasis and bone metabolism in the fetus and neonate. Curr Opin Endocrinol Diabetes Obes 17(1):25–30
19. Care AD (1996) Unique aspects of calcium and vitamin D metabolism in the placenta and fetus. In: Gluckman PD, Heyman MA (eds) Bone and cartilage. Pediatrics and perinatology: the scientific basis. Arnold, London, p 540–542
20. Kovacs CS (2011) Bone development in the fetus and neonate: role of the calciotropic hormones. Curr Osteoporos Rep 9(4):274–283
21. Misra M et al (2008) Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics 122(2):398–417
22. Salle BL et al (2000) Perinatal metabolism of vitamin D. Am J Clin Nutr 71(5 Suppl):1317S–1324S
23. Miller SC et al (1983) Studies on the role of vitamin D in early skeletal development, mineralization, and growth in rats. Calcif Tissue Int 35(4–5):455–460
24. Halloran BP, De Luca HF (1981) Effect of vitamin D deficiency on skeletal development during early growth in the rat. Arch Biochem Biophys 209(1):7–14
25. Brommage R, De Luca HF (1984) Placental transport of calcium and phosphorus is not regulated by vitamin D. Am J Physiol 246(4 Pt 2):F526–F529
26. Kovacs CS et al (2005) The vitamin D receptor is not required for fetal mineral homeostasis or for the regulation of placental calcium transfer in mice. Am J Physiol Endocrinol Metab 289(1):E133–E144
27. Glazier JD, Mawer EB, Sibley CP (1995) Calbindin-D9K gene expression in rat chorioallantoic placenta is not regulated by 1,25-dihydroxyvitamin D3. Pediatr Res 37(6):720–725
28. Javaid MK et al (2006) Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 367(9504):36–43
29. Cooper C et al (2006) Review: developmental origins of osteoporotic fracture. Osteoporos Int 17(3):337–347

30. Munns C et al (2006) Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: a consensus statement. Med J Aust 185(5):268–272
31. McCloskey KM et al (2011) Neonatal vitamin D supplementation: are the protocols getting ahead of the evidence? Med J Aust 195(11–12):661
32. Hsu SC, Levine MA (2004) Perinatal calcium metabolism: physiology and pathophysiology. Semin Neonatol 9(1):23–36
33. Kruse K, Kustermann W (1987) Evidence for transient peripheral resistance to parathyroid hormone in premature infants. Acta Paediatr Scand 76(1):115–118
34. Rauch F, Schoenau E (2002) Skeletal development in premature infants: a review of bone physiology beyond nutritional aspects. Arch Dis Child Fetal Neonatal Ed 86(2):F82–F85
35. Demarini S (2005) Calcium and phosphorus nutrition in preterm infants. Acta Paediatr Suppl 94(449):87–92
36. Jacinto JS et al (1988) Renal calcification incidence in very low birth weight infants. Pediatrics 81(1):31–35
37. Zanardo V et al (1995) Methylxanthines increase renal calcium excretion in preterm infants. Biol Neonate 68(3):169–174
38. Crofton PM et al (1999) Bone and collagen markers in preterm infants: relationship with growth and bone mineral content over the first 10 weeks of life. Pediatr Res 46(5):581–587
39. Ward WE et al (1999) Bone metabolism and circulating IGF-I and IGFBPs in dexamethasone-treated preterm infants. Early Hum Dev 56(2–3):127–141
40. Ng PC et al (2002) Changes in markers of bone metabolism during dexamethasone treatment for chronic lung disease in preterm infants. Arch Dis Child Fetal Neonatal Ed **86**(1):F49–F54
41. Frost HM, Schonau E (2000) The "muscle-bone unit" in children and adolescents: a 2000 overview. J Pediatr Endocrinol Metab 13(6):571–590
42. Eliakim A et al (1993) Evidence for increased bone formation following a brief endurance-type training intervention in adolescent males. J Bone Miner Res 8:127–132
43. Myburgh KH (1998) Exercise and peak bone mass: an update. S Afr J Sport 5:3–9
44. Slemenda CW et al (1991) Role of physical activity in the development of skeletal mass in children. J Bone Miner Res 6:1227–1233
45. Mazess RB, Whedon GD (1983) Immobilization and bone. Calcif Tissue Int 35:265–267
46. Rodriguez JI et al (1988) Changes in the long bones due to fetal immobility caused by neuromuscular disease: a radiographic and histological study. J Bone Joint Surg Am 70:1052–1060
47. Eliakim A et al (2002) Spontaneous activity in premature infant affects bone growth. J Perinatol 22(8):650–652
48. Mayne PD, Kovar IZ (1991) Calcium and phosphorus metabolism in the premature infant. Ann Clin Biochem 28(Pt 2):131–142
49. Amir J et al (1988) Fractures in premature infants. J Pediatr Orthop 8(1):41–44
50. Koo WW et al (1982) Skeletal changes in preterm infants. Arch Dis Child 57(6):447–452
51. Hall FM (1999) Demise of generic terms "osteoporosis" and "osteopenia" in radiology reporting. AJR Am J Roentgenol 173(4):1127–1128
52. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis (1994) Report of a WHO Study Group. World Health Organ Tech Rep Ser 843:1–129
53. Hung YL et al (2011) Serial measurements of serum alkaline phosphatase for early prediction of osteopaenia in preterm infants. J Paediatr Child Health 47(3):134–139
54. Backstrom MC et al (2000) Bone isoenzyme of serum alkaline phosphatase and serum inorganic phosphate in metabolic bone disease of prematurity. Acta Paediatr 89(7):867–873
55. Crofton PM, Hume R (1987) Alkaline phosphatase isoenzymes in the plasma of preterm and term infants: serial measurements and clinical correlations. Clin Chem 33(10):1783–1787
56. Koo WW, Succop P, Hambidge KM (1989) Serum alkaline phosphatase and serum zinc concentrations in preterm infants with rickets and fractures. Am J Dis Child 143(11):1342–1345
57. Tomlinson C et al (2006) Longitudinal changes in bone health as assessed by the speed of sound in very low birth weight preterm infants. J Pediatr 148(4):450–455

58. Fewtrell MS et al (2008) Quantitative ultrasound (QUS): a useful tool for monitoring bone health in preterm infants? Acta Paediatr 97(12):1625–1630
59. Ashmeade T et al (2007) Longitudinal measurements of bone status in preterm infants. J Pediatr Endocrinol Metab 20(3):415–424
60. Lothe A, Sinn J, Stone M (2011) Metabolic bone disease of prematurity and secondary hyperparathyroidism. J Paediatr Child Health 47:550–553
61. Pohlandt F (1994) Prevention of postnatal bone demineralization in very low-birth-weight infants by individually monitored supplementation with calcium and phosphorus. Pediatr Res 35(1):125–129
62. Hellstern G, Poschl J, Linderkamp O (2003) Renal phosphate handling of premature infants of 23–25 weeks gestational age. Pediatr Nephrol 18(8):756–758
63. Tsukahara H et al (1999) Assessment of bone turnover in term and preterm newborns at birth: measurement of urinary collagen crosslink excretion. Early Hum Dev 53(3):185–191
64. Tsukahara H et al (1998) High-turnover osteopenia in preterm infants: determination of urinary pyridinium cross-links of collagen. Metabolism 47(3):333–335
65. Done SL (2012) Fetal and neonatal bone health: update on bone growth and manifestations in health and disease. Pediatr Radiol 42(Suppl 1):S158–S176
66. Lachmann E (1955) Osteoporosis: the potentialities and limitations of its Roentgenologic diagnosis. Am J Roentgenol Radium Ther 74:712–715
67. Adams J (2009) Imaging evaluation of osteoporosis. In: Weitzman BN (ed) Imaging of arthritis and metabolic bone diseases. Elsevier, Philadelphia, p 608
68. Eliakim A, Nemet D (2005) Osteopenia of prematurity—the role of exercise in prevention and treatment. Pediatr Endocrinol Rev 2(4):675–682
69. Eriksson S, Mellstrom D, Strandvik B (2009) Volumetric bone mineral density is an important tool when interpreting bone mineralization in healthy children. Acta Paediatr 98(2):374–349
70. Visser F, Sprij AJ, Brus F (2012) The validity of biochemical markers in metabolic bone disease in preterm infants: a systematic review. Acta Paediatr 101(6):562–568
71. McDevitt H et al (2007) Changes in quantitative ultrasound in infants born at less than 32 weeks' gestation over the first 2 years of life: influence of clinical and biochemical changes. Calcif Tissue Int 81(4):263–269
72. Litmanovitz I et al (2007) Assisted exercise and bone strength in preterm infants. Calcif Tissue Int 80(1):39–43
73. Agostoni C et al (2010) Enteral nutrient supply for preterm infants: commentary from the European society of paediatric gastroenterology, hepatology and nutrition committee on nutrition. J Pediatr Gastroenterol Nutr 50(1):85–91
74. Atkinson SA, Tsang R (2005) Recommended reasonable range for parenteral and enteral nutritio for preterm infants during hospitalisation. In: Tsang R et al (eds) Nutrition of the preterm infant: scientific basis and practical guidelines. Digital Educational Publishing, Cincinati, p 245–275
75. Atkinson SA (1994) Calcium and phosporus needs of premature infants. Nutrition 10:66–68
76. Koo WW, Steichen JJ (1998) Osteopenia and rickets of prematurity. In: Polin RA, Fox WW (eds) Fetal and neonatal physiology. WB Saunders, Philadelphia, p 2235–2249
77. Stennett DJ et al (1988) Precipitate analysis from an indwelling total parenteral nutrition catheter. JPEN J Parenter Enteral Nutr 12(1):88–92
78. Flurkey H (1994) A case presentation: precipitate in the central venous line: what went wrong? Neonatal Netw 13(1):51–55
79. Lumpkin MM (1994) Safety alert: hazards of precipitation associated with parenteral nutrition. Am J Hosp Pharm 51(11):1427–1478
80. Hanning RM, Atkinson SA, Whyte RK (1991) Efficacy of calcium glycerophosphate vs conventional mineral salts for total parenteral nutrition in low-birth-weight infants: a randomized clinical trial. Am J Clin Nutr 54(5):903–908
81. Allwood MC, Kearney MC (1998) Compatibility and stability of additives in parenteral nutrition admixtures. Nutrition 14(9):697–706

82. Eggert LD et al (1982) Calcium and phosphorus compatibility in parental nutrition solutions for neonates. Am J Hosp Pharm 39(1):49–53
83. Raupp P et al (1991) Glycero- vs glucose-phosphate in parenteral nutrition of premature infants: a comparative in vitro evaluation of calcium/phosphorus compatibility. JPEN J Parenter Enteral Nutr 15(4):469–473
84. Pelegano JF et al (1991) Effect of calcium/phosphorus ratio on mineral retention in parenterally fed premature infants. J Pediatr Gastroenterol Nutr 12(3):351–355
85. Pelegano JF et al (1989) Simultaneous infusion of calcium and phosphorus in parenteral nutrition for premature infants: use of physiologic calcium/phosphorus ratio. J Pediatr 114(1):115–119
86. Lemons JA et al (1982) Differences in the composition of preterm and term human milk during early lactation. Pediatr Res 16:113–117
87. Ziegler EE et al (1976) Body composition of reference fetus. Growth 40:329–341
88. Schanler RJ (2001) The use of human milk for premature infants. Pediatr Clin North Am 48(1):207–219
89. Atkinson SA, Bryan MH, Anderson GH (1981) Human milk feeding in premature infants: protein, fat and carbohydrate balances in first two weeks of life. J Pediatr 99:617
90. Lucas A et al (1989) High alkaline phosphatase activity and growth in preterm neonates. Arch Dis Child 64(7 Spec No):902–909
91. Faerk J et al (2000) Diet and bone mineral content at term in premature infants. Pediatr Res 47:148–156
92. Kuschel CA, Harding JE (2004) Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev (1): CD000343. DOI:10.1002/14651858.pub2.
93. Hillman LS (1990) Mineral and vitamin D adequacy in infants fed human milk or formula between 6 and 12 months of age. J Pediatr 117(2 Pt 2):S134–S142
94. Kovacs CS (2008) Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr 88(2):520S–528S
95. Schanler RJ et al (1999) Feeding strategies for premature infants: randomized trial of gastrointestinal priming and tube-feeding method. Pediatrics 103(2):434–439
96. Weiler HA et al (2006) Minimal enteral feeding within 3 days of birth in prematurely born infants with birth weight $\leq$ 1,200 g improves bone mass by term age. Am J Clin Nutr 83(1):155–162
97. Dunn L et al (1988) Beneficial effects of early hypocaloric enteral feeding on neonatal gastrointestinal function: preliminary report of a randomized trial. J Pediatr 112(4):622–629
98. Bonaiuti D et al (2002) Exercise for preventing and treating osteoporosis in postmenopausal women. Cochrane Database Syst Rev (3):CD000333
99. Heinonen A et al (1996) Randomised controlled trial of effect of high-impact exercise on selected risk factors for osteoporotic fractures. Lancet 348(9038):1343–1347
100. Moyer-Mileur L et al (1995) Effect of physical activity on bone mineralization in premature infants. J Pediatr 127(4):620–625
101. Moyer-Mileur LJ et al (2000) Daily physical activity program increases bone mineralization and growth in preterm very low birth weight infants. Pediatrics 106(5):1088–1092
102. Chen HL et al (2010) Assisted exercise improves bone strength in very low birthweight infants by bone quantitative ultrasound. J Paediatr Child Health 46(11):653–659
103. Specker BL, Mulligan L, Ho M (1999) Longitudinal study of calcium intake, physical activity, and bone mineral content in infants 6–18 months of age. J Bone Miner Res 14(4):569–576
104. Schulzke SM, Trachsel D, Patole SK (2007) Physical activity programs for promoting bone mineralization and growth in preterm infants. Cochrane Database Syst Rev (2):CD005387
105. Wei C et al (2012) Fractures in a tertiary Neonatal Intensive Care Unit in Wales. Acta Paediatr 101(6):587–90
106. Bulloch B et al (2000) Cause and clinical characteristics of rib fractures in infants. Pediatrics 105(4):E48–E

107. Barsness KA et al (2003) The positive predictive value of rib fractures as an indicator of nonaccidental trauma in children. J Trauma 54(6):1107–1110
108. Bishop N, Sprigg A, Dalton A (2007) Unexplained fractures in infancy: looking for fragile bones. Arch Dis Child 92(3):251–256
109. Kleinman PK et al (1996) Rib fractures in 31 abused infants: postmortem radiologic-histopathologic study. Radiology 200(3):807–810
110. Kleinman PK, Schlesinger AE (1997) Mechanical factors associated with posterior rib fractures: laboratory and case studies. Pediatr Radiol 27(1):87–91
111. Dyer C (2012) Prosecution of parents over baby's death raises controversy over diagnosing child abuse. BMJ 344:e2932
112. Lucas PJ, Jessiman T, Cameron A (2012) Don't ignore preventive message of baby Jayden's case. BMJ 344:e3386
113. Marquardt ML et al (2012) Copper deficiency presenting as metabolic bone disease in extremely low birth weight, short-gut infants. Pediatrics 130(3):e695–e698
114. Kurl S et al (1998) Determinants of bone mineral density in prematurely born children aged 6–7 years. Acta Paediatr 87(6):650–653
115. Fewtrell MS et al (2009) Early diet and peak bone mass: 20 year follow-up of a randomized trial of early diet in infants born preterm. Bone 45(1):142–149
116. Fewtrell MS (2006) Osteoporosis: is primary prevention possible? Nestle Nutr Workshop Ser Pediatr Program 57:135–146; discussion 146–151
117. Hori C et al (1995) Bone mineral status in preterm-born children: assessment by dual-energy X-ray absorptiometry. Biol Neonate 68(4):254–258

Chapter 7
Gastro-Esophageal Reflux in Neonatology

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Abstract Gastro-esophageal reflux (GER) is a near universal phenomenon in newborn infants. Regurgitation is so frequent that its complete absence may be considered as evidence of inadequate milk intake. GER occurs usually in association with transient relaxations of the lower esophageal sphincter, which may be triggered by entry of milk into the stomach, and is not associated with delayed gastric emptying. GER can be quantified using monitoring techniques which require prolonged presence of a foreign body in the esophagus. Either just pH (for possible complications related to acid exposure) monitoring or pH with multiple intraluminal impedance monitoring are available. Other investigations are rarely helpful. The definitions and diagnostic criteria of GER disease (GERD) are controversial and there is no consensus regarding which clinical signs may be caused by GER. In particular behavioural changes, feeding difficulties and significant apnea have not been clearly shown to be increased in infants with higher frequency of GER episodes, or to be temporally related to them. Prokinetic agents are ineffective. Anti-acid medications may effectively reduce gastric acid production, but have not been shown to improve clinical symptoms, and are associated with increased infectious risk, and may reduce the absorption of some micronutrients. In cases of acid related disease a therapeutic trial may be indicated if the benefits are thought to outweigh the risks. Reflux spontaneously improves over time in the preterm infant, but may be prolonged in those with surgical conditions such as esophageal atresia and diaphragmatic hernia.

Key points

- Gastro-esophageal reflux is universal in newborn infants, but serious clinical consequences are very unusual.
- There is no clear method to clinically diagnose reflux which is causing clinical disease
- Documentation of increased numbers and duration of episodes can be done with prolonged pH monitoring for acid reflux, or with combined multiple intraluminal impedance and pH monitoring for both acid and non-acid reflux.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_7,

- There is no proven effective medical therapy for reflux disease. Thickening feeds has a modest effect on reflux, but is not proven to work in reflux disease, and other medications are both ineffective and may have side effects.
- Some patients with surgical diagnoses or serious neurologic impairment do have major clinical consequences of reflux. The only proven effective therapy for them is surgery.

1 Introduction

Almost all human babies have reflux, regurgitation, possiting, vomiting, or throwing up. The wide variety of terms (and there are many others) is evidence of the ubiquitous nature of the phenomenon. Baby mammals take in relatively huge volumes of liquid nutrition (equivalent to over 15 liters a day if extrapolated to the size of an adult) and regurgitate some of it. Even when not external and obvious, reflux of stomach contents into the esophagus (gastro-esophageal reflux-GER) is a nearly universal phenomenon in the newborn. Preterm and sick newborn infants also may have GER, and they often have clinical signs and worse outcomes attributed to GER. In some NICUs a large proportion of preterm infants receive therapy aimed at GER during their hospitalization, and they may be discharged from hospital while still receiving these drugs or other interventions.

2 Epidemiology of GER and Objective Diagnosis

The epidemiology of GER and GERD depends on how they are defined. Quantifying how often GER occurs is done either with pH-metry, continuously measuring the pH in the esophagus to detect the presence of gastric acid, or with the multiple intraluminal impedance (MII) technique, which can detect reflux of any pH, and can also determine the relative height of the reflux in the esophagus [1]. Both techniques require a foreign body in the esophagus, which might by itself increase the prevalence of GER episodes.

As mentioned before, some degree of GER is almost universal, and sometimes it will occur when the gastric pH is acidic. Therefore statistically normal standards for acid reflux in the lower esophagus have been developed [2]. Usually these depend on detection of a pH < 4 in the lower esophagus; the number of episodes of pH < 4 per hour, the proportion of time the esophageal pH is < 4, and the average duration of pH < 4 episodes have all been used as indicators of GER; using these data various reflux indices have been constructed. The studies which have tried to define normal values for GER have demonstrated that it is quite unusual to have no reflux whatsoever, confirming the initial statements introducing this chapter. In the preterm infant there are fewer normative studies, but they confirm the universality of GER in the preterm. The small amount of data available shows

fewer episodes of acid [2] reflux, and shorter total duration of acid exposure of the esophagus compared to the term infant. How much these data are influenced by the lower gastric acid production in the preterm is uncertain, but even very preterm infants produce some gastric acid in the first days of life, and are able to produce a gastric pH less than 4 shortly after birth [3], so acid reflux should be detectable. Infants with surgical or neurologic problems who were born at term are appropriately compared to term normal values from healthy infants.

Clearly, defining GER according to a statistical norm will determine how frequently it occurs, a value above the 90th percentile for the number of episodes of GER will occur in 10 % of infants. There are several limitations to this type of statistical definition of GER, mainly that the frequency of reflux and the presence of symptoms or signs due to reflux are not closely correlated with each other, either in the newborn or in older infants [4].

In adults the phenomenon of high pH, alkaline, reflux is well recognized, and may be of clinical importance, whether such non-acid reflux might have clinical consequences in the newborn is uncertain. Alkaline reflux however appears to be uncommon in the preterm [5]. More importantly in the newborn the usual feed, milk, is a reasonably good buffer of acid. This means that episodes of GER occurring immediately after a feed may not have a low enough pH to drop the esophageal pH below 4. This has been very well studied by Omari and Davidson [6] who showed an increase in gastric pH to almost neutral pH within 30 min of a milk feed. They further demonstrated that by comparing gastric to esophageal pH they could still detect GER during the period of time that the pH was buffered, but many of those GER episodes were not associated with a fall of esophageal pH to below 4. Some investigators have suggested replacing a feed of milk with apple juice for this reason, apple juice having a mildly acidic pH. However, this too is problematic as apple juice is not affected by gastric enzymes in the same way as milk, therefore remains very fluid in the stomach and seems to be much easier to reflux, therefore biasing the results.

These are some of the reasons behind the development of the MII technique; this newer technique detects both acid and non-acid reflux. The MII technique requires a catheter with multiple electrical impedance electrodes to be appropriately placed in the esophagus an antimony pH electrode is also integrated into the catheter; MII produces multi-channel recordings which are laborious to interpret, although automated techniques are being developed. Liquid passing the catheter increases impedance, whereas air decreases impedance. Therefore the nature, height, and direction of fluid movements can be described. More recently the baseline impedance value has been promoted as a way of determining mucosal integrity [7]. Most episodes are detected by the MII, some by both MII and pH, and relatively rarely an episode of GER can occur which is detected by the pH probe, but does not satisfy GER criteria by MII [8]. Normal values for the preterm have been developed. The proportion of GER which is non-acid, therefore not recognized by pH probe alone has varied between studies, but is usually at least 50 %.

In the same way as for pH-metry, definitions of abnormal are made on a statistical basis, so 10 % of infants will have “abnormal” combined MII/pH results beyond the

90th percentile. These may be a different 10 % to those defined by pH alone. Again the major problem with using this statistical definition of GER is the poor association between reflux frequency or duration and the presence of symptoms attributed to reflux.

Other methods of diagnosis have been used. They include short term evaluations such as a radiographic contrast study, which only informs the caregivers of GER during the few minutes that the images are taken, an ultrasound study which can be more prolonged [9] (but is unlikely to last several hours) and can eliminate other pathologies, or scintigraphic studies, which can determine if the radiolabelled tracer is detected in the lungs, and therefore are one way of diagnosing pulmonary aspiration, but only aspiration occurring during that particular feed. Finally esophageal manometry (usually combined with pH monitoring) which is a technique used very effectively by some groups [10], but requires a great deal of expertise, and a tube that passes through the lower esophageal sphincter, which may itself increase GER [11].

3 Pathophysiology of GE Reflux

GER is normally prevented by tonic activity of the lower esophageal sphincter. Relaxations of the LES are required for normal swallowing, but may also occur spontaneously and are known as "transient lower esophageal sphincter relaxations". This phenomenon has been well studied in the preterm newborn. Transient LES relaxations occur between swallows most commonly as a component of reflux episodes [12], about 3/4 of transient LES relaxations are associated with GER episodes. The converse is also true, the large majority of GER episodes occur when there are transient LES relaxations [13, 14]. Such relaxations and reflux episodes are triggered by milk entering the stomach, even at relatively small volumes that do not cause significant gastric distension [15]. The horizontal position of the newborn infant also contributes to the occurrence of GER, and when an infant begins to stand, and then walk, reflux becomes much less of a clinical problem. LES relaxations are associated with both acid and non-acid reflux. It was previously thought that gastric emptying was prolonged in infants experiencing GER, however direct measurement of gastric emptying has shown no delay in newborn infants with GER [13, 16].

4 GER Disease

Reflux becomes a disease or disorder when the refluxed liquid leads to clinical consequences of importance, either because of the nature of the liquid (such as in acid erosive esophagitis) or because of pathophysiologic consequences of responses to the liquid, such as when liquid is aspirated into the lungs.

Non-acid regurgitation without aspiration is generally of no consequence other than being messy; however the presence of regurgitations or the suspected presence

of GER may lead to other investigations to determine whether there is an abnormally high number of GER episodes. Which pathologic consequences of GER, that is GERD, actually occur in the newborn, in contrast, has proven so difficult to define in the neonatal population (see below) that its prevalence is unknown and it is uncertain what consequences exist.

What disease entities might be triggered by GER, and thus warrant the term GERD? They might be due to reflux of acid, or reflux of gastric contents with non-acid-related physiologic consequences.

Serious consequences of reflux in healthy term or preterm infants are rare. Most clinical signs thought to be associated with GER have been minor. Neurologically impaired infants with reduced airway protection, on the other hand, may suffer from recurrent pulmonary aspiration. The presence of regurgitation or a history of pulmonary aspiration in an infant with neurological compromise warrants an MII test, and may require therapy, including surgery, to prevent complications. Some infants with surgical disorders (in particular diaphragmatic hernia [17] or tracheo-oesophageal fistula [18]) also may have very prominent reflux and may develop erosive esophagitis.

GERD is so frequent after repair of diaphragmatic hernia that some authorities have suggested that initial repair of the defect should be accompanied by surgical anti-reflux measures [19], however, the latest data suggest that the benefits are short-lived, as clinical signs of reflux decrease by 24 months. Potent acid suppression medications may well be required for these infants, as most of the reported complications have been due to acid esophagitis, it is likely that only pH metry is required for diagnosis and monitoring, rather than MII. After trachea-esophageal fistula/esophageal atresia repair, esophageal dysmotility is universal. The clinical importance of GER in these patients in the very long term is only recently being clearly reported.

5 Acid Related

Symptoms of acid related disease in the preterm are unclear. It is uncertain whether aversion/refusal to feed and signs of discomfort occur during reflux associated with erosive esophagitis in the preterm, as is occasionally the case in the older infant. Studies evaluating various clinical signs have not been able to clearly define any, which are good indicators of the presence of acid esophagitis [20]. Common behavioral changes ascribed to GER include grimacing, fussiness, frequent crying or irritability, feeding refusal or poor progress in establishing oral feeds One study with combined pH and MII monitoring found that there was no correlation between symptom scores and findings on objective monitoring, furthermore the timing of the symptoms which were ascribed to GER by the caregivers bore no relation to the actual timing of the GER episodes. [21] Feeding difficulties are very frequent in the former preterm infants approaching term, but to ascribe them to reflux in an individual case is always questionable. If feeding aversion is thought to be due to erosive acid related esophagitis, the diagnostic process in an older patient would

normally require ph-metry and an esophago-gastroscopy. However endoscopy of the upper GI is rarely practiced in the small newborn infant, and biopsies have very rarely been reported, so ph-metry and a therapeutic trial of acid blocking medication may be considered. Such a therapeutic trial should use a medication which is effective, safe, of known kinetics, and be time limited with a trial off medication if there is a clinical response (to confirm that the improvement is not simply coincidental with increasing maturation), and stopping the medication completely if there is no response, or if the infant remains free of signs during the trial off period. However it should be noted that randomized trials of acid blocking medications in supposedly symptomatic infants have not shown any improvement in clinical signs compared to placebo [22].

6 Non-Acid Related

6.1 Micro-Aspiration

Frequent aspiration of small quantities of refluxed gastric contents has been suggested as a risk factor for the development of bronchopulmonary dysplasia. Chronically intubated preterm infants often have evidence of gastric fluid aspiration if their endotracheal fluid is examined, looking for extraneous components such as lactose or pepsin [23], or macrophages that have engulfed the lipid contained in milk that was aspirated into the lungs. Some older studies suggested that lipid laden macrophages were indeed more common in endo-tracheal aspirates of infants who went on the develop BPD, however recent studies have found no association between GER and BPD [24]. It seems therefore, that although recurrent aspiration is frequent among ventilated preterm infants, it probably does not relate in a causative fashion to bronchopulmonary dysplasia. The studies however are relatively low powered to detect even a strong association, and further work might clarify this.

6.2 Macro-Aspiration

Macro-aspiration events following large regurgitations do occasionally occur; desaturation and bradycardia during airway occlusion may require immediate resuscitation and direct therapy. These events sometimes occur in preterm infants, or in term infants with neurological compromise or large regurgitation. Therapy after initial resuscitation is unclear, the role of antibiotics, or of steroids have been suggested, but it is not clear if any benefit is gained by using them. Recurrent macro-aspiration is clearly an indication for effective therapy, acid blockade is unlikely to be sufficient and surgery may be required.

6.3 Apnea

Recurrent apnea of prematurity has often been considered to be a possible sign of reflux. The evidence to support this contention is controversial, but generally negative [25]. Most studies have failed to find any association between reflux and apnea [26, 27] Although it is possible that in individual cases there is a link between GER episodes and apneic spells, the preponderance of the evidence suggests that this is unusual either for acid or non-acid reflux [28–31]. Di Fiore et al have shown no difference in the frequency of apneas of at least 15 s before, during or after an episode of GER, they also noted no difference in the characteristics of the apneas which happened to occur immediately after an episode of GER compared to those which occurred distant from an episode [32].

Corvaglia and his group in contrast have published a few studies which have consistently reported a significant increase in apnea during the 30 s after GER episodes [33]. The reason for the difference between their results and most other groups is uncertain, those authors suggest that Peters' inability to show a correlation between GER and apnea is because they only used MII and did not measure distal pH which is actually inaccurate [34]. They also suggest that Di Fiore's inability to show such an association is because they did not use MII and only measured pH, although this has also now been addressed, a more recent study by their group used combined pH and MII [35] and also showed no association. There is also no known mechanism whereby distal acidification of the esophagus could trigger apnea, nor is there one for non-acid reflux. References which have sometimes been quoted to suggest that such reflex apnea is a possibility actually refers to laryngeal instillation of liquid, which leads to apnea while the infant is clearing the liquid [36], in fact the pH of that liquid is irrelevant to the reflex [37]. In contrast pharyngeal instillation of liquid does not lead to apneas [38] in either term or preterm infants (or indeed in piglets [39]), and GER in the preterm does not usually enter the larynx [38]. In contrast acid infusion into the lower esophagus can cause reflex increases in airway resistance and wheezing in certain susceptible older individuals, but this is of questionable relevance to apnea in the preterm. One difference between Corvaglia's studies and other groups is the inclusion of all apneas over 5 s duration [33]. He has not reported whether more prolonged apneas, or apneas associated with bradycardia or hypoxia are increased after reflux in his studies. Other authors have only analyzed apneas of over 10 [35] or over 15 s. So one possible explanation of the difference could be that very short apneas occur more frequently immediately after the onset of an episode of GER, but more prolonged significant apneas are unrelated to GER. Corvaglia also notes that he measured airflow, rather than using respiratory inductance, although he does not give enough detail of methodology to be sure it seems likely that he used a face mask, and prolonged use of face masks actually change respiratory characteristics. Other groups that have reported GER related apnea have only reported apneas occurring after an episode of GER, and there has been no evaluation of the relative incidence of apnea during non-GER periods, and no description of the apnea monitoring method used [40].

Confirmatory evidence of the relative unimportance of GER as a cause of apnea is the realization that studies attempting to treat apnea with anti-reflux medication have been universally unsuccessful (see below).

7 Clinical Diagnosis of GERD

Clinical features of GER are uncertain. Although there are beliefs regarding certain clinical features and their association with GER among various members of staff in our NICUs, objective assessment of the correlation of the signs with objective evidence of GER has shown that many episodes of reflux occur without such signs. Very often signs such as apparent discomfort and crying and so-called "Reflux-specific behavior" such as regurgitation or spitting, yawning, mouthing, hiccupping, sneezing, coughing and gagging, thumb sucking, and head retraction occur when there is no GER [21]. The only definite clinical sign of reflux is regurgitation. The importance and predictive value of other clinical signs are therefore quite unclear, and they are unreliable for the diagnosis of GER.

Signs of GERD have not been adequately evaluated in the newborn, and particularly not in the preterm, a study in slightly older infants found that many babies with no evidence of GER on ph-metry had signs commonly supposed to be associated with GER, and was unable to define clinical signs that were closely associated with either abnormal pH-metry or abnormal esophageal biopsy results [41]. Even in older children the relationship between symptoms and both ph-metry and MII differs enormously according to the scoring system used [4]. Similarly in the former preterm infants at term, results from the Symptom Severity Index, and the Symptom Index are quite different [42], this study from Jadcherla and co-workers noted than 50 % of the GER episodes were not associated with any clinical signs in a group of 30 infants referred for suspected clinically important GER, but he did not report how many symptoms occurred when the infants were not having reflux.

In summary there is no clear clinical sign, which differentiates between infants with and without reflux, and no validated scoring system for the newborn infant, either term or preterm. If GER is clinically suspected to be the cause of clinical signs in an individual baby then confirmation that the child has an abnormal amount of reflux can be obtained by combined MII and pH-metry, however, determination of "excessive" GER does not mean that the child's clinical signs are necessarily due to GER. To make such a determination a trial of an effective therapy may be the only option.

8 Treatment of GERD

8.1 Non-Pharmacological

8.1.1 Positioning

Changing the position of the preterm infant, especially after feeds, can reduce the number of episodes of GER, specifically placing the infant in a left lateral position [43]. There is however, no evidence of efficacy for GERD, such as healing

of esophagitis. The maneuver might be worthwhile if the baby tolerates the manipulation involved, and if parents are sufficiently concerned about regurgitation to warrant it.

8.1.2 Thickening Feeds

Feeds can be thickened in a number of different ways, with gums, cereals, starches or other agents. The efficacy of this approach is limited, some studies in infants show no effect, some show a reduction in obvious regurgitation but not esophageal acid exposure, and some show a reduction in many indices of reflux, both the number and duration of episodes [44]. Whether these different results are due to different thickeners, patient population, or monitoring methodology is not clear [44]. Few studies have been performed in a specifically neonatal population, but one study showed that thickening feeds with starch [45] was ineffective in a sample of preterm infants. There is also no evidence of enhanced healing or other clinically important effects of thickeners in the setting of newborn infants with GERD. Thickening feeds may not be innocuous. There are reports of interference with the absorption of various nutrients, increases in cough, and even GI obstruction. Recent case reports suggest that some thickeners at least (specifically xanthan gum) may be associated with the development of necrotizing enterocolitis [46, 47]. These reports reinforce the importance of performing studies in preterm infants, in whom clinical responses and potential toxicities are unique.

8.1.3 Small Frequent Feeds

Families of older infants with reflux are frequently counseled to give them smaller more frequent feeding. There is no evidence to support the use of such an intervention in the newborn. As newborn infants are often fed every 3–4 h in any case, feeding them more frequently would be a significant burden on their families, or on the nursing staff in the NICU; evidence of efficacy is therefore essential. A recent observational study found an association between longer feeding duration and slower flow rate and fewer episodes of GER [48], but as an observational study causation cannot be determined.

8.2 Pharmacological

8.2.1 Acid Suppression

Suppressing acid production should only be considered if there is evidence of GERD caused by acid. In particular, evidence of erosive esophagitis. In such a circumstance the risks of such treatment should be taken into consideration, and acid-blockade

should be continued for as short a period as possible. Acid-blockers remove an important barrier to bacterial and fungal colonization of the preterm infant. Although there are no large RCTs, several observational studies have demonstrated an association between the use of acid suppressants and increased systemic nosocomial infections, in particular candida infections as well as with necrotizing enterocolitis [49, 50] and with mortality. This indeed is consistent with findings in older patients in intensive care and even among asthmatic children, who have more pulmonary infections if randomized to acid suppression [51]. This toxicity has been demonstrated with both histamine blockers and proton pump inhibitors. So, in view of the paucity of evidence of benefit, care should always be taken when prescribing these kinds of agents. In addition, many commonly used agents have unknown pharmacokinetics in the newborn, and even more particularly the small preterm infant, increasing the risks of therapeutic misadventures. Histamine receptor blockade is associated in older infants with numerous side effects, particularly agitation and headache [52].

Acid blockade, most importantly with the more effective proton pump inhibitors, is known to interfere with calcium absorption, and increases the risk of osteoporotic fractures in the elderly [53]. The effects of acid blockade on calcium absorption in the newborn with GER does not appear to have been studied, As preterm infants are already at risk of reduced bone mineralization and osteopenia of prematurity, and in particular those preterm infants with BPD or who have had an episode of necrotizing enterocolitis, are more likely to be treated for GER and are more likely to develop osteopenia, further caution is advised in prescribing these agents. Gastric acid is also important for the absorption of iron and vitamin B12, the potential that acid blockade may interfere with the absorption of these micronutrients has not been adequately investigated in the newborn [54].

Proton pump inhibitors are more effective in older patients in suppressing acid production than are H2 blockers. Nevertheless a large placebo controlled randomized trial in older infants showed no benefit from a PPI in improving symptoms that were ascribed to GER [55]. This points out the difficulty in clinical diagnosis of GER and reinforces the need for caution in prescription of drugs that may have side effects when the diagnosis is unclear. The pharmacodynamics of the PPIs have also been little studied in the preterm infant. A recent trial showed very ineffective acid suppression with oral lansoprazole in preterm infants, with a pH that remained below 2 despite treatment [56]. Oral omeprazole was in contrast shown to effectively reduce gastric acidity and esophageal acid exposure, in a randomized cross-over trial, without any effect on apnea, bradycardia, vomiting or behavioral changes [57]. A randomized cross-over study in older infants showed effective reduction in esophageal acid exposure, but no improvement in symptoms attributed to GER [22].

In summary although there is some efficacy of histamine receptor blockade and proton pump inhibitors for reducing gastric acid production, there are significant risks with either class of medication, particularly in the infant at risk of nosocomial infections, and no clear evidence that any clinical symptom is benefited by their use. They should in general be avoided in the newborn.

8.2.2 Alginates

A mixture of sodium and magnesium alginate is available formulated as Gaviscon, which also contains sodium bicarbonate, (however it should be noted that Gaviscon Infant in the USA contains no bicarbonate). When it comes into contact with gastric acid the alginate precipitates to form a gel. It is thought that this gel is more difficult to reflux, and the sodium bicarbonate adds an antacid characteristic to the formulation. The efficacy of this preparation is questionable; some studies have found no effect on the frequency or duration of GER, one study did show that the height of reflux was reduced, but the clinical significance of this for treating GERD is uncertain. A single study with MII/pH methodology in the preterm infant seemed to show a reduction in acid GER, but not non-acid or overall reflux episodes [58]. Interestingly another study by the same group found the same changes in GER, with a significant reduction in acid GER, but no changes in apnea [59].

8.3 Prokinetic Agents

There is little evidence that increasing intestinal motility with medical interventions improves GER, or GERD. Indeed it could be questioned why this would even be considered. As gastric emptying is not delayed in infants with GER, and the mechanism of GER is closely tied to the occurrence of transient LES relaxations; only if there were evidence that an agent had a specific effect that reduces LES relaxations would it be likely to be effective at decreasing GER. It has not been demonstrated that any agent has such an effect on transient LES relaxations.

8.3.1 Metoclopramide

The most commonly prescribed prokinetic agent currently is metoclopramide, however, a recent systematic review showed that there is no reliable evidence that metoclopramide reduces reflux [60]. Metoclopramide is a prokinetic agent which blocks D2 receptors in the gut and brain; it has a modest effect as an antiemetic agent in some circumstances due to the cerebral D2 blockade, but there is little evidence of effect on GER or GERD. Indeed, an increase in gastric contractility from a prokinetic agent such as metoclopramide without any change in LES function could easily increase reflux. This has actually been shown in one study. Machida et al showed more reflux episodes during treatment with metoclopramide compared to control periods in a cross-over study [61]. In addition metoclopramide has extra-pyramidal effects, leading to a high risk of side effects, and the therapeutic index is narrow.

Wheatley and Kennedy showed in a randomized cross-over study that during the periods of metoclopramide treatment the babies had more bradycardias than during the control periods [62]. There are 2 potential explanations for this. Either reflux causes bradycardias and there are increased numbers of reflux episodes during

metoclopramide treatment than during control: or the bradycardic episodes are not due to reflux, but metoclopramide acts directly to increase these episodes (or it was just a random effect in a small study).

Indeed there is no evidence of benefit of metoclopramide for reflux in preterm infants. In a recent systematic review [60] it was noted that there were several patients (in the minority of studies that reported adverse effects) who had increased irritability, or other side effects which were potentially of extra-pyramidal origin. Several commentators have suggested that metoclopramide is contra-indicated as a GER medication in infants because of the high incidence of side-effects [63]. In older patients in whom the effects of metoclopramide on GER have been more extensively investigated, there is little or no efficacy.

8.3.2 Cisapride

Although now removed from the market in most jurisdictions because of a risk of arrhythmia, associated with prolongation of the QT interval, cisapride may still be available under special access programs and was for a period one of the most prescribed medications in neonatal units in the USA. This situation occurred despite the complete lack of evidence of efficacy or safety in the neonatal population. Even in older children there is no good evidence of efficacy, the Cochrane review of use for GER in children, updated in 2010 [64], found no clear evidence of benefit, despite evidence of publication bias favoring the publication of positive studies. In addition short term physiologic studies show that cisapride delays gastric emptying in the preterm infant [65]. There are no controlled data showing a beneficial effect of cisapride in newborn infants.

8.3.3 Domperidone

This agent is a D2 blocking compound that has been suggested as a therapy for GER. There are no RCTs showing a beneficial effect of domperidone. On the contrary, one small blinded cross-over trial showed that indices of reflux as determined by MII with pH monitoring were worse during dopmeridone treatment compared to during placebo treatment [66]. Domperidone also has effects at a potassium channel and prolongs the QT interval [67]. It also may cause extra-pyramidal side effects. It is not licensed for use in some countries, and does not appear to be licensed for use as a therapy for GER in any jurisdiction.

8.4 Erythromycin

Erythromycin is a macrolide antibiotic that also has effects on bowel motility. It is an agonist at motilin receptors and in mature animals and humans is an effective

prokinetic. The maturation of the motilin receptors is however uncertain. Systematic review of erythromycin use as a prokinetic to improve feeding tolerance in the newborn has suggested efficacy, but the most immature infants may not benefit [68]. In these studies feeding tolerance was often assessed by the volume of gastric aspirate, and feeding advancement was allowed when aspirate volumes were low. As there is no known correlation between aspirate volumes and adverse outcomes in the preterm infant, this methodology is suspect. There has never been a controlled trial of erythromycin for GER, so its use in this situation is very questionable, especially given the lack of evidence to support the use of other prokinetic agents for treating GER. In older infants erythromycin use increases the risk of developing pyloric stenosis, and it may induce arrhythmias. Erythromycin has an effect on the inward potassium channel known as I(Kr), and prolongs the QT interval on the ECG, increasing the risk of ventricular tachydysrhythmias, especially in patients with other risk factors. This potential has not been studied in the newborn.

There is no evidence to support the use of erythromycin for GER in the newborn.

9 Surgery

Fundoplication has been performed for many years as a "definitive" therapy for GER. A number of different open and laparoscopic surgical techniques have been described [69]. Because of its invasive nature, surgery has been confined to those infants whose problems are not controlled by good acid control with PPIs, or those in whom their clinical problems are not caused by acid, such as in recurrent macroaspiration. This may occur in infants with serious neurodevelopmental difficulties. Although generally considered effective, complications are not infrequent, and some doubts regarding efficacy have been raised [70]. Laparoscopic fundoplication has been performed in infants as small as full term neonates, with a complication rate similar to open techniques.

10 Future Research

There is a clear need to determine if there are any clinical signs which can be used to diagnose GER or GERD in the newborn. A study to investigate a potential clinical sign could use a similar methodology to Dr Snel's publication [21], comparing the incidence and timing of signs to the findings on objective measures of GER using MII combined with pH-metry. If signs can be defined, then RCTs, adequately powered to detect potential complications, could be performed to try and determine for once and for all if any therapy other than surgery helps to heal GERD, and improve clinical outcomes.

11 Summary

Gastro-esophageal reflux is a common phenomenon in the preterm and term infant. It is unclear how to diagnose it clinically, and the correlation between GER and any particular clinical sign is unproven. When increased GER is diagnosed there is no good quality evidence to support any intervention, rather, all the medications that have been studied are ineffective in reducing GER, and are associated with potential or proven side effects. Clear evidence of acid related disease is currently the only indication for therapy, and in such a clinical situation, proton pump inhibitors could be given a trial of therapy. More research in this area may assist in developing an evidence base for rational treatment in the future.

References

1. Wenzl TG, Silny J, Schenke S, Peschgens T, Heimann G, Skopnik H (1999) Gastroesophageal reflux and respiratory phenomena in infants: status of the intraluminal impedance technique. J Pediatr Gastroenterol Nutr 28(4):423–428
2. Jeffery HE, Page M (1995) Developmental maturation of gastro-oesophageal reflux in preterm infants. Acta Pædiatrica 84(3):245–250
3. Kelly EJ, Newell SJ, Brownlee KG, Primrose JN, Dear PRF (1993) Gastric acid secretion in preterm infants. Early Hum Dev 35(3):215–220
4. Luthold SC, Rochat MK, Bahler P (2010) Disagreement between symptom-reflux association analysis parameters in pediatric gastroesophageal reflux disease investigation. World J Gastroentero: WJG 16(19):2401–2406
5. Lopez-Alonso M, Moya MJ, Cabo JA et al (2006) Twenty-four-hour esophageal impedance-pH monitoring in healthy preterm neonates: rate and characteristics of acid, weakly acidic, and weakly alkaline gastroesophageal reflux. Pediatrics 118(2):e299–e308
6. Omari TI, Davidson GP (2003) Multipoint measurement of intragastric pH in healthy preterm infants. Arch Dis Child Fetal Neonatal Ed 88(6):F517–F520
7. Loots CM, Van Wijk MP, Smits MJ, Wenzl TG, Benninga MA, Omari TI (2011) Measurement of mucosal conductivity by MII Is a potential marker of mucosal integrity restored in infants on acid-suppression therapy. J Pediatr Gastroenterol Nutr 53(1):120–123
8. Di Fiore JM, Arko M, Churbock K, Hibbs AM, Martin RJ (2009) Technical limitations in detection of gastroesophageal reflux in neonates. J Pediatr Gastroenterol Nutr 49(2):177–182
9. Savino A, Cecamore C, Matronola M et al (2012) US in the diagnosis of gastroesophageal reflux in children. Pediatr Radiol 42(5):515–524
10. Jadcherla SR, Hoffmann RG, Shaker R (2006) Effect of maturation of the magnitude of mechanosensitive and chemosensitive reflexes in the premature human esophagus. J Pediatr 149(1):77–82
11. Peter CS, Wiechers C, Bohnhorst B, Silny J, Poets CF (2002) Influence of nasogastric tubes on gastroesophageal reflux in preterm infants: a multiple intraluminal impedance study. J Pediatr 141(2):277–279
12. Omari TI, Miki K, Davidson G et al (1997) Characterisation of relaxation of the lower oesophageal sphincter in healthy premature infants. Gut 40(3):370–375
13. Omari TI, Barnett CP, Benninga MA et al (2002) Mechanisms of gastro-oesophageal reflux in preterm and term infants with reflux disease. Gut 51(4):475–479
14. Omari TI, Barnett C, Snel A et al (1998) Mechanisms of gastroesophageal reflux in healthy premature infants. J Pediatr 133(5):650–654

15. Omari TI, Rommel N, Staunton E et al (2004) Paradoxical impact of body positioning on gastroesophageal reflux and gastric emptying in the premature neonate. J Pediatr 145(2):194–200
16. Ewer AK, Durbin GM, Morgan MEI, Booth IW (1996) Gastric emptying and gastro-oesophageal reflux in preterm infants. Arch Dis Child 75(2 Sp. Iss.):F117–F121
17. Kawahara H, Okuyama H, Nose K et al (2010) Physiological and clinical characteristics of gastroesophageal reflux after congenital diaphragmatic hernia repair. J Pediatr Surg 45(12):2346–2350
18. Legrand C, Michaud L, Salleron J et al (2012) Long-term outcome of children with oesophageal atresia type III. Arch Dis Child 97(9):808–811
19. Maier S, Zahn K, Wessel LM, Schaible T, Brade J, Reinshagen K (2011) Preventive antireflux surgery in neonates with congenital diaphragmatic hernia: a single-blinded prospective study. J Pediatr Surg 46(8):1510–1515
20. Maki M, Ruuska T, Kuusela AL, Karikoski-Leo R, Ikonen RS (1993) High prevalence of asymptomatic esophageal and gastric lesions in preterm infants in intensive care. Crit Care Med 21(12):1863–1867
21. Snel A, Barnett CP, Cresp TL et al (2000) Behavior and gastroesophageal reflux in the premature neonate. J Pediatr Gastroenterol Nutr 30:18–21
22. Moore DJ, Tao BS-K, Lines DR, Hirte C, Heddle ML, Davidson GP (2003) Double-blind placebo-controlled trial of omeprazole in irritable infants with gastroesophageal reflux. J Pediatr 143(2):219–223
23. Farhath S, He Z, Nakhla T et al (2008) Pepsin, a marker of gastric contents, is increased in tracheal aspirates from preterm infants who develop bronchopulmonary dysplasia. Pediatrics 121(2):e253–e259
24. Akinola E, Rosenkrantz TS, Pappagallo M, McKay K, Hussain N (2004) Gastroesophageal reflux in infants < 32 weeks gestational age at birth: lack of relationship to chronic lung disease. Am J Perinatol 21(2):57–62
25. Mousa H, Woodley FW, Metheney M, Hayes J (2005) Testing the association between gastroesophageal reflux and apnea in infants. J Pediatr Gastroenterol Nutr 41(2):169–177
26. Barrington KJ, Tan K, Rich W (2002) Apnea at discharge and gastro-esophageal reflux in the preterm infant. J Perinatol 22(1):8–11
27. Poets CF, Brockmann PE (2011) Myth: gastroesophageal reflux is a pathological entity in the preterm infant. Semin Fetal Neonatal Med 16(5):259–263
28. de Ajuriaguerra M, Radvanyi-Bouvet MF, Huon C, Moriette G (1991) Gastroesophageal reflux and apnea in prematurely born infants during wakefulness and sleep. Am J Dis Child (1960) 145(10):1132–1136
29. Wenzl TG, Schenke S, Peschgens T, Silny J, Heimann G, Skopnik H (2001) Association of apnea and nonacid gastroesophageal reflux in infants: Investigations with the intraluminal impedance technique. Pediatr Pulmonol 31(2):144–149
30. Poets CF (2004) Gastroesophageal reflux: a critical review of its role in preterm infants. Pediatrics 113(2):e128–e132
31. Newell SJ, Booth IW, Morgan ME, Durbin GM, McNeish AS (1989) Gastro-oesophageal reflux in preterm infants. Arch Dis Child 64(6):780–786
32. Di Fiore JM, Arko M, Whitehouse M, Kimball A, Martin RJ (2005) Apnea is not prolonged by acid gastroesophageal reflux in preterm infants. Pediatrics 116(5):1059–1063
33. Corvaglia L, Zama D, Gualdi S, Ferlini M, Aceti A, Faldella G (2009) Gastro-oesophageal reflux increases the number of apnoeas in very preterm infants. Arch Dis Child Fetal Neonatal Ed 94(3):F188–F192
34. Peter CS, Sprodowski N, Bohnhorst B, Silny J, Poets CF (2002) Gastroesophageal reflux and apnea of prematurity: no temporal relationship. Pediatrics 109(1):8–11
35. Di Fiore J, Arko M, Herynk B, Martin R, Hibbs AM (2010) Characterization of cardiorespiratory events following gastroesophageal reflux in preterm infants. J Perinatol 30(10):683–687
36. Perkett EA, Vaughan RL (1982) Evidence for a laryngeal chemoreflex in some human preterm infants. Acta Paediatr Scand 71:969

37. Kovar I, Selstam U, Catterton WZ, Stahlman MT, Sundell HW (1979) Laryngeal chemoreflex in newborn lambs: respiratory and swallowing response to salts, acids, and sugars. Pediatr Res 13(10):1144–1149
38. Page M, Jeffery HE (1998) Airway protection in sleeping infants in response to pharyngeal fluid stimulation in the supine position. Pediatr Res 44(5):691–698
39. Page M, Jeffery HE, Marks V, Post EJ, Wood AK (1995) Mechanisms of airway protection after pharyngeal fluid infusion in healthy sleeping piglets. J Appl Physiol 78(5):1942–1949
40. Magistà AM, Indrio F, Baldassarre M et al (2007) Multichannel intraluminal impedance to detect relationship between gastroesophageal reflux and apnoea of prematurity. Digest Liver Dis 39(3):216–221
41. Salvatore S, Hauser B, Vandemaele K, Novario R, Vandenplas Y (2005) Gastroesophageal reflux disease in infants: how much is predictable with questionnaires, pH-metry, endoscopy and histology? [Article]. J Pediatr Gastroenterol Nutr 40(2):210–215
42. Jadcherla SR, Peng J, Chan CY et al (2011) Significance of gastroesophageal refluxate in relation to physical, chemical, and spatiotemporal characteristics in symptomatic intensive care unit neonates. Pediatr Res 70(2):192–198
43. van Wijk MP, Benninga MA, Dent J et al (2007) Effect of body position changes on postprandial gastroesophageal reflux and gastric emptying in the healthy premature neonate. J Pediatr 151(6):585–590.e2
44. Horvath A, Dziechciarz P, Szajewska H (2008) The effect of thickened-feed interventions on gastroesophageal reflux in infants: systematic review and meta-analysis of randomized, controlled trials. Pediatrics 122(6):e1268–e1277
45. Corvaglia L, Ferlini M, Rotatori R et al (2006) Starch thickening of human milk is ineffective in reducing the gastroesophageal reflux in preterm infants: a crossover study using intraluminal impedance. J Pediatr 148(2):265–268
46. Beal J, Silverman B, Bellant J, Young TE, Klontz K (2012) Late onset necrotizing enterocolitis in infants following use of a Xanthan gum-containing thickening agent. J Pediatr 161(2):354–356
47. Woods CW, Oliver T, Lewis K, Yang Q (2012) Development of necrotizing enterocolitis in premature infants receiving thickened feeds using SimplyThick[reg]. J Perinatol 32(2):150–152
48. Jadcherla SR, Chan CY, Moore R, Malkar M, Timan CJ, Valentine CJ (2012) Impact of feeding strategies on the frequency and clearance of acid and nonacid gastroesophageal reflux events in dysphagic neonates. J Parenter Enteral Nutr 36(4):449–455
49. Terrin G, Passariello A, De Curtis M et al (2012) Ranitidine is associated with infections, necrotizing enterocolitis, and fatal outcome in newborns. Pediatrics 129(1):e40–e45
50. Graham PLr, Begg MD, Larson E, Della-Latta P, Allen A, Saiman L (2006) Risk factors for late onset gram-negative sepsis in low birth weight infants hospitalized in the neonatal intensive care unit. Pediatr Infect Dis J 25(2):113–117
51. Writing Committee for the American Lung Association Asthma Clinical Research C, Holbrook JT, Wise RA et al (2012) Lansoprazole for children with poorly controlled asthma: a randomized controlled trial. JAMA: J Am Med Assoc 307(4):373–381
52. Orenstein SR, Shalaby TM, Devandry SN et al (2003) Famotidine for infant gastro-oesophageal reflux: a multi-centre, randomized, placebo-controlled, withdrawal trial. Aliment Pharm Therap 17(9):1097–1107
53. Yang YX (2012) Chronic proton pump inihibitor therapy and calcium metabolism. Curr Gastroenterol Rep 14(6):473–479
54. Ito T, Jensen RT (2010) Association of long-term proton pump inhibitor therapy with bone fractures and effects on absorption of calcium, vitamin B12, iron, and magnesium. Curr Gastroenterol Rep 12(6):448–457
55. Orenstein SR, Hassall E, Furmaga-Jablonska W, Atkinson S, Raanan M (2009) Multicenter, double-blind, randomized, placebo-controlled trial assessing the efficacy and safety of proton pump inhibitor lansoprazole in infants with symptoms of gastroesophageal reflux disease. J Pediatr 154(4):514–520.e4
56. Tham SY, Rogers IM, Samuel KF, Singh A, Ong KK (2012) Does oral lansoprazole really reduce gastric acidity in VLBW premature neonates? Med J Malaysia 67(3):284–288

57. Omari TI, Haslam RR, Lundborg P, Davidson GP (2007) Effect of omeprazole on acid gastroesophageal reflux and gastric acidity in preterm infants with pathological acid reflux. J Pediatric Gastroenterol Nutr 44(1):41–44
58. Corvaglia L, Aceti A, Mariani E, De Giorgi M, Capretti MG, Faldella G (2011) The efficacy of sodium alginate (Gaviscon) for the treatment of gastro-oesophageal reflux in preterm infants. Aliment Pharm Therap 33(4):466–470
59. Corvaglia L, Spizzichino M, Zama D et al (2011) Sodium alginate (Gaviscon®) does not reduce apnoeas related to gastro-oesophageal reflux in preterm infants. Early Hum Dev 87(12):775–778
60. Hibbs AM, Lorch SA (2006) Metoclopramide for the treatment of gastroesophageal reflux disease in infants: a systematic review. Pediatrics 118(2):746–752
61. Machida HM, Forbes DA, Gall DG, Scott RB (1988) Metoclopramide in gastroesophageal reflux of infancy. J Pediatr 112(3):483–487
62. Wheatley E, Kennedy KA (2009) Cross-over trial of treatment for bradycardia attributed to gastroesophageal reflux in preterm infants. J Pediatr 155(4):516–521.e1
63. Vandenplas Y, Salvatore S, Hauser B (2005) The diagnosis and management of gastro-oesophageal reflux in infants. Early Hum Dev 81(12):1011–1024
64. Maclennan S, Augood C, Cash-Gibson L, Logan S, Gilbert RE (2010) Cisapride treatment for gastro-oesophageal reflux in children. Cochrane Database Syst Rev (4):CD002300
65. McClure RJ, Kristensen JH, Grauaug A (1999) Randomised controlled trial of cisapride in preterm infants. Arch Dis Child Fetal Neonatal Ed 80(3):F174–F177
66. Cresi F, Marinaccio C, Russo MC, Miniero R, Silvestro L (2008) Short-term effect of domperidone on gastroesophageal reflux in newborns assessed by combined intraluminal impedance and pH monitoring. J Perinatol 28(11):766–770
67. Vieira MC, Miyague NI, Van Steen K, Salvatore S, Vandenplas Y (2012) Effects of domperidone on QTc interval in infants. Acta Paediatrica 101(5):494–496
68. Ng E, Shah VS (2008) Erythromycin for the prevention and treatment of feeding intolerance in preterm infants Cochrane Database Syst Rev (3):CD001815
69. Pacilli M, Chowdhury MM, Pierro A (2005) The surgical treatment of gastro-esophageal reflux in neonates and infants. Semin Pediatr Surg 14(1):34–41
70. Hassall E (2005) Outcomes of fundoplication: causes for concern, newer options. Arch Dis Child 90(10):1047–1052

Chapter 8
Breast Milk Additives and Infant Formula

Jill Sherriff and Gemma McLeod

Abstract Breast milk is recommended for very preterm infants but fortification is required to increase its nutrient density in order to promote growth and development. Even with fortification, those born extremely preterm and those who are fluid restricted may not achieve intrauterine growth targets. Thus, fortification beyond routine amounts may be necessary for some infants. Further study is necessary to determine optimal methods, types and amounts of fortification, as well as upper limits of osmolality, so as to ensure avoidance of feeding intolerance and necrotizing enterocolitis whilst achieving appropriate rate of weight gain and accretion of nutrients. The efficacy of new formulations of fortifiers and infant formulae needs further study.

Key points

- Breast milk requires fortification to meet the nutrient needs of preterm infants; monitoring protein intake via blood urea nitrogen to guide fortification looks promising.
- Breast milk fortifiers have variable effects on osmolality of fortified expressed breast milk.
- Randomised controlled trials are required to assess the efficacy of recently revised formulations of breast milk fortifier and preterm formulae in the context of their ability to meet nutritional requirements for growth and effect on osmolality and feeding intolerance.
- Use of gum-based thickening agents for treatment of regurgitation is associated with increased risk of necrotising enterocolitis in preterm and term infants.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_8,

- Large, longitudinal cohort studies are required to explore the relationship between early nutrition, catch-up growth, metabolic health and neurodevelopmental outcomes.

Mother's own milk (MOM), fresh or frozen, is first recommended for feeding preterm infants and if unavailable, pasteurized donor milk (DM) is preferred over infant formula (IF) [1]. Whilst the undisputed benefits of feeding breast milk (BM) justify this recommendation, BM cannot be used as the reference standard for preterm nutrition because its nutrient content does not meet the estimated needs of the preterm infant. Fortification is therefore recommended [1], and common practice for infants with birth weights below 1,500 g. A Cochrane review from 2004 [2] of ten randomized controlled trials (> 600 infants) demonstrated that fortification of expressed breast milk (EBM) with either a commercial breast milk fortifier (BMF) or at least two individual components was associated with short-term increases in weight (2.33 g/kg/day; 95 % CI 1.73–2.93, $p = 0.00001$), length (0.12 cm/week; 95 % CI 0.07–0.18) and head growth (0.12 cm/week; 95 % CI 0.07–0.16, $p = 0.000037$, $p = 0.00001$), increased nitrogen retention and plasma urea levels. The authors of this Cochrane review conceded that whilst it was unlikely that further studies would be conducted to ratify the need for fortification, future research was required to compare different proprietary, multicomponent preparations and evaluate both short- and long-term outcomes in search of the optimal composition of fortifiers.

1 Limitations of Fortifying Breast Milk

In the past decade, it has become common practice at volumes between 80 and 150 mL/kg/day, to add proprietary, multicomponent BMF to EBM as directed by manufacturers (some initiate fortification at half strength) [3]. The efficacy of this practice is complicated by the variable protein and fat content of BM evident between mothers and across the course of lactation [4] and the differences in composition between preterm and term milk [5–8] and that of pasteurised DM [9]. In addition, the concentration of some micronutrients [10] and the fatty acid profile [4], including long chain polyunsaturated fatty acids incorporated into brain and retinal cell membranes [11], are influenced by maternal diet. These are important considerations when fortifying milk, as rate of weight gain is dependent on absolute protein and energy intakes (and presumably a healthy micronutrient status) and the quality of the weight gain (protein accreted and fat deposited) is dependent on the ratio of protein and energy (PER) [17]; both must be adapted to preterm infants of different gestational ages [12]. Despite best efforts, compared to term infants at the equivalent adjusted age, preterm infants have altered body composition with significantly greater abdominal adipose tissue [13] and intra-hepatocellular lipid (IHCL) [14], a profile that extends into adult life [15]. Ex-preterm adults have been shown to have significantly increased whole-body adiposity, altered adipose tissue partitioning, higher systolic and diastolic blood pressure, and increased IHCL and intra-musculocellular lipid [15]. The concern is that poor nutrition and subsequent poor postnatal growth and

then acceleration of growth beyond intrauterine and normal term-born rates may be associated with detrimental metabolic programming that will influence later health outcomes. Indeed, multiple premorbid biomarkers that have recently been identified in ex-preterm young adults by Thomas et al. [15] are predictive of risk to later metabolic health. Of equal concern is the potential association between suboptimal preterm nutrition in the early postnatal period and impaired neurodevelopment [16–18] which may persist into childhood [19] and beyond [20].

In 2006, Arslanoglu et al. [21] turned their attention to fortification design, and compared the efficacy of an adjustable, EBM fortification feeding regimen (ADJ) to routine fortification. The level of fortification in the ADJ group ($n = 16$) was upgraded or downgraded according to the infant's blood urea nitrogen (BUN) levels, either by using different amounts of a bovine, whey protein powder concentrate (providing an additional 0.3 g or 0.6 g of protein) or by adjusting the amount of breast milk fortifier (BMF) that was routinely added (routine: 5 g BMF/100 mL; adjusted range: 2.5–6.25 g/100 mL), or both. The mean PER was greater in weeks two and three in the ADJ group and weight and head circumference increased at a faster rate during this period (weight (g/kg/day): 17.4 *vs* 14.4, $p < 0.01$; head circumference (cm/kg/day): 1.4 *vs* 1.0, $p < 0.05$).

Retrospective analysis revealed that recommendations for fat and total energy intakes were met. However, protein content of the fortified milk feeds was consistently lower than was assumed resulting in actual protein intakes being significantly lower than anticipated (0.5–0.8 g/kg/day) and smaller in the group receiving routine fortification. Differences in intakes among individual infants ranged from −0.2 to as much as 1.5 g/kg/day [22]. The mean rate of weight gain achieved by the ADJ infants reflected that reported for the fetus [23]. This fortification model holds much promise, but further fine-tuning of the ADJ method is necessary in order to optimise PER and ensure quality of growth for all infants. Measuring the temporal response of BUN levels to different levels of fortification will likely be necessary. Incorporating body composition as a measured outcome in future studies will also be crucial to determining optimal and safe upper limits of protein fortification and to facilitating change in current practice. This is pertinent given the 2010 ESPGHAN-CON commentary [24] suggesting that daily protein intakes as high as 4.5 g/kg and energy intakes between 110–135 kcal/kg (PER 3.2–4.1) are necessary to achieve growth targets. Relative to 2005 Guidelines [25], the Committee also recommends substantial changes to several micronutrient intakes.

Since 2010, the compositions of some proprietary fortifiers have been modified to better optimize fortification levels; studies are required to evaluate the efficacy of these revised formulations. Protein and energy fortification beyond that which these and other formulations are able to provide can be achieved using one or more of the following options (i) an increased amount of proprietary multicomponent fortifier, (ii) hind BM, (iii) individual or combined macronutrient components, including protein powder, glucose polymers, medium chain triglyceride oil, medium chain or long chain fat emulsions and a carbohydrate/fat blend. Further attention to fortification with individual micronutrients may be required and of course, the osmolality of feeds in context of the preterm gut must be considered when exploring optimal upper limits of fortification.

2 Osmolality

Poor motility of the preterm gut limits the volume and osmolality of feeds in order to avoid feeding intolerance which is characterised by a number of issues including poor gastric emptying and abdominal distension.

If medications are used, their addition to small volume feeds can contribute to the osmolality, as does the use of fortification (Table 8.1). Hyperosmolality of feeds is one of the factors proposed to increase the risk of necrotising enterocolitis (NEC) [26] and the current recommendation (cited in Pearson et al. [27]) suggests that the osmolality of enteral feeds should not exceed 450 mOsm/kg. Pearson et al. [27] reviewed the historical consensus and experimental evidence for this upper limit.

In its simplest terms osmolality (osmolar concentration) is a measure of the number of particles in a solution. In more technical terms osmolar concentration is the number of osmoles of solute/kg of solvent. In clinical practice solute concentration is expressed per litre of solution rather than kg of solvent.

Osmolality can be measured either by the depression of freezing point using an osmometer (more common) or by vapour pressure. Alternatively it can be calculated from the following:

1. Non-polar solutes: 1 mol = 1 Osm
2. For salts that dissociate completely to release 2 ions, 1 mol = 2 Osm.

Confusion occurs because there is a difference between solutions measured in a laboratory and *effective in vivo* osmolality (tonicity). The latter is a measure of the movement of H_2O across a semi-permeable membrane. Caffeine, for example permeates cells freely and while it increases measured osmolality, it has no effect on tonicity. Srinivasan et al. [28] created a format to calculate the volume of EBM required as solvent for various additives, an appropriate strategy for salts.

The osmolality of fortified EBM climbs by $< 10\,\%$ after standing for $\sim 24\,h$ (likely due to the amylase activity in BM [29]). Carbohydrate-containing supplements increase the osmolality of milk feeds more than the addition of protein and fat supplements (Table 8.1). The addition of medications to milk feeds will increase osmolality but the effect of each on tonicity will depend upon the chemical nature of each substance.

3 Proprietary Fortifiers

Availability of different brands and compositions of liquid- and powdered-based BMF is dictated to some extent by regional location. One advantage of powdered BMF is that there is minimal dilution of EBM [30]. These fortifiers vary in composition across brands and depending on the brand of fortifier used, add between 1.0 g and 1.2 g of whole or hydrolysed whey bovine protein, 14–17 kcal/100 mL of EBM, and as well, additional calcium, phosphate and varying amounts of other vitamins and minerals. All brands of fortifier fail to meet the Vitamin D guideline, some do not incorporate all nutrients (e.g., fat, iron, selenium, iodine), and various other nutrients

Table 8.1 Osmolality of milk fortified with different fortifiers (range) and of common medications

Product	mOsm/kg H_2O	
Pentavite with Vitamin A (Bayer)	8081	
Vitamin D Solution (Biological)	3614	
[a]Sodium Bicarbonate (AUSPAM)	2071	
Calcium carbonate (AUSPAM)	3456	
Caffeine BP (AUSPAM)	3307	
Ferrous Sulphate (FERRO-LIQUID)	4913	
Dexarnethasone (AUSPAM)	16043	
Sodium Chloride (KEMH)	1861	
Sodium phosphate (KEMH)	627	
Cotrimoxazole (Bactrim) (ROCHE)	3956	
Nystatin (Omegapharm)	1192	
	2 h post preparation	23 h
EBM	290	296
Breast milk fortified with human miIk fortifier (as directed)	416–446	423–479
Fortified breast milk and 1.0 g protein supplement	414–453	420–483
Fortified breast milk and 1.0 g protein supplement and 1.0 mL long chain fat emulsion	417–455	421–479
Fortified breast milk and 1.0 g protein supplement and 1 g fat/carbohydrate supplement	443–489	455–516
Fortified breast milk and 1.0 g protein supplement and 1 g carbohydrate supplement	437–490	453–509

[a] Equipment: Model 3320, Osmometer Advanced Instruments, INC.

are limiting, or found in excess at different volume intakes. Based on our estimates, milk fortified with these products must be fed at least at volumes of 170 mL/kg to meet the *lower* range of protein intakes for infants weighing < 1 kg. Achieving these volumes may be difficult for fluid-restricted preterm infants.

In some brands, fat replaces a portion of the carbohydrate component, thus reducing final osmolality. It is unclear if this strategy and the use of hydrolysed protein in place of intact protein have an impact on growth. Carbohydrate is thought to be the main determinant of growth when protein intake is adequate [31] and use of hydrolysed protein in formula-fed preterm infants to reduce risk of feeding intolerance, has been associated with slower growth [32].

Mineral compounds of differing solubility are used in the manufacture of breast milk fortifiers and therefore both nutrient-nutrient interactions and bioavailability in fortified milk may fluctuate according to the compounds utilised. For example, calcium gluconate and calcium glycerophosphate are highly soluble salts relative to calcium phosphate tribasic and calcium carbonate and infants fed the latter in fortified milk were found to have lower serum concentrations of calcium and phosphorus, and higher levels of alkaline phosphatase and magnesium, compared to those fed fortifier containing the soluble calcium salts [30].

Osmolality can be affected by the extent of protein hydrolysis and the sources and form of the micronutrient component. It should be noted that the osmolality of fortified feeds may be contingent not only on the choice of fortifier/s and the lapse

of time between preparing and then feeding a fortified feed to an infant (Table 8.1), but also by the amounts of weighed powder that is contained within the human milk fortifier sachets, which often exceeds the amounts claimed by the manufacturers (by our calculation, upwards to 22 %).

There is a lack of outcome data on the efficacy of current formulations of BMF and large, well-designed studies are necessary to determine the safe and optimal upper limits of fortification that will promote target growth and metabolic health.

4 Protein Supplements

In a recent randomized trial, Miller et al. [33] compared a specially formulated commercial multicomponent fortifier (1.4 g of protein/100 mL of EBM) with routine fortifier (1 g protein/100 mL). The former yielded better weight gain in the primary analysis, but no difference in head circumference and a difference in length was only demonstrated in the secondary sub-analysis that adjusted for sibling clustering and gestational age [33], indicating perhaps that a different form, or even more protein is required and/or that the nutritional issue is more complex than just that of protein.

5 Energy Supplements

5.1 Fat Supplements

Calogen (SHS, Nutricia) is a high energy, water-soluble fat emulsion containing canola and sunflower oils, provides 0.5 g of fat and 4.5 kcal/mL, is a source of essential fatty acids (EFA) and at 18 mOsm/kg H_2O, has little effect on osmolality. Calogen can be added to infant feeds in 1 % increments as tolerated.

Liquigen (SHS, Nutricia) is a white emulsion comprising medium chain triaclyglycerides (MCT) produced by fractionation of coconut oil, and contains no EFA. Medium chain triaclyglycerides are more easily digested and absorbed. It has an osmolality of 275 mOsm/kg H_2O, provides 0.5 g of fat and 4.5 kcal/mL and as directed, is suitable for children over 1 year of age and should be introduced slowly and upgraded over a period of days, according to tolerance.

5.2 Mixed Fat and Carbohydrate Supplement

Doucal (SHS, Nutricia) is a mixed energy supplement, free from gluten, protein and lactose, containing dried glucose syrup (59 %) and fractionated coconut, safflower and canola oils (41 %) providing medium (35 %) and long chain triacylglycerides (65 %). One gram of the off-white, soluble powder provides 5 kcal of energy and

the supplement can be added to breast milk or formula feeds initially in amounts of 1 g/100 mL and graded daily by 1 % increments up to 3 %, as tolerated. The carbohydrate in Duocal raises the osmolality of feeds.

5.3 Glucose Polymers

Carbohydrate supplements provide a readily digestible source of energy in the form of a neutral flavoured, fine white powder containing maltodextrins. Carbohydrate supplements are free of protein, fat, lactose, gluten and fibre and have a very low electrolyte content, provide around 3.8 kcal/g of soluble powder, and can be added to breast milk or formula feeds when necessary, initially at 1 g powder/100 mL and then upgrading by 1 % daily increments as tolerated. As mentioned, the addition of glucose polymers to EBM raises osmolality.

5.4 Breast Milk-Based Fortification

In 1999, Polberger et al. [34] conducted a Swedish multicentre trial where 32 preterm infants (birth weight: 900 g−1,750 g) were randomised to receive EBM fortified with breast milk protein (BMP) ($n = 16$) or a bovine whey protein fortifier (BF) ($n = 16$) for a period of 25.1 d ± 7.7 and 22.9 d ± 7, respectively. The BMP concentrate was derived from the ultra-filtration of defatted, pasteurized DM and consisted of 68 % protein. The BF provided 1 g protein and 13 kcal/100 mL EBM. Fortification to provide a targeted protein intake of 3.5 g/kg/day was based on infant weight, volume intake (150 mL/kg/day to 170 mL/kg/day), feed tolerance and *measured* protein content of the milk. A multivitamin preparation and additional calcium, phosphorous and iron were also provided depending on birth weight and type of fortifier.

Mean protein intake did not differ significantly between groups (BMP: 3.13 g/kg/day ± 0.14, BF: 3.05 g/kg/day ± 0.15) and the bovine whey protein fortifier attained similar biochemical and growth outcomes similar to those in infants exclusively fed breast milk protein.

5.5 Hind Milk

EBM has been fractionated into fore- and hind milk by mothers using either a time parameter [35] or by observing colour and viscosity change in the pumped milk [36] and hindmilk, which is significantly more energy dense (1.3-fold) than foremilk, has successfully been used to increase the energy density of EBM to promote weight gain in preterm infants [37]. The implication of this practice on body composition is not known but requires study [37]. Consideration of the higher fat-soluble vitamin content of hind milk may also be warranted if used in conjunction with newly formulated fortifiers.

6 Selected Vitamins and Minerals

6.1 Vitamin D

The secosteroid hormone version of vitamin D, 1,25 $(OH)_2D$, plays an undisputed role in calcium homeostasis and bone mineralization, with an as yet incomplete understanding of its roles in immune function and long-term health [38]. It is now recognised that many tissues are capable of forming this active metabolite with renal formation probably being the source of that which participates in calcium homeostasis and bone mineralization [39]. This renal hydroxylation at least, develops by 26 weeks gestation [40], and thus synthesising 1,25 $(OH)_2D$ probably isn't a limitation for the preterm infant.

Circulating maternal unbound 25OHD crosses the placenta, and preterm as well as term infants are born with cord 25OHD levels that are correlated with those of the mother [39, 41]. Vitamin D status is assessed using the concentration of 25OHD, and this circulating substrate for hydroxylation represents the neonate's main store of vitamin D. Although controversial, the currently recommended value for adequacy is $>$ 50 nmol/L 25OHD (20 ng/mL) [42]. Novakovic et al. [41], using a classical twin study design, found that the impact of the maternal 25OHD at 28 weeks on neonatal heel prick concentrations was far greater than that of genetic factors. There is a discrepancy between older studies suggesting that neonatal levels are *lower* than maternal levels [38] and more recent studies showing that they are *higher* particularly when maternal levels are $<$ 50 nmol/L [41]. This discrepancy may relate to changes in analytical methods as some detect the 3-OH epimer which appears to be higher in neonates [43].

There is evidence to suggest that several causes of prematurity are linked to poor maternal vitamin D status [39, 44]. In Australia it is recommended that pregnant women be screened and those with 25OHD values $<$ 50 nmol/L be supplemented [45]. McCloskey et al. [46] have warned against the use of unnecessary vitamin D supplementation (i.e., if 25OHD $>$ 50 nmol/L) in pregnant women, given the lack of evidence for its safety and the observational evidence that suggests a link with later asthma and hay fever in the offspring. This is particularly relevant given the suggestion that the fetus has some capacity to regulate 25OHD levels [43].

The levels of vitamin D in breast milk from vitamin D replete women are generally low [47], and given that the hospitalised preterm infant will not be in a position to synthesise any cutaneous vitamin, further dietary vitamin D is required. An intake of 800–1,000 IU/day is recommended [24] which in practice comes from a combination of sources e.g., fortifier and multivitamin supplement for those being fed breast milk. Raising 25OHD levels is more efficiently done by supplementing the neonate with vitamin D than supplementing lactating mothers who have low vitamin D levels [47].

To date the predominant concern for attaining robust vitamin D status in preterm infants has been in relation to maximising bone mineralisation, for example, in the preterm infant calcium uptake from the intestine appears to be a combination of 1,25 $(OH)_2D$-enhanced uptake and passive diffusion [48]. Clearly the immunological

roles of 1,25 $(OH)_2D$ are of paramount importance to the relatively immunocompromised preterm infant but further research is required to determine if higher levels of vitamin D are required to optimise these functions.

6.2 Calcium and Phosphate

The in utero environment during 3rd trimester fosters extensive bone mineral accretion. Over this trimester the fetus is increasingly able to maintain higher blood calcium and phosphorus levels than the mother [49] and is exposed to a high estrogen environment which allows for a progressive increase in volumetric bone mineral density as demonstrated by longitudinal Dual Energy X ray absorptiometry (DEXA) analysis [50]. After term birth, serum calcium concentrations fall from the high fetal levels as a consequence of the removal of the transplacental calcium supply. The parathyroid glands respond to the fall in ionized calcium but it is initially a deficient response [49]. After reaching the low point within the first 48 h, calcium levels gradually reach adult levels over several days [47]. The reduction in calcium supply and changed hormonal environment result in a rapid but physiological fall in bone mineral apparent density over the next 3–4 months which is not associated with an increase in bone fragility [50].

Preterm birth shortens or even completely removes the unique opportunity for heightened bone mineralization described above. Furthermore, a preterm infant experiences a delayed PTH response because of the immaturity of the parathyroid glands [49]. This, together with the relatively poorer absorptive capacity of the preterm infant's gut, means that early neonatal hypocalcaemia is more likely in preterm babies. Rigo and Sarterre [50] describe a sharp postnatal decrease in bone mineral apparent density from birth to discharge. Length and skeletal growth will continue to occur which contributes further to the reduction in bone density. In contrast to the outcome in term infants, the sequence of events in preterm infants can result in an increase in bone fragility and fracture risk, with those born with birth weight under 1,500 g being particularly vulnerable.

Since the longitudinal DEXA work of Rigo and Senterre [50] it has become apparent that it is not appropriate to expect the preterm infant to match the retention levels of calcium and phosphorus achieved by the fetus. Furthermore the postnatal adaptation that occurs regardless of the timing of birth contributes some of the mineral requirement and highlights the importance of mechanical stimulation. Thus the target retention rate of calcium for preterm infants promoted by ESPGHAN [24] (i.e., 60–90 mg/kg/day retained calcium, assuming adequate protein intake) moved from that required to match fetal rates to one which suppresses the risk of fracture and clinical symptoms of osteopenia. When given calcium (100–160 mg/kg/day) and phosphorus (60–90 mg/kg/day) at these levels, catch-up mineralization seems to occur so that by 6 months corrected age, spine and total bone mineral density, expressed in terms of their weight and height, are in the range of term infants. Abrams [51] highlighted the need for research regarding nutrition requirements for bone health

of extremely preterm babies (gestation < 25 week and birth weight < 800 g). Long-term outcomes, and the influence of maternal vitamin D status, are currently being investigated.

Breast milk provides ~260 mg calcium and 140 mg phosphorus/L in highly bioavailable forms [51]. Despite the enhanced bioavailability of calcium in particular, levels in BM do need to be increased with BMF or supplements.

6.3 Iron

Iron is essential to neural development and > 66 % of an infant's total body iron is acquired during the final trimester of pregnancy. Thus preterm infants are at higher risk of iron deficiency anaemia than term infants because of reduced iron, haemoglobin and ferritin levels at birth, but also because of iron losses due to phlebotomy (estimated at 6 mg/kg/week in VLBW infants), and increased requirement for rapid growth (preterm infants double birth weight within ~2 months). Whilst red blood cell transfusions, erythropoietin treatment and reasonable uptake of exogenous sources of iron offset the impact of these factors, iron supplementation and/or blood transfusions are routinely used to manage anemia of prematurity [52].

In 2010, EPSGHAN recommended a daily iron intake of 2–3 mg/kg (previous 2005 Consensus guideline was 2–4 mg/kg/day [25]), corresponding to 1.8–2.7 mg/100 kcal for preterm infants, a dose also reinforced as being adequate by Mills and Davies [53] in 2012. These authors conducted a Cochrane systematic review of 2,726 low birth weight, predominantly preterm infants from 26 trials to primarily evaluate the effect of prophylactic enteral iron supplementation on growth and neurodevelopmental outcomes and secondly, to determine whether iron supplementation resulted in improved haematological parameters and prevention of other causes of morbidity and mortality. The source and form of iron supplement varied widely (e.g., ferric ammonium citrate, ferrous sulphate, iron edetate and ferrous succinate). Mills and Davies concluded that infants who receive iron supplementation have a slightly higher haemoglobin level, improved iron stores and a lower risk of developing iron deficiency anaemia, compared to non-supplemented infants. No haematological benefit from exceeding a 2–3 mg/kg/day supplemental dose of iron was apparent but the authors determined that further clarity relating to demonstrated long term neurodevelopmental and growth benefits, and the optimum timing and duration of iron supplementation, was required.

Breast milk contains about 0.3–0.4 mg/L of elemental iron [54–56]. Generally, the iron content of preterm formulae, usually ferrous sulphate, ranges from 1.4–1.8 mg/100 mL. Some powdered BMF do not contain iron, but others provide < 1.7 mg of iron when added as directed to 100 mL of EBM (see Appendix 11). Thus, at volume intakes between 150–180 mL/kg/day, iron intake from EBM fortified with a powdered BMF can range from as little as 0.6–0.7 mg/kg/day to 2.6–3.1 mg/kg/day and in some cases, an iron supplement may be necessary for infants who receive milk that is fortified with an iron-free fortifier. This needs consideration when applying

the current recommendation for iron [24], which is to commence prophylactic enteral iron supplementation (given as a separate iron supplement, in preterm formula or in fortified EBM) at 2–4 weeks of age for ELBW infants and at 2–6 weeks of age for VLBW infants. Further supplementation may be required for some infants (e.g., those receiving erythropoietin treatment), but as the human body lacks any mechanism for regulated iron excretion, caution must be taken to ensure excessive iron supplementation does not occur. Iron supplementation should continue after discharge at least until 6–12 months of age, depending on adequacy of iron intake once solid diet is commenced.

6.4 Feed Thickener

The process of stomach contents refluxing into the oesophagus is most commonly due to inappropriate relaxation of the lower oesophageal sphincter and while about half of healthy infants aged 3–4 months regurgitate at least once a day [57], the condition occurs more frequently in neonates and at higher rates in those born preterm [58]. The clinical presentation of neonatal gastroesophageal reflux (GOR), though variable, classically includes regurgitation, posseting and vomiting and sometimes as well, haematemesis, respiratory symptoms, apnoeas, recurrent oxygen desaturation and bradycardias. In 2009, NASPHGN and ESPGHAN [57] jointly recommended that in addition to parental education, consideration be given to using thickened feeds to manage uncomplicated, recurrent regurgitation in otherwise healthy infants.

Feed thickening agents have been developed based on indigestible (cellulose, pectins and gums), and digestible polysaccharides (rice, corn and potato starch). Feed thickener has been advocated [59] and used in some neonatal nurseries to thicken breast milk and formula feeds for preterm infants.

A 2002 Cochrane review [58] focusing on full term infants < 28 days and preterm infants up to 44 weeks post menstrual age did not find any evidence from randomized studies to support or refute the efficacy of feed thickeners in newborn infants with GOR. In 2008, Horvath et al. [60] conducted a systematic review and meta-analysis of 14 RCT evaluating the efficacy of using thickened formula feeds for at least several days to treat GOR in otherwise healthy infants < 6 months of age. The authors reported that use of thickened formulas compared with standard formula significantly increased the percentage of infants with no regurgitation, slightly reduced the number of daily episodes of regurgitation and vomiting and increased weight gain.

A variety of thickening agents are used to develop proprietary anti-regurgitant (AR) formulae and when consumed in normal volumes, these products contain a similar energy density, osmolality, protein, calcium and fatty acid content as standard formula. Excessive energy intake is potentially a concern with long-term intake of formula thickened with rice cereal due to its higher energy density. In one study, use of rice cereal to thicken the feeds of formula-fed infants increased caloric intake by as much as 25 % and promoted significant gains in weight and length compared to infants whose regurgitation was managed solely with postural technique [61].

The experience of some neonatologists suggests that gum is a more effective thickener of BM than rice cereal [62]. However, the effects of thickening agents on metabolic and physiologic responses during infancy have not been adequately elucidated; some may compromise bioavailability of nutrients, including calcium [63]. More alarmingly, there is increasing concern that use of gum-based thickening agents may be linked to fatal NEC. In 1984, Mercier et al. [64] reported a possible association between intestinal occlusion and a pectin and cellulose-based thickener. In 2004, Clarke and Robinson [65] described two cases where infants each born 25 weeks gestation who were fully established on enteral feeds with EBM by postnatal day 12 and 18, respectively, received *carob-thickened* EBM on postnatal day 12 and 24 with onset of NEC day 26 and 30, followed one day later by death. In May 2011, the US Food and Drug Administration (FDA) became aware of 15 cases of preterm infants with NEC possibly associated with use of a xanthan gum-based thickener, and subsequently warned health providers and consumers against the use of the thickener in preterm infants [66]. The manufacturer of the thickener voluntarily recalled their product from one of its plants because of a failure to ensure that bacteria of possible public health significance were destroyed in the manufacturing process at that plant. Subsequent to this and including those noted by the FDA, Beal et al. [67] described 22 cases (preterm $n = 21$) where onset of NEC had occurred after infants well established on enteral feeds (median: 43 days, range: 18–73 days; EBM and formula $n = 11$; formula $n = 10$; EBM $n = 1$) began ingesting xanthan-thickened feeds to manage GOR or feeding dysfunction. Sixteen infants commenced thickened feeds after 37 week post menstrual age (PMA) and the thickening agent was consumed for a median of 13 days (range: 1–31 days) prior to NEC onset. The median PMA and chronological age at NEC onset were 39.7 weeks and 66 days of life, respectively; 14 cases required surgery and 7 infants died.

A set of case series does not prove cause and effect but there is now a growing concern that use of gum thickeners for treatment of GOR in preterm infants may be a risk for NEC. The authors of these case series identified several mechanisms, extrapolated from either the rat model or adult human studies, by which gum-based thickeners might predispose to mucosal injury and NEC: (i) through accumulation of SCFA's (produced by bacterial metabolism of the soluble fibres in the gum component) [68], (ii) bacterial overgrowth and increased enzyme activity [69], (iii) accumulation of intestinal bile acids [70, 71] and (iv) substantial increases in intraluminal water and sugars [71].

Notably, the maximum level of carob bean gum (410) or guar gum (412) permitted as an additive to infant formula in Australia by Food Standards Australian and New Zealand (FSANZ) [72] is 1,000 mg/L (0.1 g/100 mL). A maximum of 300 mg/L of carrageenan (407) is permitted as an additive to liquid infant formula. FSANZ do not permit xanthan gum as an additive to infant formula in Australia. The U.S. FDA [73] has recently extended its warning against the use of xanthan gum-based thickener to include all infants and suggests that further study investigating the link between food thickeners and NEC is necessary. Further investigation evaluating the physiological dose-response of the preterm gut to ingestion of different thickeners is warranted.

7 Preterm Formula

Early in the 20th century when breast milk was unavailable, it was common practice to feed preterm infants buttermilk or diluted, boiled cow's milk with added sugar and to supplement with orange juice, cod liver oil and iron supplements at 3–4 weeks postnatal age [74]. This artificial feed has been replaced over time with more complex formulae that commonly have low osmolality and contain whey/casein protein, glucose polymers, lactose, oligosaccharides, long chain polyunsaturated fatty acids, MCT, EFA, minerals, fat-soluble vitamins and nucleotides.

In 2002, Klein edited a report by an expert panel of the Life Sciences Research Office (LSRO) of the American Society of Nutritional Sciences (ASNS) summarising recommendations for minimum and maximum amounts of protein (2.5–3.6 g/100 kcal) and energy (110–135 kcal/kg/day) and 45 nutrient components of enteral formulas for preterm low-birth-weight infants [75]. This report, together with the 2005 Consensus Nutrition Guidelines [25] and the 2010 ESPGHAN Commentary for enteral nutrient supply [24] have prompted some companies to reformulate their preterm products to provide more than the conventional 2–2.5 g protein/100 mL (PER $\leq$ 3.0 g/100 kcal) and to modify some other components, including micronutrient profiles.

In 2006, using a cross-over design, Cooke et al. [76] compared nitrogen balance, metabolic status and growth in 18 infants fed a standard (3.0 g/100 kcal: RegPro) and high (3.6 g/100 kcal; HiPro) protein infant formula for a week in a crossover design. The protein in both formulae was fully hydrolysed bovine whey protein. Weight gain, paralleled by increased protein accretion, was greater in infants fed the HiPro formula, growth exceeded intrauterine rates and none of the infants developed uremia or metabolic acidosis. This study was limited by the small cohort size and the short duration of the intervention, and clarity is urgently required around longer-term metabolic health and growth outcomes in ELBW preterm infants fed these formulae for longer periods of time.

8 Hydrolysed vs Intact Protein

Hydrolysed infant formulae were primarily developed for infants with cow's milk allergy and were later adapted for primary allergy prevention, the concept being that hydrolysation reduces the antigenicity and allergenicity of milk proteins. The allergenicity of a protein is influenced by its molecular complexity, its solubility and stability and its concentration; the level to which a protein is hydrolysed depends upon its degree of exposure to enzymatic hydrolysis, ultra-heating and ultra-filtration [77]. According to a 2008 AAP clinical report [78], various industry sources have defined partially hydrolysed (PH) formulae as containing fewer oligopeptides that have a molecular weight generally of $<$ 5,000 daltons and extensively hydrolysed (EH) formula as containing only peptides that have a molecular weight $<$ 3,000 daltons.

The Food and Drug Authority determined the relationship between whey partially hydrolysed formula and the reduced risk of atopic dermatitis as uncertain, and has required companies to state the following qualified health claim: "very little scientific evidence suggests that, for healthy infants who are not exclusively breastfed and who have a family history of allergy, feeding a 100 % Whey-Protein Partially Hydrolysed infant formula from birth up to 4 months of age instead of a formula containing intact cow's milk proteins may reduce the risk of developing atopic dermatitis throughout the 1st year of life and up to 3 years of age. Partially hydrolysed formulas should not be fed to infants who are allergic to milk or to infants with existing milk allergy symptoms" [79].

Preterm infants are known to have increased gut permeability compared with term infants [80] in the first few days of life, which appears to rapidly adapt after birth, regardless of gestational age or birth weight [81]. Thus, it seems logical that the risk of preterm infants developing allergic disease is similar to that of any term infant who has at least one first-degree relative (parent or sibling) with documented allergic disease.

Preterm infants are considered high risk for developing feeding intolerance and NEC. Use of hydrolysed, compared to intact protein, in formula-fed term [82] and preterm infants [83, 84] has been shown to accelerate gastrointestinal transit time [83] and to accelerate feeding advancement [84]. The degree to which the protein is hydrolysed may moderate the rate of gastric emptying with increased rates demonstrated with use of extensively, rather than partially, hydrolysed formula [82]. It is unclear whether utilization of the hydrolysed protein is compromised by the tendency towards higher frequency of stools that appear to be generated through its use [83]. Slower weight gain, lower mean change in z-scores for weight and head circumference and higher renal excretion of essential amino acids have been demonstrated in preterm infants randomized to receive a hydrolysed cow's milk preterm formula compared to those who received intact protein [32]. Compensation for these associated outcomes is often made by adjusting (increasing) the nitrogen content in the hydrolysed-protein formula [85]. The benefits or otherwise of feeding a hydrolysed compared to intact protein formula to preterm infants remain unclear and further studies with larger groups are needed to.

9 Summary and Future Research

There is a lack of outcome data on the efficacy of current formulations of BMF and large, well-designed studies are necessary to determine the safe and optimal upper limits of fortification necessary to promote target growth and metabolic health. Further attention to fortification (dose, timing, duration) with individual micronutrients (e.g., vitamin D, calcium, phosphorus) is required and osmolality of fortifiers and fortified feeds must be considered when exploring formulations and optimal upper limits of fortification. The effects of feeding preterm infants hydrolysed *vs* intact protein on gut function, nitrogen absorption/retention and growth needs further study. Alternative methods than those utilising gum-based thickeners for management of regurgitation in otherwise healthy preterm infants are required.

Acknowledgements The authors acknowledge the ongoing support of Professors Karen Simmer & Peter Hartmann, & the expertise on medications provided by Ms Judith Kristensen.

References

1. Johnston M, Landers S, Noble L, Szucs KLV (2012) Breastfeeding and the use of human milk. Pediatrics 129(3):e827–e841. Epub 2012 Feb 27
2. Kuschel CA, Harding JE (2004) Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev 2:CD000343
3. Klingenberg C, Embleton ND, Jacobs SE, O'Connell LA, Kuschel CA (2012) Enteral feeding practices in very preterm infants: an international survey. Arch Dis Child Fetal Neonat Ed 97(1):F56–F61
4. Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL, Hartmann PE (2002) Variation in fat, lactose and protein in human milk over 24 h and throughout the first year of lactation. Br J Nutr 88(1):29–37
5. Gross SJ, Geller J, Tomarelli RM (1981) Composition of breast milk from mothers of preterm infants. Pediatrics 68(4):490–493
6. Lai C (2007) Production and composition of milk from 10–60 days of lactation in mothers who delivered prematurely. University of Western Australia, Nedlands
7. Anderson GH, Atkinson SA, Bryan MH (1981) Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. Am J Clin Nutr 34(2):258–265
8. Lemons JA, Moye L, Hall D, Simmons M (1982) Differences in the composition of preterm and term human milk during early lactation. Pediatr Res 16(2):113–117
9. Wojcik KY, Rechtman DJ, Lee ML, Montoya A, Medo ET (2009) Macronutrient analysis of a nationwide sample of donor breast milk. J Am Diet Assoc 109(1):137–140
10. Allen LH (2005) Multiple micronutrients in pregnancy and lactation: an overview. Am J Clin Nutr 81:1206S–1212S
11. Guesnet P, Alessandri JM (2011) Docosahexaenoic acid (DHA) and the developing central nervous system (CNS)—implications for dietary recommendations. Biochimie 93(1):7–12
12. Rigo J (2005) Protein, amino acid and other nitrogen compounds. In: Tsang R, Uauy R, Koletzko B, Zlotkin S (eds) Nutrition of the preterm infant scientific basis and practical guidelines, 2nd edn. Digital Educational Publishing Inc, Cincinati
13. Uthaya S, Thomas EL, Hamilton G, Dore CJ, Bell J, Modi N (2005) Altered adiposity after extremely preterm birth. Pediatr Res 57(2):211–215
14. Thomas EL, Uthaya S, Vasu V, McCarthy JP, McEwan P, Hamilton G et al (2008) Neonatal intrahepatocellular lipid. Arch Dis Child Fetal Neonat Ed 93:F382–F383
15. Thomas EL, Parkinson JR, Hyde MJ, Yap IK, Holmes E, Dore CJ et al (2011) Aberrant adiposity and ectopic lipid deposition characterize the adult phenotype of the preterm infant. Pediatr Res 70(5):507–512
16. Lucas A, Morley R, Cole TJ, Gore SM, Lucas PJ, Crowle P et al (1990) Early diet in preterm babies and developmental status at 18 months. Lancet 335(8704):1477–1481
17. Stephens BE, Walden RV, Gargus RA, Tucker R, McKinley L, Mance M et al (2009) First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123(5):1337–1343
18. Isaacs EB, Gadian DG, Sabatini S, Chong WK, Quinn BT, Fischl BR et al (2008) The effect of early human diet on caudate volumes and IQ. Pediatr Res 63(3):308–314
19. Lucas A, Morley R, Cole TJ (1998) Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ 317(7171):1481–1487
20. Ehrenkranz R, Dusick A, Vohr B, Wright L, Wrage L, Poole WK (2006) Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 117:1253–1261

21. Arslanoglu S, Moro GE, Ziegler EE (2006) Adjustable fortification of human milk fed to preterm infants: does it make a difference? J. Perinatol 26(10):614–621
22. Arslanoglu S, Moro G, Ziegler E (2009) Preterm infants fed fortified human milk receive less protein than they need. J Perinatol 29:489–492
23. Ziegler E, O'Donnell A, Nelson S, Fomon S (1976) Body composition of the reference fetus. Growth 40:329–341
24. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T et al (2010) Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr 50(1):85–91
25. Tsang RC, Uauy R, Koletzko B, Zlotkin SH (eds) (2005) Nutrition of the preterm infant. Scientific basis and practical guidelines, 2nd edn. Digital Educational Publishing, Inc., Cincinnati, Ohio
26. Patole S (2005) Strategies for prevention of feed intolerance in preterm neonates: a systematic review. J Maternal-Fetal Neonat Med 18(1):67–76. (The official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet)
27. Pearson F, Johnson MJ, Leaf AA (2011) Milk osmolality: does it matter? Arch Dis Child Fetal Neonat Ed 10.1136/adc.2011.300492
28. Srinivasan L, Bokiniec R, King C, Weaver G, Edwards AD (2004) Increased osmolality of breast milk with therapeutic additives. Arch Dis Child Fetal Neonat Ed 89(6):F514–F517
29. Fenton TR, Belik J (2002) Routine handling of milk fed to preterm infants can significantly increase osmolality. J Pediatr Gastroenterol Nutr 35(3):298–302
30. Reis BB, Hall RT, Schanler RJ, Berseth CL, Chan G, Ernst JA et al (2000) Enhanced growth of preterm infants fed a new powdered human milk fortifier: A randomized, controlled trial. Pediatrics 106(3):581–588
31. Collins CT, Gibson RA, Miller J, McPhee AJ, Willson K, Smithers LG et al (2008) Carbohydrate intake is the main determinant of growth in infants born < 33 weeks′ gestation when protein intake is adequate. Nutrition 24(5):451–457
32. Maggio L, Zuppa AA, Sawatzki G, Valsasina R, Schubert W, Tortorolo G (2005) Higher urinary excretion of essential amino acids in preterm infants fed protein hydrolysates. Acta Paediatr 94(1):75–84
33. Miller J, Makrides M, Gibson RA, McPhee AJ, Stanford TE, Morris S et al (2012) Effect of increasing protein content of human milk fortifier on growth in preterm infants born at < 31 week gestation: a randomized controlled trial. Am J Clin Nutr 95(3):648–655
34. Polberger S, Raiha NC, Juvonen P, Moro GE, Minoli I, Warm A (1999) Individualized protein fortification of human milk for preterm infants: comparison of ultrafiltrated human milk protein and a bovine whey fortifier. J Pediatr Gastroenterol Nutr 29(3):332–338
35. Bishara R, Dunn MS, Merko SE, Darling P (2008) Nutrient composition of hindmilk produced by mothers of very low birth weight infants born at less than 28 weeks' gestation. J Hum Lact 24(2):159–67. (official journal of International Lactation Consultant Association)
36. Slusher T, Hampton R, Bode-Thomas F, Pam S, Akor F, Meier P (2003) Promoting the exclusive feeding of own mother's milk through the use of hindmilk and increased maternal milk volume for hospitalized, low birth weight infants (< 1800 g) in Nigeria: a feasibility study. J Hum Lact 19(2):191-198. (official journal of International Lactation Consultant Association)
37. Ogechi AA, William O, Fidelia BT (2007) Hindmilk and weight gain in preterm very low-birthweight infants. Pediatr Int Off J Jpn Pediatr Soc 49(2):156–160
38. Lucas RM, Ponsonby AL, Pasco JA, Morley R (2008) Future health implications of prenatal and early-life vitamin D status. Nutr Rev 66(12):710–720
39. Wagner CL, Taylor SN, Dawodu A, Johnson DD, Hollis BW (2012) Vitamin D and its role during pregnancy in attaining optimal health of mother and fetus. Nutrients 4(3):208–230
40. Salle BL, Delvin EE, Lapillonne A, Bishop NJ, Glorieux FH (2000) Perinatal metabolism of vitamin D. Am J Clin Nutr 71(5 Suppl):1317S-1324S

41. Novakovic B, Galati JC, Chen A, Morley R, Craig JM, Saffery R (2012) Maternal vitamin D predominates over genetic factors in determining neonatal circulating vitamin D concentrations. Am J Clin Nutr 96(1):188–195
42. Institute of Medicine (2010) Dietary Reference Intakes for Calcium and, Vitamin D
43. Thomas S, Fudge A, Whiting M, Coates P (2011) The correlation between third-trimester maternal and newborn-serum 25-hydroxy-vitamin D in a selected South Australian group of newborn samples. BMJ Open 1:e000236. doi;10.1136/bmjopen-2011-000236
44. Lewis S, Lucas RM, Halliday J, Ponsonby AL (2010) Vitamin D deficiency and pregnancy: from preconception to birth. Mol Nutr Food Res 54(8):1092–1102
45. Munns C, Zacharin MR, Rodda CP, Batch JA, Morley R, Cranswick NE et al (2006) Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: a consensus statement. Med J Aust 185(5):268–272
46. McCloskey KM, Wright N, Ponsonby AL, Vuillermin PJ (2011) Neonatal vitamin D supplementation: are the protocols getting ahead of the evidence? Med J Aust 195(11–12):661
47. Kovacs CS (2008) Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr 88(2):520S–528S
48. Rigo J, Pieltain C, Salle B, Senterre J, Rigo J, Pieltain C et al (2007) Enteral calcium, phosphate and vitamin D requirements and bone mineralization in preterm infants. Acta Paediatr 96(7):969–974
49. Hsu SC, Levine MA. Perinatal calcium metabolism: physiology and pathophysiology. Semin Neonatol SN 9(1):23–36
50. Rigo J, Senterre J (2006) Nutritional needs of premature infants: current isues. J Pediatr 149:S80–S88
51. Abrams SA, Abrams SA (2007) In utero physiology: role in nutrient delivery and fetal development for calcium, phosphorus, and vitamin D. Am J Clin Nutr 85(2):604S–607S
52. Lonnerdal B, Hernell O (2010) Homeostatic regulation of iron and its role in normal and abnormal iron status in infancy and childhood. In: Hernell O (ed) Iron in infancy and childhood. Newstec Ltd. Vevey/S Karger AG, Basel, pp 96–104
53. Mills RJ, Davies MW (2012) Enteral iron supplementation in preterm and low birth weight infants. Cochrane Database Syst Rev 14(3):CD005095
54. Domellof M, Lonnerdal B, Dewey KG, Cohen RJ, Hernell O (2004) Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. Am J Clin Nutr 79(1):111–115
55. National Health and Medical Research Council (2006) Nutrient reference values for Australia and New Zealand including Recommended Dietary Intakes. Canberra, Commonwealth of Australia
56. Shaw JC (1982) Iron absorption by the premature infant. The effect of transfusion and iron supplements on the serum ferritin levels. Acta Paediatr Scand Suppl 299:83–89
57. Vandenplas Y, Rudolph CD, Di Lorenzo C, Hassall E, Liptak G, Mazur L et al (2009) Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). J Pediatr Gastroenterol Nutr 49(4):498–547
58. Huang RC, Forbes DA, Davies MW (2002) Feed thickener for newborn infants with gastro-oesophageal reflux. Cochrane Database Syst Rev (3):CD003211 (revised 2004; reprinted 2009)
59. Birch JL, Newell SJ (2009) Gastrooesophageal reflux disease in preterm infants: current management and diagnostic dilemmas. Arch Dis Child Fetal Neonat Ed 94(5):F379–F383
60. Horvath A, Dziechciarz P, Szajewska H (2008) The effect of thickened-feed interventions on gastroesophageal reflux in infants: systematic review and meta-analysis of randomized, controlled trials. Pediatrics 122(6):e1268–e1277
61. Chao HC, Vandenplas Y (2007) Effect of cereal-thickened formula and upright positioning on regurgitation, gastric emptying, and weight gain in infants with regurgitation. Nutrition 23(1):23–28

62. Woods CW, Oliver T, Lewis K, Yang Q (2012) Development of necrotizing enterocolitis in premature infants receiving thickened feeds using SimplyThick®. J Perinatol 32(2):150–152
63. Bosscher D, Van Caillie-Bertrand M, Van Dyck K, Robberecht H, Van Cauwenbergh R, Deelstra H (2000) Thickening infant formula with digestible and indigestible carbohydrate: availability of calcium, iron, and zinc in vitro. J Pediatr Gastroenterol Nutr 30(4):373–378
64. Mercier JC, Hartmann JF, Cohen R, Tran H, Biriotti V, Kessler A. (1984) [Intestinal occlusion and enterocolitis caused by Gelopectose]. Arch Fr Pediatr 41(10):709–710
65. Clarke P, Robinson MJ (2004) Thickening milk feeds may cause necrotising enterocolitis. Arch Dis Child Fetal Neonat Ed 89(3):F280
66. U.S. Food and Drug Administration (2011) [homepage on the internet]. Silver spring: US food and drug administration [updated 2011 Sep 9; cited 2012 July 22]. FDA Warns Not to Feed SimplyThick to Premature Infants; [1 screen] [cited Available from: http://www.fda.gov//ForConsumers/ConsumerUpdates/ucm256250.htm]
67. Beal J, Silverman B, Bellant J, Young TE, Klontz K (2012) Late onset necrotizing enterocolitis in infants following use of a xanthan gum-containing thickening agent. J Pediatr
68. Lin J, Nafday SM, Chauvin SN, Magid MS, Pabbatireddy S, Holzman IR et al (2002) Variable effects of short chain fatty acids and lactic acid in inducing intestinal mucosal injury in newborn rats. J Pediatr Gastroenterol Nutr 35(4):545–550
69. Mallett AK, Wise A, Rowland IR (1984) Hydrocolloid food additives and rat caecal microbial enzyme activities. Food Chem Toxicol 22(6):415–418. (An international journal published for the British Industrial Biological Research Association)
70. Gunness P, Gidley MJ (2010) Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. Food Funct 1(2):149–155
71. Trout DL, Ryan RO, Bickard MC (1983) The amount and distribution of water, dry matter, and sugars in the digestive tract of rats fed xanthan gum. Proc Soc Exp Biol Med Soc Exp Biol Med (New York, NY) 172(3):340–345
72. Foods Standard Australia New Zealand (2011) Australian and New Zealand foods standards code – standard 1.3.1 – food additives – F2011C00892 Infant formula products http://www.comlaw.gov.au/Details/F2011C00892/Html/Volume_2. 2011 [cited Available from: http://www.foodstandards.gov.au/foodstandardscode/]
73. U.S. Food and Drug Administration (2012) FDA expands caution about SimplyThick. 2012 September 18 2012 [cited 2012 October 11 2012]; Available from: http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm256250.htm
74. Hess JH (1922) Premature and congenitally diseased infants. Lea & Febiger, Philadelphia and New York http://www.neonatology.org/classics/hess1922/hess.html
75. Klein CK (2002) Nutrient requirements for preterm infant formulas. J Nutr 132:1395S–1577S
76. Cooke R, Embleton N, Rigo J, Carrie A, Haschke F, Ziegler E (2006) High protein pre-term infant formula: effect on nutrient balance, metabolic status and growth. Pediatr Res 59(2):265–270
77. von Berg A (2006) The concept of hypoallergeniciy for atopy prevention. In: Cooke RJ VY, Wahn U (eds) Nestle nutrition workshop series pediatric program nutrition support for infants and children at risk. Nestec Ltd, Vevey, Switzerland, pp 11–13
78. Greer FR, Sicherer SH, Burks AW (2008) Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics 121(1):183–191
79. Chung CS, Yamini S, Trumbo PR (2012) FDA's health claim review: whey-protein partially hydrolyzed infant formula and atopic dermatitis. Pediatrics 130(2):e408–e414
80. Roberton DM, Paganelli R, Dinwiddie R, Levinsky RJ (1982) Milk antigen absorption in the preterm and term neonate. Arch Dis Child 57(5):369–372
81. van Elburg RM, van den Berg A, Bunkers CM, van Lingen RA, Smink EW, van Eyck J et al (2004) Minimal enteral feeding, fetal blood flow pulsatility, and postnatal intestinal permeability in preterm infants with intrauterine growth retardation. Arch Dis Child Fetal Neonat Ed 89(4):F293–F296

82. Staelens S, Van den Driessche M, Barclay D, Carrie-Faessler AL, Haschke F, Verbeke K et al (2008) Gastric emptying in healthy newborns fed an intact protein formula, a partially and an extensively hydrolysed formula. Clin Nutr 27(2):264–268
83. Mihatsch WA, Hogel J, Pohlandt F (2001) Hydrolysed protein accelerates the gastrointestinal transport of formula in preterm infants. Acta Paediatr 90(2):196–198
84. Mihatsch WA, Franz AR, Hogel J, Pohlandt F (2002) Hydrolyzed protein accelerates feeding advancement in very low birth weight infants. Pediatrics 110(6):1199–1203
85. Picaud JC, Rigo J, Normand S, Lapillonne A, Reygrobellet B, Claris O et al (2001) Nutritional efficacy of preterm formula with a partially hydrolyzed protein source: a randomized pilot study. J Pediatr Gastroenterol Nutr 32:555–561

Chapter 9
Post-Discharge Nutrition for High-Risk Preterm Neonates

Gemma McLeod, Jill Sherriff and Sanjay Patole

Abstract Preterm infants may be nutritionally compromised at discharge, due to unrecovered early protein and energy deficits accumulated during hospital stay and because exclusive breastfeeding is not well established prior to going home. The strategy of enriching breast milk and infant formula to accelerate and catch-up growth must be weighed against the current evidence relating to these practices and in the context of the preterm phenotype at discharge, which persists into adulthood and which differs from that of term-born infants. Commencing the transition from liquid food to nutrient-dense solid foods and then progressing through a variety of textures should be considered in the context of gross motor development.

Key points

- Preterm phenotype at discharge differs from that of term babies and identified pre-morbidity biomarkers suggest that preterm young adults are at increased risk of later, adverse metabolic complications, compared to term-born peers.
- Use of enriched formula should be carefully considered in light of lack of evidence for its use and in relation to the preterm phenotype at discharge; it may be that protein and/or other micronutrients and not energy, are limiting for some infants after discharge.
- Mothers and babies may need extra support in the home after discharge to facilitate successful breastfeeding and to avoid its early cessation.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_9, © Springer Science+Business Media Dordrecht 2013

- Whilst ensuring optimal nutritional intake from liquid diet, a reasonable guide for the introduction of solids appears to be after at least a corrected age of 3 months, when gross motor development should enable safe eating.
- High-risk infants may need individualised nutrition support with regular monitoring of growth after discharge to ensure adequate nutritional intakes.
- Research is necessary to clarify the optimal length, quantity, and method of providing supplemental minerals and vitamin D after hospital discharge for preterm infants, especially for those who are breastfed.

Preterm infants are born with immature organs and compromised immunity and those born small for gestational age (SGA) and/or growth-restricted are at increased risk of mortality and morbidity compared to their appropriately grown, gestation-matched peers [1]. During hospital stay, neurological insults, early sepsis, need for respiratory support, unstable glucose levels, a patent ductus arteriosus, risk of necrotising enterocolitis and feeding intolerance impact on prescribed feeding regimes. More specifically, restricted fluid volumes, increased nutrient requirements, pharmacologically-related nutrient losses, and delayed enteral feeding and milk fortification are frequently encountered. Early nutritional deficits accumulate [2], resulting in poor postnatal growth, coupled potentially with associated, impaired neurodevelopment [3–6].

Extra-uterine growth restriction (EUGR), defined as either a decrease in z-score greater than two standard deviations (SD) between birth and 36 weeks post menstrual age (PMA) [7] or as a weight below the 10th percentile at 36 weeks PMA [8], is common in the preterm population [9]. Whilst at least 45 % of its prevalence has been attributed to nutrition, the true extent of EUGR is difficult to determine, with variations in its reported prevalence [2, 10–14] likely due to (i) the various definitions employed to quantify it [7, 8] (ii) the reference populations against which it is classified [15–19], and (iii) the postnatal ages at which it is assessed.

Avoiding growth restriction in the neonatal intensive care unit with ‘aggressive’ early nutritional strategies, rather than attempting to ‘catch up’ growth after discharge has been the focus of nutrition research and a priority of neonatal clinicians for some years. Aggressive parenteral nutrition has largely focused on the introduction of earlier and higher infusions of amino acids (± lipid) than previously administered [20–23], promoting early, positive nitrogen balance. However, the short- and long-term risks of this strategy have not been extensively assessed and there is little evidence to suggest that aggressive parenteral support in the first days of life confers any long-term benefit.

Unfortunately, recommended early parenteral amino acid intakes of 3.5–4 g/kg/day and energy intakes of around 90 kcal/kg/day are not always sufficient to recover the lean mass that very preterm infants lose before recovering birth weight [24]. Early introduction of trophic enteral feeds, faster achievement of full enteral feeding, earlier and increased fortification of milk feeds and increased nutrient density of formula feeds are the latest enteral feeding strategies aimed at encouraging rates of growth and nutrient accretion that mimics that of the gestational age-matched fetus, without causing metabolic stress. Unfortunately, reported intakes are often

lower than expected [25], and despite best efforts to avoid EUGR, preterm infants are commonly discharged at much earlier gestations than before, with body weights and lengths far below those typical for healthy, term-born infants. Whilst bone mineralization has been shown to normalize in preterm infants between 6 and 12 months of age, and to reach values similar to term born infants, after adjusting for body size [26, 27], recent data suggest preterm infants remain smaller than their peers for some years after discharge [28]. These outcomes are concerning, given the epidemiological evidence in term infants at least, that low birth weight [29] and low weight at one year [30] increases risk of cardiovascular [29] and coronary heart disease [30] and that low growth rates up to one year are associated with increased prevalence of known cardiovascular risk factors, including blood pressure [31].

1 Preterm Phenotype at Discharge

It is alarming too that around discharge, preterm infants have altered adiposity [32–34] and increased intra-hepatocellular lipid [35] compared to their term peers, a phenotype that persists into adulthood [36]. Thomas et al. [36] measured intra-hepatocellular (IHCL) and intra-myocellular (IMCL) lipid in 18–27 years olds born at or below 33 weeks gestation and compared results to those adults of similar age who were born healthy at term. They found that ex-preterm adults had greater (i) total and abdominal adipose tissue; (ii) higher blood pressure; and (iii) more ectopic lipid. It is highly probable that nutrition contributed to these outcomes as it has been demonstrated both in animal models [37–39], and clinically in preterm infants [40–42] that macronutrient composition of the diet influences composition of weight gain. Notably, potential nutritional determinants of fatty liver in the preterm infant population include excessive and extended use of parenteral nutrition and carbohydrate/lipid rich diet in the face of protein deficiency (i.e., excess energy and low protein energy ratio-"PER"). There is growing appreciation that composition of growth must be considered alongside weight, length and head circumference as measures of nutrition adequacy. This realization has prompted nutrition companies to alter the formulation of their available fortifiers and formulas and to introduce new formulas over recent years. Nowadays, when used as directed, human milk fortifiers double the protein content of human milk, conventional preterm formulae (PTF) have an energy range from 75–82 kcal and a protein content of 2–2.5 g/100 mL (PER of < 3) and formula products containing as much as 2.9 g protein per 100 mL (PER of 3.6) [43] have recently become available on the market. It is unclear if these latest formulations improve the quality of growth and the metabolic profile of infants in the short-term and/or if they are efficacious in the long term. What is apparent, is that they widen even further the gap between hospital and home nutrition as infants are forced at discharge to transition from fortified milk feeds (estimated PER < 3.0) and/or PTF (PER ~2.7–3.6) to unfortified breast milk (estimated PER < 2.0) and/or term (PER ~2.2) or 'enriched' formula (PER ~2.5). This transition translates to a

substantial reduction in the amount of protein per unit of energy that infants consume. It seems logical therefore to hypothesise that unhealthy fat deposition (with or without accompanying accelerated weight gain) and further risk to metabolic health may result if high-risk preterm infants are fed diets relatively low in protein and high in energy after discharge. The challenge therefore is to ensure protein and other nutrient intakes are adequate to promote maintenance, recovery or 'catch-up' of lean mass and nutrient accretion, as pertinent to each infant's needs after they go home.

2 Post Discharge Nutrition in Preterm Neonates

Seven years ago, The European Society of Paediatric, Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition (CON) published a medical position paper on feeding preterm infants after hospital discharge [44]. The Committee acknowledged the importance of early nutritional support in influencing long-term health and development, but noted the difficulty in documenting associated neurodevelopmental outcomes because genetics, early neurological insults, and chronic lung disease also impact on neurodevelopment in this population [44]. The Committee encouraged regular monitoring of weight, length and head circumference (HC) in hospital and beyond, to assist clinicians in identifying infants with poor growth who may need additional, individually tailored, nutritional support. The paper classified four typical, preterm growth patterns observed at discharge (see below) and identified infants displaying growth patterns two or three as being at increased risk of long-term growth failure:

1. Appropriate growth for gestational age at birth and discharge (AGA: 10th–90th percentile; or, within 2 SD of reference median birth weight/length for gestational age);
2. Appropriate growth for gestational age at birth (AGA) but below reference growth at discharge ($<$ 10th percentile; $>$ 2 SD below the reference median weight/length for gestational age);
3. Growth restriction at birth (SGA: $<$ 10th percentile, $>$ 2 SD below the reference median birth weight/length for gestational age) and discharge weight below reference growth ($<$ 10th percentile; $>$ 2 SD below the reference median weight/length for gestational age); and
4. Growth restriction at birth (SGA) and recovery to reference growth by discharge (10th–90th percentile; within 2 SD of reference median weight/length for gestational age).

However the long-term anthropometric outcomes of preterm infants have been reported differently in other studies, not according to patterns described by ESPGHAN. For example, a recent, community-based cohort study in the Netherlands followed preterm infants with gestational age (GA): 25 to $<$ 32 weeks, $n = 612$, birth weight (BW): 1,297 $\pm$ 362; GA: 32 $\leq$ 36 weeks, $n = 1{,}123$, BW: 2241 $\pm$ 467) and a random sample of term control infants (GA: 38 $\leq$ 42 weeks, $n = 605$, BW: 3,549 $\pm$ 503)

from birth to 4 years of age and assessed absolute measurements and distribution of weight, length and HC, stratified by GA and gender [28]. Thirty percent of the sample contained multiples of twins (96 %) and some triplets and quadruplets (4 %). In this study, SGA was defined as weight > 2SD below the mean birth weight for GA, based on Kloosterman curves (i.e., < 2nd percentile: (preterm SGA: 3.6 %; term SGA: 2 %). Weight gain was inversely related to GA. Compared with term-born infants, median height and weight of preterm children were lower at all ages and on the absolute scale, there was no catch up in weight or height. There was however early catch up in HC, with measurements being similar to term-born infants by one-year of age. Notably, there was greater variability of growth in boys, suggesting a greater vulnerability to the complications of preterm birth that influence growth. Neurodevelopmental outcomes were not measured.

In a British study [45], although some catch up growth was evident, growth restriction persisted into school age in children who were born at 25 weeks gestation or less. Compared with their classmates, they were 1–1.3 SD lower for all growth parameters. In another study, this time of 1,300 German infants (mean birth weight and gestation: 1.1 kg, and 29 weeks, respectively), rapid catch-up growth was observed in the first year, followed subsequently by gradual height increases during preschool age [46]. At 5 years of age, only 14 % of the cohort were > 2 SD below reference population mean for height; these infants were born of short parents and had shown slow length gain during infancy.

It is difficult therefore, for clinicians to direct nutritional management of preterm infants after discharge when there are no clear nutrition recommendations to guide them and the evidence of poor growth outcomes is so variably reported and interpreted. Nash et al. [47] recently highlighted this difficulty, by demonstrating the potential variance in outcomes obtained when different growth references are employed to assess growth and development. These authors used both the World Health Organisation Growth Standards (WHO-GS) and the Centers for Disease Control and Prevention reference growth charts (CDC-RGC) to assess whether the pattern of growth of AGA very low birth weight (VLBW) infants ($n = 289$) during the first 18–24 months CA was associated with neurodevelopment. The investigators classified growth as sustained (change in z-score ≤ 1 SD), decelerated (decline > 1 SD), or accelerated (incline > 1 SD). Development was assessed using the Bayley Scales of Infant and Toddler Development–III (BSID-III) at 18–24 months corrected age (CA). Twice as many VLBW infants were classified as having decelerated weight gain from birth to 18–24 months CA with the CDC-RGC (35 %), compared with the WHO-GS (17 %). Using the WHO-GS, (a prescriptive set of growth charts that describe how term infants should grow under ideal environmental conditions), children with a decelerated pattern of weight gain had lower cognitive (10 points), language (6 points) and motor (4 points) scores than infants with sustained weight gain ($p < 0.05$), even after adjustment for morbidities. No association was found using the CDC-RGC. Notably, infants who had an accelerated pattern of weight gain, assessed using the WHO-GS (24 %) and CDC-RGC (12 %), had cognitive, language and motor composite scores that did not differ from infants with either sustained or decelerated patterns of weight gain, suggesting an accelerated pattern of growth in the first

2 years may not be associated with a developmental advantage. The authors concluded that a decelerated pattern of weight gain, determined with the WHO-GS, but not the CDC-GRC, is associated with poorer neurodevelopment scores on the BSID-III than a pattern of sustained growth. They hypothesised that many infants classified as having a decelerated pattern of growth, using the CDC-RGC, were actually growing at a rate that supported optimal neurodevelopment. It is yet to be determined if these growth standards are also a more discerning tool for identifying growth patterns associated with adverse metabolic outcomes.

Global consensus on standardising the reference population against which preterm growth outcomes are measured (i.e., WHO-GRC) would facilitate data synthesis and better assist researchers in determining the association between nutrition, postnatal growth patterns and neurodevelopment and help direct clinicians in their nutritional management of infants after discharge.

ESPGHAN-CON have directed clinicians to provide additional nutritional support up until at least 40 weeks PMA and possibly up until about 3 months CA to infants displaying sub-optimal growth at discharge (i.e., growth patterns two and three) [44]. Specifically, it has been recommended that human milk-fed infants should receive supplemented human milk (e.g., with human milk fortifier) and formula fed infants should receive post-discharge formula (PDF).

3 Enriched Human Milk Feeding After Discharge

In 2010, McCormack et al. [48] systematically reviewed the [then] only randomized trial designed to assess the efficacy of fortifying breast milk after discharge to improve the nutrient intakes of preterm infants [49]. Canadian investigators randomised 39 breast-milk fed infants receiving at least 80 % of feeds as breast milk at discharge to receive fortification to half of their total daily milk intake ($n = 19$; birth weight 1253 ± 242; birth gestation weeks: 28.9 ± 1.2) or to continue with unfortified breast milk feeding ($n = 20$; birth weight: 1322 ± 332; birth gestation weeks: 29.8 ± 1.7), from breast or expressed milk, for 12 weeks [49]. They estimated that intervention infants would receive nutrition similar in content to the amount of protein and energy provided by a discharge formula.

At 12 months of age, weight gain ($p = 0.0035$), length ($p = 0.001$) and head circumference (infants born $\leq 1{,}250$ g; $p < 0.0001$) measurements and whole-body bone mineral content ($p = 0.02$) were all significantly greater in the infants who had received fortified human milk. At 4–6 months, their visual acuity was higher ($p = 0.02$) [50] and their estimated intakes of calcium ($p < 0.0001$) and phosphorus ($p < 0.0001$) were greater [49]. Notably, their mean energy intake didn't differ from that of infants drinking unfortified milk, which suggests that the infants receiving fortified milk down regulated their intake according to the nutrient density of the milk—regulating intake to energy density has also been reported in formula-fed infants [51] and in animal models [39]. As percentage fat mass was not significantly

different at 12 months PMA, it's possible that the better growth achieved by the fortified group was due to higher bone mineral and protein content [52].

The authors of this 2010 Cochrane systematic review concluded that growth was improved with milk fortification after discharge but that long-term effects on neurodevelopment and growth were unclear. They also cautioned that fortifying breast milk for breastfed infants is logistically difficult, that it has the potential to interfere with breastfeeding, and that if researchers plan in the future to conduct further trials of this nature, they will first need to ascertain the degree to which this type of intervention is supported by mothers [48].

Subsequent to this Cochrane review, Zachariassen et al. [53] reported a Danish study conducted between July 2004 and August 2008. It involved 207 breast-fed infants who were randomly assigned shortly before discharge to receive either breastfeeding without intervention ($n = 102$) or breastfeeding with intervention ($n = 105$) until 4 months CA. The intervention differed from that employed by O'Connor et al. [49] and consisted of bottle-feeding 20–50 mL/day of expressed mother's own milk, fortified with five sachets of human milk fortifier, providing an additional 17.5 kcal and 1.38 g protein. The remaining infants who were not breastfed ($n = 113$) were fed PTF at discharge. Birth gestation and birth weights ranged from 24–32 weeks and 535–2,255 g, respectively. Weight, length and head circumference (HC) were primary outcomes with some secondary, biochemical outcomes (i.e., haemoglobin, serum phosphate and urea). The study had high attrition rates, with 88 % ($n = 283$) remaining in their assigned nutrition groups until term, 66 % ($n = 211$) remaining in their assigned groups until 2 months CA and only 34 % ($n = 108$) of the original cohort still participating in their assigned nutrition groups at 4 months CA (i.e., unfortified milk $n = 38$; fortified milk $n = 20$; PTF $n = 37$). On the basis of individual assessment, term formula (TF) or PTF was introduced in the first two months after discharge if breastfeeding ceased or needed supplementation, and thereafter, only TF was used for this purpose. Duration of breastfeeding after term was not influenced by fortification (control: 11.8 ± 7.7 *vs.* intervention: 10.6 ± 7.5 weeks). Boys had greater weight, length and HC than girls within all three nutrition groups from term until 1 year of age. In the intention-to-treat (ITT) analyses, PTF-fed infants increased significantly more in length and weight z-scores compared with those who were breastfed. At 2 and 4 months, PTF-fed boys had significantly increased length z-scores, compared with those who were breastfed, whereas a higher increase in length z-scores was evident in PTF-fed girls, compared only to breastfed girls who received no intervention. Furthermore, weight at 2–6 months and HC from term to 4 months were significantly higher in girls in the intervention group, compared to breastfed controls. A second 'per protocol' analysis was conducted in the 211 infants who were participating in the study at 2 months CA. These analyses showed that PTF-fed infants increased significantly more in length and weight z score until 4 months CA compared with breastfed infants. Preterm formula fed-girls increased significantly more in weight z-score compared with breast fed-girls at term and increased significantly more in length z-score compared only with breast fed girls who did not receive the intervention (2–6 months). Since any positive outcomes associated with fortified breast milk were not observed beyond 6 months CA, the authors

concluded that fortifying mother's milk after hospital discharge while breastfeeding very preterm infants did not significantly influence growth parameters at 1 year of age, compared with unfortified mother's milk. Perhaps further trials comparing the efficacy of these two intervention types, with increased levels of fortification, may be useful; however, lactation and breastfeeding support should be provided for mothers and high attrition rates should be anticipated.

4 Enriched Formula Feeding After Discharge

The effect of feeding nutrient-enriched formulas to preterm infants after discharge has frequently been studied [27, 51, 54–64] and often found to improve growth, primarily in males and in infants weighing < 1,251 g [65]. However, in 2012, Young et al. [66] published the results of a systematic review and meta-analysis of 15 randomised controlled trials [27, 51, 54, 57–61, 63, 64, 67–75] of varying methodological quality, exploring the effects of feeding a nutrient-enriched, versus standard TF after preterm discharge. In these trials, infants were fed ad libitum from one month up to 12 months. The control feeds (TF) contained < 73 kcal and < 1.8 g protein/100 mL. There were 2 levels of enriched feeds. The energy and protein content of the first (PDF) contained 73–75 or more kcal and > 1.7 g protein/100 mL ($n = 10$ trials; $n = 762$ infants). The second was classified as PTF and contained at least 76 kcal and at least 2.1 g protein ($n = 5$ trials; $n = 366$ infants). The major measured outcomes included weight, length and HC up to 12–18 months CA. Three trials assessed neurodevelopmental outcomes at 18 months using Bayley Scales of Infant Development and one trial assessed Griffith's Developmental Scales at 6, 9 and 12 months CA. Only at 9 months did the meta-analysis of data from four trials indicate that infants receiving PDF were significantly heavier [weighted mean difference (WMD): 244 (95 % CI 17–471) g; $p = 0.04$] and longer [WMD: 7.3 (95 % CI 1.8–12.9) mm; $p = 0.009$] than infants fed TF. This effect was not seen before or after 9 months of age and meta-analyses did not detect any statistically significant differences at 3–4, 6, 9 or 12 months of age in HC measurement. However, a different outcome was seen in the meta-analyses of PTF versus TF. At 12 [WMD: 540 (95 % CI 255–824) g; $p = 0.0002$] and 18 months [WMD: 491 (95 % CI 142–839) g; $p = 0.006$], meta-analysis of data from 4 and 2 trials, respectively, found preterm infants fed PTF after discharge were significantly heavier than controls who were fed TF. At 18 months [WMD: 11 (95 % CI 1.9–20) mm; $p = 0.02$], meta-analysis of data from two trials found a statistically significant higher length in the PTF group than controls fed TF. Similarly with reference to HC, each meta-analyses of the included trials at 6 [MWD: 5.9 (95 % CI 1.3–10.3) mm; $p = 0.01$], 9 [MWD: 8.0 (95 % CI 0.9–15.2) mm; $p = 0.03$], 12 [MWD: 6.1 (95 % CI 1.1–11.1) mm; $p = 0.02$] and 18 [MWD: 5.4 (95 % CI 0.7–10.1) mm; $p = 0.02$] months found statistically larger HC in the PTF group relative to controls fed TF. Of the four trials that measured neurodevelopmental outcomes, no differences between groups were evident. The Cochrane reviewers'

concluded that their findings did not support expert group and consensus recommendations that formula-fed preterm infants should be fed PDF for up to 12 months post-discharge. However, the reviewers felt that the data did indicate that feeding with PTF, which is only usually solely available throughout the world for hospital use, may increase weight, length and HC up to 12–18 months CA. Cautionary remarks were made by reviewers about the applicability and interpretation of the review findings, given the available data were limited by the short duration of follow-up and by the methodological weaknesses evident in the study designs. They encouraged a systematic review of all studies that have employed post-discharge nutrition strategies to improve growth of preterm infants and they recommended further research to determine what PER and which specific nutrients are key to promoting lean mass and linear growth and to improving developmental outcomes. Since completion of this meta-analysis, Roggero and colleagues [76] have published the final results of their RCT. These investigators randomised 207 preterm infants at term-CA to receive TF (per 100 mL–68 kcal and 1.4 g protein) or PDF (per 100 mL–75 kcal and 2.0 g protein) up to 6 months CA, using a computer-generated randomization list for AGA infants (TF: $n-64$; PDF: $n=59$) and another for SGA infants (TF: $n=43$; PDF: $n=41$). Thirteen infants (AGA $n=8$; SGA $n=5$) withdrew and 115 AGA and 79 SGA infants completed the study. Anthropometric parameters and body composition (measured using an air displacement plethysmograph), were assessed at term and 1, 3 and 6 months CA and anthropometry was again assessed at 12 months CA. Whilst energy intakes were not different between groups, infants fed PDF had significantly higher protein intakes than infants fed the TF at each study point (PDF vs TF AGA: 1 month—3.2 vs. 2.5 $p<0.001$, 3 months—2.6 vs 2.0 $p<0.001$, 6 months—2.6 vs 1.9 $p<0.001$; SGA: 1 month—3.3 vs 2.8 $p<0.05$, 3 months—2.7 vs 2.2 $p<0.05$, 6 months—2.5 vs 2.0 $p<0.05$) and their volume intakes were also significantly lower at 1 and 3 months (and 6 months for SGA infants). No differences in weight and length SD scores existed between either AGA or SGA group. Mean HC values were higher in AGA infants receiving PDF at 6 and 12 months, than in AGA infants fed TF whereas at 6 months, % FM measured using air displacement plethysmography, was lower. No difference in body composition was detected among SGA infants through the study. The authors concluded that feeding a PDF to AGA infants for 6 months after discharge conferred beneficial gains in HC growth and fat-free mass. They also concluded that the growth pattern of SGA preterm infants was not affected by feeding a PDF [74]. Perhaps an explanation for this second conclusion could be that SGA infants require more protein for the same or an even greater period of time than AGA infants after discharge, to improve growth outcomes. Studies employing latest formulas with increased protein content (and perhaps other nutrients) are therefore required to further explore this possibility. Assessing body composition beyond 6 months CA would also be beneficial. These results published by Roggero et al. [76] need to be included in the next Cochrane update.

In summary, nutritional intakes and growth of all high risk breast and formula fed preterm infants after discharge should be regularly monitored and nutrients, including protein, should be supplemented if nutritional status and growth are found to be sub-optimal. *Notably, breastfed preterm infants commonly receive at least an iron supplement after discharge until solid intake is well-established* [77].

5 Feeding Difficulties and Challenges

Feeding difficulties, that potentially develop as a consequence of medical procedures and treatments administered in the NICU, sometimes continue after discharge and have the potential to compromise nutritional status and to impact growth. For example, oral hypersensitivity and a disorganized suck and swallow have been associated with laryngeal trauma from prolonged use of endotracheal tubes. Frequent placement and prolonged use of nasogastric and orogastric tubes is associated with nasal and pharyngeal irritation, conditioned dysphagia and poor response to sensory input [78]. Learned oral aversions that interfere with feeding progress can stress the parents of preterm infants who tend to become over-anxious and employ maladaptive strategies in an attempt to correct the feeding behaviors.

The immediate feeding concern around discharge is that breastfeeding is rarely well established, and its duration after discharge [79, 80], is lower for preterm than for term infants [81]. Limited muscle strength and endurance make the task of breastfeeding very challenging for some infants [82, 83]. Regular follow-up by specialist health professionals is therefore recommended after discharge to help mothers and their infants protect breastfeeding in the home whilst ensuring adequate growth and development. An awareness of infant- and maternal-related factors that influence a mother's early decision to cease breastfeeding is important. These include (i) poor attachment and a disorganised sucking pattern; (ii) a 'sleepy' infant who is not interested in the breast; (iii) slow weight gain; (iv) fussy and unsettled behaviour (v) poor milk supply; (vi) sore cracked nipples, (vii) mastitis, (viii) thrush, (ix) return to work and (x) lack of support in the home, especially when continuing to express milk after discharge [82–84]. Other 'red flags' that have been identified in the NICU as being indicative of future early weaning from the breast, include low birth gestation, low birth weight, extended time to recover birth weight and to reach full feeds, and most indicative, a protracted nursery stay [79]. In addition to those for whom breast feeding is not well established, individualised feeding advice after discharge from specialised members of a multidisciplinary feeding team is strongly encouraged for infants with chronic lung disease and short gut, for those who have reflux and/or are slow feeders and for those whose growth is maintaining a downward trajectory at discharge.

6 Introduction to Solid Foods

As part of normal development, infants are introduced to solid diet when they're unable to consume adequate volume of breast milk or infant formula to meet their nutritional needs [85]. Starting solids provides an opportunity for infants to practice their feeding skills at appropriate developmental stages to minimise risk of later feeding problems. It also provides opportunity for infants to touch and play with food and to be exposed to different flavours, which may promote gradual acceptance of a wide variety of foods in their diet. Chewing is a skill that is developing from early

childhood and starting solids begins the progression from liquid to more textured foods. Delaying timely introduction to solid foods may lead to nutritional compromise and impact on growth. Further, delayed introduction of solids may place an infant at risk of speech delay, due to under-utilisation of tongue and jaw muscles [86]. Data are limited and mostly observational studies inform current thinking and common practices around the introduction of solids for preterm infants.

The World Health Organisation (WHO) [87] recommended in 2001 that mothers should breast-feed their infants exclusively for the first six months of life and introduce complementary foods thereafter, with continued breast-feeding until at least twelve months of age. This policy did not differentiate between healthy term infants and the special needs of preterm infants and those born SGA. More recently, in 2012, the National Health and Medical Research Council in Australia (NHMRC) [88], released new infant feeding guidelines for healthy term infants and recommended that solids should be introduced around six months of age and breastfeeding should continue until 12 months and beyond, for as long as the mother and child desire. The NHMRC cautioned that although many of the described principles of infant feeding could be applied to low birth weight infants, specific medical advice should be sought for preterm infants and underweight infants. The WHO Global Strategy for Infant and Child Feeding [89] states that the introduction of complementary foods must occur when exclusive and frequent breast-feeding can no longer meet the energy and nutrient needs of the infant and should be provided according to a child's signals of satiety and appetite.

In a study investigating the adequacy of protein and energy intakes of preterm infants with chronic lung disease after discharge, McLeod et al. [84] reported that mothers ($n = 26$) introduced solids to their infants at a median corrected age of 3.6 months (range: 2.2–6.0) and a median uncorrected age of 7.0 months (range: 4.0–8.8). The three main reasons cited by mothers for introducing solids were (i) because their infant appeared ready, (ii) because it was recommended and/or (iii) to promote weight gain. Commonly, the first food introduced was rice cereal, with single or mixed vegetables, fruit and porridge being the alternative first foods. In the majority, mothers introduced a variety of vegetables and fruits before incorporating meat or dairy into their infants' diets. As noted in an earlier review [90], Norris et al. [91] conducted structured interviews on milk and complementary feeding practices with 217 mothers of 253 (139 males; 114 females) preterm infants from three hospitals in the United Kingdom. The sample population was skewed towards the higher socio-economic status and older maternal age. Ninety-five percent of the infants were introduced to complementary foods before four months corrected GA. The mean age at which solids were introduced from term was 11.5 ± 0.21 weeks (chronological age: 17.1 ± 0.23 weeks). Formula fed-infants were introduced to solids significantly earlier than both human milk-fed (11.9 ± 0.49 weeks; $p < 0.05$) and combined milk-fed infants (11.9 ± 0.25 weeks; $p < 0.005$). First foods offered included baby rice (84.6 %), baby cereal (4.0 %) rusks (3.4 %) vegetables (3.2 %) and fruit (2.8 %), pureed meat and vegetables (1.2 %) and other dessert, including egg custard (0.8 %). Morgan et al. [92] retrospectively reviewed data from five prospective randomised dietary trials involving a mixed group of 1600 infants, including term AGA, term

SGA and preterm infants. These authors found that infants who were introduced to solids at ≤ 12 weeks were heavier at 12 weeks of age than those who were introduced later. However, by 18 months of age there were no significant differences in size between the two groups, largely due to catch up growth between 9 and 18 months. No significant effects or interactions from introducing solids were observed for measured health outcomes, including diarrhea, vomiting, lower respiratory chest infections, atopy and sleep patterns to 18 months post term. Marriott et al. [93] assessed the effect on growth and iron status in preterm infants of a specially devised 'introduction to solids' strategy that recommended early introduction of foods with a higher energy and protein content than standard milk formula and foods that were rich sources of iron and zinc, compared with current best practices in infant feeding. Provided the infant weighed at least 3.5 kg and was at least 13 weeks post-natal age, the recommendation was to introduce high energy and protein foods as soon as the infants appeared ready. The preterm 'early introduction to solids' strategy significantly influenced dietary intakes with consequent beneficial effects on growth in length and iron status. Potential or actual adverse effects of the early intervention were not discussed in this study.

Generally, evidence is limited and there has been uncertainty by health professionals as to how best direct families about when to introduce solids to their preterm infants. Recently however evidence-based guidelines have been published [85, 86], that underline the importance of good head control for the safe and successful transition to solid foods [94], and document that their introduction should begin around 4–7 [85] months or 5–8 [86] months of uncorrected age, provided that the infant is at least 3 months CA (i.e., when gross motor development should enable safe eating). According to Palmer and Makrides [85], reasonable advice often given to parents of preterm infants is that children are developmentally ready for solid food when they have a reduced tongue thrust (protrusion) reflex, can sit in a stable supported position, can hold their heads up well, open their mouths, and lean forward towards the spoon. This is not thought to be the case until at least 3 months after term corrected age. When commencing solids, it is recommended for otherwise healthy preterm infants, to begin with high-protein, energy and nutrient-dense, texture appropriate, solid foods [85]. Indeed, it could be reasonable to adopt the same recommendation for preterm infants as that recently recommended by the NHMRC for term infants [88], which is to start with iron-containing foods, including iron-enriched infant cereals, pureed meat, poultry and fish (all sources of haem iron), or cooked tofu and legumes and then include vegetables, fruits, and dairy products such as full-fat yoghurt, cheese and custard. Solid foods should be of acceptable taste, without added salt, honey or sugar and be introduced at a rate that suits the infant [88].

Parents of preterm infants are encouraged to be guided by infant cues (e.g., signs of food refusal; infant keeping mouth closed; turning away when food is offered; actively spitting food out; becoming distressed when food is offered), and to consider progressing from puree and mash to minced, lumpy and more textured foods before 9 months uncorrected age. Lack of appearance of teeth is no reason to delay progression to more textured foods [85].

Of note, 'baby-led weaning' is a term used to describe the philosophy of allowing term-born infants the independence to take and eat food themselves whenever they're ready. This can mean that first foods are quite highly textured. Since preterm infants may not have achieved the developmental skills to commence with highly textured foods, it may be necessary (and is encouraged) to first wait until puree and soft foods are being accepted before this philosophy is integrated into the continued progression towards accepting the family diet.

In summary, preterm infants are lighter and shorter than term-born infants at discharge and their altered phenotype of increased abdominal adiposity and fatty liver has been shown to persist into young adulthood, with many preterm adults having increased levels of both, suggesting they are more at risk of metabolic disease later in life, relative to their peers. Benefits of accelerating growth to catch up hospital-accrued nutritional deficits, by using enriched formula feeds to promote appropriate growth, must be carefully weighed against associated evidence and risks. When transitioning from hospital to home, nutritional needs of infants after preterm discharge should be assessed in relation to their medical and clinical history and growth outcomes. Mothers and babies may need extra support to facilitate and to maximise duration of exclusive breastfeeding. Motor development of head control is necessary for safe and successful transition to solid foods and the decision to introduce (at around 3 months CA) and progress solids needs to be considered in the context of developmental cues. Infants identified as being at risk of nutritional deficiency may need individual assessment and multidisciplinary management. Research to clarify the optimal length, quantity, quality and method of providing nutritional support after hospital discharge for preterm infants is necessary.

Acknowledgements The authors acknowledge the continued support of Professors Karen Simmer and Peter Hartmann.

References

1. Damodaram M, Story L, Kulinskaya E, Rutherford M, Kumar S (2011) Early adverse perinatal complications in preterm growth-restricted fetuses. Aust NZ J Obstet Gyn 51(3):204–209
2. Embleton NE, Pang N, Cooke RJ (2001) Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 107(2): 270–273
3. Lucas A, Morley R, Cole TJ, Gore SM, Lucas PJ, Crowle P et al (1990) Early diet in preterm babies and developmental status at 18 months. Lancet 335(8704):1477–1481
4. Stephens BE, Walden RV, Gargus RA, Tucker R, McKinley L, Mance M et al (2009) First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123(5):1337–1343
5. Isaacs EB, Gadian DG, Sabatini S, Chong WK, Quinn BT, Fischl BR et al (2008) The effect of early human diet on caudate volumes and IQ. Pediatr Res 63(3):308–314
6. Lucas A, Morley R, Cole TJ (1998) Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ 317(7171):1481–1487

7. Shah PS, Wong KY, Merko S, Bishara R, Dunn M, Asztalos E et al (2006) Postnatal growth failure in preterm infants:ascertainment and relation to long-term outcome. J Perinat Med 34(6):484–489
8. Ehrenkranz RA (2000) Growth outcomes of very low-birth weight infants in the newborn intensive care unit. Clin Perinatol 27(2):325–345
9. De Curtis M, Rigo J (2004) Extrauterine growth restriction in very-low-birthweight infants. Acta Paediatr 93:1563–1568
10. Clark R, Thomas P, Peabody J (2003) Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics 111:986–990
11. Lemons JA, Bauer CR, Oh W, Korones SB, Papile LA, Stoll BJ et al (2001) Very low birth weight outcomes of the National Institute of Child Health and Human Development Neonatal Research Network, January 1995 through December 1996. NICHD Neonatal Research Network. Pediatrics 107(1):E1
12. Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL et al (1999) Longitudinal growth of hospitalised very low birth weight infants. Pediatrics 104(2 Pt 1): 280–289
13. Kitchen W, Robinson H, Dickinson A (1983) Revised intrauterine growth curves for an Australian hospital population. Aust Paediatr J 19(3):157–161
14. Usher R, McLean F (1969) Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74(6):901–910
15. Lubchenco LO, Hansman C, Boyd E (1966) Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26–42 weeks. Pediatrics 37:403
16. Lubchenco LO, Hansman C, Dressler M, Boyd E (1963) Intrauterine growth as estimated from liveborn birth-weight data at 24–42 weeks of gestation. Pediatrics 32:793–800
17. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M (1996) A United States national reference for fetal growth. Obstet Gynecol 87(2):163–168
18. Thomas P, Peabody J, Turnier V, Clark RH (2000) A new look at intrauterine growth and the impact of race, altitude, and gender. Pediatrics 106(2):E21
19. Babson SG, Benda GI (1976) Growth graphs for the clinical assessment of infants of varying gestational age. J Pediatr 89(5):814–820
20. Thureen PJ, Melara D, Fennessey PV, Hay WW Jr (2003) Effect of low versus high intravenous amino acid intake on very low birth weight infants in the early neonatal period. Pediatr Res 53(1):24–32
21. Ibrahim HM, Jeroudi MA, Baier RJ, Dhanireddy R, Krouskop RW (2004) Aggressive early total parental nutrition in low-birth-weight infants. J Perinatol 24(8):482–486
22. te Braake FW, van den Akker CH, Wattimena DJ, Huijmans JG, van Goudoever JB (2005) Amino acid administration to premature infants directly after birth. J Pediatr 147(4):457–461
23. Valentine CJ, Fernandez S, Rogers LK, Gulati P, Hayes J, Lore P et al (2009) Early amino-acid administration improves preterm infant weight. J Perinatol 29(6):428–432
24. Thureen P (2007) The neonatologist's dilemma: catch up growth or beneficial undernutrition in very low birth weight infants—What are optimal growth rates? JPGN 45:S152–S154
25. Arslanoglu S, Moro G, Ziegler E (2009) Preterm infants fed fortified human milk receive less protein than they need. J Perinatol 29:489–492
26. Fewtrell M, Prentice A, Jones SC, Bishop NJ, Stirling D, Buffenstein R et al (1999) Bone mineralisation and turnover in preterm infants at 8–12 years of age: The effect of early diet. J Bone Miner Res 14:810–820
27. Bishop NJ, King FJ, Lucas A (1993) Increased bone mineral content of preterm infants fed with a nutrient enriched formula after discharge from hospital. Arch Dis Child 68(5 Spec No):573–578
28. Bocca-Tjeertes IF, van Buuren S, Bos AF, Kerstjens JM, Ten Vergert EM, Reijneveld SA (2012) Growth of preterm and full-term children aged 0–4 years: integrating median growth and variability in growth charts. J Pediatr 161(3):460–465 e1

29. Barker DJ, Osmond C, Simmonds SJ, Wield GA (1993) The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. BMJ 306:422–426
30. Barker DJP, Osmond C, Winter PD, Margetts B, Simmonds SJ (1989) Weight in infancy and death from ischaemic heart disease. Lancet 334(8663):577–580
31. Barker DJ, Bull AR, Osmond C, Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. BMJ 301(6746):259–262
32. Cooke RJ, Griffin I (2009) Altered body composition in preterm infants at hospital discharge. Acta Paediatr 98(8):1269–1273
33. Roggero P, Gianni ML, Amato O, Orsi A, Piemontese P, Morlacchi L et al (2009) Is term newborn body composition being achieved postnatally in preterm infants? Early Hum Dev 85(6):349–352
34. Uthaya S, Thomas EL, Hamilton G, Dore CJ, Bell J, Modi N (2005) Altered adiposity after extremely preterm birth. Pediatr Res 57(2):211–215
35. Thomas EL, Uthaya S, Vasu V, McCarthy JP, McEwan P, Hamilton G et al (2008) Neonatal intrahepatocellular lipid. Arch Dis Child Fetal Neonat Ed 93:F382–F383
36. Thomas EL, Parkinson JR, Hyde MJ, Yap IK, Holmes E, Dore CJ et al (2011) Aberrant adiposity and ectopic lipid deposition characterize the adult phenotype of the preterm infant. Pediatr Res 70(5):507–512
37. Ozanne SE, Hales CN (2004) Lifespan: catch-up growth and obesity in male mice. Nature 427(6973):411–412
38. Widdowson EM (1974) Changes in pigs due to undernutrition before birth, and for one, two, and three years afterwards, and the effects of rehabilitation. Adv Exp Med Biol 49:165–181
39. McCance RA, Widdowson EM (1974) The determinants of growth and form. Proc R Soc Lond B Biol Sci 185:1–17
40. Kashyap S, Forsyth M, Zucker C, Ramakrishnan R, Dell RB, Heird WC (1986) Effects of varying protein and energy intakes on growth and metabolic response in low birth weight infants. J Pediatr 108(6):955–963
41. Kashyap S, Schulze KF, Forsyth M, Zucker C, Dell RB, Ramakrishnan R et al (1988) Growth, nutrient retention, and metabolic response in low birth weight infants fed varying intakes of protein and energy. J Pediatr 113(4):713–721
42. Costa-Orvay JA, Figueras-Aloy J, Romera G, Closa-Monasterolo R, Carbonell-Estrany X (2011) The effects of varying protein and energy intakes on the growth and body composition of very low birth weight infants. Nutr J 10:140
43. Fanaro S, Ballardini E, Vigi V (2010) Different pre-term formulas for different pre-term infants. Early Hum Dev 86 Suppl 1:27–31
44. Aggett PJ, Agostoni C, Axelsson I, De Curtis M, Goulet O, Hernell O et al (2006) Feeding preterm infants after hospital discharge: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 42(5):596–603
45. Bracewell MA, Hennessy EM, Wolke D, Marlow N (2008) The EPICure study: growth and blood pressure at 6 years of age following extremely preterm birth. Arch Dis Child Fetal Neonatal Ed 93:F108–F114
46. Trebar B, Traunecker R, Selbmann HK, Ranke MB (2007) Growth during the first two years predicts pre-school height in children born with very low birth weight (VLBW): results of a study of 1,320 children in Germany. Pediatr Res 62(2):209–214
47. Nash A, Dunn M, Asztalos E, Corey M, Mulvihill-Jory B, O'Connor DL (2011) Pattern of growth of very low birth weight preterm infants, assessed using the WHO Growth Standards, is associated with neurodevelopment. Appl Physiol Nutr Metab 36(4):562–569
48. McCormick F, Henderson G, Fahey T, McGuire W (2010) Multinutrient fortification of human breast milk for preterm infants following hospital discharge. Cochrane Database Syst Rev July 7;(7):DC004866 (Wiley)
49. O'Connor DL, Khan S, Weishuhn K, Vaughan J, Jefferies A, Campbell DM et al (2008) Growth and nutrient intakes of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. Pediatrics 121(4):766–776

50. O'Connor DL, Weishuhn K, Rovet J, Mirabella G, Jefferies A, Campbell DM et al (2012) Visual development of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. JPEN 36(3):349–353
51. Cooke RJ, Griffin IJ, McCormick K, Wells JC, Smith JS, Robinson SJ et al (1998) Feeding preterm infants after hospital discharge: effect of dietary manipulation on nutrient intake and growth. Pediatr Res 43(3):355–360
52. Aimone A, Rovet J, Ward W, Jefferies A, Campbell DM, Asztalos E et al (2009) Growth and body composition of human milk-fed premature infants provided with extra energy and nutrients early after hospital discharge: 1-year follow-up. J Pediatr Gastroenterol Nutr 49:456–466
53. Zachariassen G, Faerk J, Grytter C, Esberg BH, Hjelmborg J, Mortensen S et al (2011) Nutrient enrichment of mother's milk and growth of very preterm infants after hospital discharge. Pediatrics 127:e995–e1003
54. Lucas A, Bishop NJ, King FJ, Cole TJ (1992) Randomised trial of nutrition for preterm infants after discharge. Arch Dis Child 67(3):324–327 (see comment)
55. Chan GM, Borschel MW, Jacobs JR (1994) Effects of human milk or formula feeding on the growth, behavior, and protein status of preterm infants discharged from the newborn intensive care unit. Am J Clin Nutr 60(5):710–716
56. Wheeler RE, Hall RT (1996) Feeding of premature infant formula after hospital discharge of infants weighing less than 1,800 g at birth. J Perinatol 16(2 Pt 1):111–116
57. Cooke RJ, Embleton ND, Griffin IJ, Wells JC, McCormick KP (2001) Feeding preterm infants after hospital discharge: growth and development at 18 months of age. Pediatr Res 49(5):719–722
58. Lucas A, Fewtrell MS, Morley R, Singhal A, Abbott RA, Isaacs E et al (2001) Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics 108(3):703–711
59. Carver JD, Wu PYK, Hall RT, Zigeler EE et al (2001) Growth of preterm infants fed nutrient-enriched or term formula after hospital discharge. Pediatrics 107(4):683–689
60. de Curtis M, Pieltain C, Rigo J (2002) Body composition in preterm infants fed standard term or enriched formula after hospital discharge. Eur J Nutr 41(4):177–182
61. Agosti M, Vegni C, Calciolari G, Marini A (2003) Post-discharge nutrition of the very low-birthweight infant: interim results of the multicentric GAMMA study. Acta paediatrica (Oslo. Norway: 1992) Supplement 91(441):39–43
62. Lapillonne A, Salle BL, Glorieux FH, Claris O (2004) Bone mineralization and growth are enhanced in preterm infants fed an isocaloric, nutrient-enriched preterm formula through term. Am J Clin Nutr 80(6):1595–1603
63. Koo W, Hockman E (2006) Posthospital discharge feeding for preterm infants: effects of standard compared with enriched milk formula on growth, bone mass and body composition. Am J Clin Nutr 84:1357–1364
64. Picaud JC, Decullier E, Plan O, Pidoux O, Bin-Dorel S, van Egroo LD et al (2008) Growth and bone mineralization in preterm infants fed preterm formula or standard term formula after discharge. J Pediatr 153(5):616–621, 21 e1–e2
65. Cooke R (2011) Nutrition of preterm infants after discharge. Ann Nutr Metab 58(Suppl 1): 32–36
66. Young L, Morgan J, McCormick Felicia M, McGuire W (2012) Nutrient-enriched formula versus standard term formula for preterm infants following hospital discharge. Cochrane Database Syst Rev Mar 14;(3):CD004696 (Wiley)
67. Cooke RJ, McCormick K, Griffin IJ, Embleton N, Faulkner K, Wells JC et al (1999) Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res 46(4):461–464
68. Cooke RJ, Griffin IJ, McCormick K (2010) Adiposity is not altered in preterm infants fed with a nutrient-enriched formula after hospital discharge. Pediatr Res 67(6):660–664
69. Peng CC, Hsu CH, Kao HA, Hung HY, Chang JH (2004) Feeding with premature or infant formula in premature infants after discharge: comparison of growth and nutrition status. Acta Paediatr Taiwan 45(3):151–157

70. Litmanovitz I, Dofin T, Arnon S, Bauer S, Shainkin-Kestenbaum R, Eliakim L (2004) Bone strength and growth of preterm infants fed nutrient-enriched or term formula after hospital discharge. Pediar Res 274A
71. Litmanovitz I, Eliakim A, Arnon S, Regev R, Bauer S, Shainkin-Kestenbaum R et al (2007) Enriched post-discharge formula versus term formula for bone strength in very low birth weight infants: a longitudinal pilot study. J Perinat Med 35(5):431–435
72. Atkinson S, Paes B, Saigal S, Hussey T, Lee D (2004) Nutrient-enriched discharge formula compared to standard formula does not benefit growth, bone mineral accretion or trace element status in preterm small for gestational age (SGA) infants to one year corrected age: A RCT. Pediatr Res 55:383A
73. Atkinson S, Randall-Simpson J, Chang M, Paes B (1999) Randomised trial of feeding nutrient-enriched vs standard formula to premature infants during the first year of life. Pediatr Res 45:276A–A
74. Roggero P, Gianni ML, Liotto N, Taroni F, Morniroli D, Mosca F (2011) Small for gestational age preterm infants: nutritional strategies and quality of growth after discharge. J Matern-Fetal Neonat Med 24(Suppl 1):144–146 (The official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet)
75. Taroni E, Liotto N, Orsi A, Piemontese P, Amato O, Morlacchi L et al (2009) [Quality of post-discharge growth in small for gestational age preterm infants: an explorative study]. Pediatr Med Chir 31(3):121–125
76. Roggero P, Gianni ML, Amato O, Liotto N, Morlacchi L, Orsi A et al (2012) Growth and fat-free mass gain in preterm infants after discharge: a randomized controlled trial. Pediatrics 130(5):e1215–e1221
77. Greer FR (2007) Post-discharge nutrition: what does the evidence support? Semin Perinatol 31(2):89–95
78. Burklow K, McGrath A, Kaul A (2002) Management and prevention of feeding problems in young children with prematurity and very low birth weight. Inf Young Child 14(4):19–30
79. Maia C, Brandao R, Roncalli A, Maranhao H (2011) Length of stay in a neonatal intensive care unit and its association with low rates of exclusive breastfeeding in very low birth weight infants. J Matern-Fetal Neonat Med 24(6):774–777 (The official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet)
80. Landers S (2003) Maximising the benefits of human milk feeding for the preterm infant. Pediatr Ann 32(5):298–306
81. Perrella SL, Williams J, Nathan EA, Fenwick J, Hartmann PE, Geddes DT (2012) Influences on breastfeeding outcomes for healthy term and preterm/sick infants. Breastfeed Med 7:255–261
82. Wheeler BJ (2009) Human-milk feeding after NICU discharge. Neonatal Netw 28(6):381–389
83. Wheeler J, Chapman C, Johnson M, Langdon R (2000) Feeding outcomes and influences within the neonatal unit. Int J Nurs Pract 6(4):196–206
84. McLeod G, Simmer K, Benninger H, Mitoulas L, Doherty D, Sherriff J (2011) Preterm infants with chronic lung disease: Are protein and energy intakes after discharge sufficient for optimal growth? J Paediatr Child Health 47:127–133
85. Palmer DJ, Makrides M (2012) Introducing solid foods to preterm infants in developed countries. Ann Nutr Metab 60(Suppl 2):31–38
86. King C (2009) An evidence-based guide to weaning preterm infants. Paediatr Child Health 19:403–414
87. World Health Organisation (2001) Global strategy for infant and young child feeding: The optimal duration of exclusive breastfeeding. Fifty-fourth World Health Assembly, Provisional agenda item 131, A54/INF.DOC./4
88. National Health and Medical Research Council. Infant Feeding Guidelines (2012) Canberra:. National Health and Medical Research, Council
89. World Health Organisation (2003) Global strategy for infant and young child feeding. 2003: Geneva

90. McLeod G (2006) Feeding the preterm infant with chronic lung disease post-discharge. Curtin University, Perth
91. Norris FJ, Larkin MS, Williams CM, Hampton SM, Morgan JB (2002) Factors affecting the introduction of complementary foods in the preterm infant. Eur J Clin Nutr 56(5):448–454
92. Morgan JB, Lucas A, Fewtrell MS (2004) Does weaning influence growth and health up to 18 months? Arch Dis Child 89(8):728–733
93. Marriott L, Foote K, Bishop J, Kimber A, Morgan J (2003) Weaning preterm infants: a randomised controlled trial. Arch Dis Child Fetal Neonat Ed 88(4):F302–F307
94. van Haastert IC, de Vries LS, Helders PJ, Jongmans MJ (2006) Early gross motor development of preterm infants according to the Alberta Infant Motor Scale. J Pediatr 149(5):617–622

Part III
Parenteral Nutrition

Chapter 10
The History, Principles, and Practice of Parenteral Nutrition in Preterm Neonates

Stanley J. Dudrick and Alpin D. Malkan

Abstract The history of the successful development of Total Parenteral Nutrition (TPN), first in beagle puppies in the basic science laboratories, and its subsequent clinical translations initially to adults, and shortly thereafter, to a newborn infant, is recounted by the original developer of the techniques, data, and results that have led to its widespread application and acceptance throughout the world. The principles, practices, standards, techniques, observations, technology, and several of the countless details which were so essential in guiding this dream to reality, are woven throughout the narrative. The advances and milestones are traced along this passionate, relentless journey to the present day, when preterm infants are actually expected to live and thrive. The precision and conscientious attention which are essential to the judicious, safe, efficacious use of TPN in preterm neonates throughout all aspects of solution formulation and delivery, together with appropriate monitoring and assessment of outcomes, are described and discussed briefly. The multiple risks and complications associated with this complex life-saving technique are extensively tabulated, with the intention to teach, in order to avoid, prevent, or overcome them. Moreover, attention has been directed toward pointing out many of the persisting shortcomings of the technique which remain to be prevented, overcome, or corrected by future research efforts and experiences. Finally, the costs, philosophy, humanity, and future advancements necessary to apply TPN to the care of preterm infants in developing countries are stated with optimism and hope.

Key points

- The concept of parenteral nutrition (PN) was advocated and attempted long before its first successful achievement almost five decades ago.
- The goal of PN is to support nutrition for optimal growth and development of the infant when the gastrointestinal tract needs time to mature anatomically and functionally to allow enteral feedings without complications.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_10, © Springer Science+Business Media Dordrecht 2013

- PN should be started as soon as possible after birth (within 24 h) to minimize weight loss and endogenous protein catabolism, to maximize growth and development in preterm infants, especially those born under 28 weeks' gestation.
- Thorough knowledge of its principles and practice is necessary for optimizing the benefits and minimising/avoiding the risks of PN.

1 Historical Background

It is obvious and generally acknowledged that the best and most physiologic means for satisfying nutritional requirements is the alimentary tract [1–26]. However, when its use is significantly compromised or precluded for lengthy periods of time, special techniques of nutritional support, including Total Parenteral Nutrition (TPN), are indicated and may become indispensable if the morbidity and mortality of the precipitating situation or condition, its treatment, its sequelae, and its complications, are to be minimized or obviated. The concept of feeding patients entirely parenterally by injecting nutrient substances or fluids directly intravenously, was advocated and attempted long before its first successful achievement almost five decades ago. The realization of this seemingly fanciful 400 year-old dream initially required centuries of fundamental investigation and discovery, coupled with basic technological developments and advancements, and judicious clinical applications. Although most clinicians in the 1950s were aware of the negative impact of starvation on morbidity, mortality, and outcomes, especially following the horrid experiences during World War II, only a relatively small percentage of them understood the necessity for providing adequate nutritional support to their malnourished patients if optimal clinical results were to be achieved. An even smaller number were aware of the multiple important contributions by our many predecessors that were the scientific, technical, and clinical essentials to the eventual development of TPN (Table 10.1) [1]. The fundamental prerequisites to rational clinical studies and improved results in this challenging, but vital area are still true today, and are outlined in Table 10.2 [2]. Although currently available knowledge, components and techniques of parenteral nutritional support have been shown to be utilitarian and life-saving in a wide variety of clinical conditions, TPN support today is still not ideal [3–13]. Much basic and clinical investigation remains to be accomplished and must be stimulated, encouraged, and supported if this technique is to be perfected in order to achieve the ultimate goal of providing optimal nutrition to all patients, under all conditions, at all times.

In the words of the eminent biochemist and nutritionist, Sir David Cuthbertson, "Lest we forget, I would remind you that we all owe our foetal life till parturition to the passage of the nutrients we require from the blood vessels of our mothers into our blood vessels as they traverse the chorionic villi in close relation [14]." It is an important fundamental fact for us to recall that we all began our lives as human beings *in utero*, receiving our nourishment entirely by vein, and we must continue our quest to attempt to emulate that ideal model of intravenous feeding for the support of those who might require a period of TPN for sustaining post-natal life, especially

Table 10.1 Milestones in the development of Total Parenteral Nutrition. (Adapted from [1])

Year	Accomplishment	Investigators
1913	Intravenous infusion of hydrolyzed proteins in animals (dogs) with demonstration of use for nutrition	VanSlyke/Meyer
1915	Intravenous infusion of fat in animals with demonstration of use for nutrition	Murlin/Riche
1924	First continuous intravenous drip infusion of glucose in humans	Matas
1935	First intravenous infusion of cottonseed oil emulsions in humans	Holt
1938	Identification of the essential amino acids and their requirements in humans	Rose
1939	Demonstration of requirements of intravenous amino acids and protein hydrolysates in humans	Elman/Weiner
1940	Demonstration of utilization of crystalline amino acids infused intravenously in humans	Shohl/Blackfan/Dennis
1944	First complete intravenous feeding (water, saline, fat, carbohydrate, amino acids) for 5 days in a 5-month old infant with Hirschsprung's disease	Helfrick/Abelson
1945	Development of first polyethylene catheters for intravenous infusions in humans	Zimmermann
1949	Development of first continuous delivery technique for long-term intravenous infusion of nutrients in dogs	Rhoads/Parkins/Vars
1952	First description of percutaneous subclavian venipuncture to achieve rapid transfusion in severely injured war victims	Aubaniac
1956	Demonstration that intravenous infusion of plasma as the sole protein source in dogs fed a protein-free diet orally could support growth	Allen/Stemmer/Head
1961	Development of first, safe, standardized, and stable intravenous fat emulsion (soybean oil stabilized by egg phosphatides)	Schuberth/Wretlind
1966	Demonstration of long-term normal growth and development in Beagle puppies receiving total parenteral nutrition by central vein	Dudrick/Vars/Rhoads
1967	Infraclavicular, percutaneous subclavian catheterization for central venous pressure monitoring in humans	Mogil/DeLaurentis/Rosemond
1968	First documentation of normal growth and development in an infant nourished entirely by central venous total parenteral nutrition	Dudrick/Wilmore
1968	First comprehensive technique for long-term total parenteral nutrition in human adults and infants	Dudrick/Wilmore/Vars/Rhoads

the preterm neonates, whose feeding by this miraculous mechanism ends abruptly with the clamping and transection of the umbilical cord. The general prerequisites which had to be met for developing safe and effective TPN are listed in Table 10.3 [2, 15].

Additionally, the requirements for nutrients given intravenously to achieve total parenteral nutrition had to be determined or estimated from existing oral nutritional

Table 10.2 The fundamental prerequisites to the initial development of TPN [2]

Comprehensive knowledge of the:	1. anatomy and physiology of the circulation 2. basic biochemical nature of nutrient substrates 3. interrelationships of the nutrient substrates with microbiology, immunology, asepsis, and antisepsis
Relevant knowledge of the complex interactions of nutrient substrates:	1. during normal metabolism, growth, and development 2. under various and/or multiple pathophysiological conditions 3. together with pharmacologic agents and surgical interventions

Table 10.3 The general prerequisites to development of safe and effective TPN [2, 15]

1.	To formulate complete parenteral nutrient solutions
2.	To concentrate the nutrient substrate components up to 5–6 times isotonicity without adverse interactions or precipitation in order not to exceed tolerable fluid limitations
3.	To demonstrate and maintain the utility and safety of central venous catheterization access
4.	To demonstrate and maintain the practicality, efficacy, and safety of long-term continuous central venous infusion of hypertonic nutrient solution
5.	To maintain meticulous asepsis and antisepsis throughout the entire continuum of solution preparation, admixture, and infusion
6.	To anticipate, avoid and correct nutritional and metabolic imbalances, derangements, or adverse reactions

data because precise, comprehensive parenteral nutrient requirements or recommendations were not known or available. The compatibility of the individual components of the intravenous nutrient regimen had to be determined, assured, and maintained under a number of variable situations, including a wide range of ambient temperature changes, exposure to light, time from formulation to infusion, instability during transportation, duration of shelf life, etc. The risk of infection had to be eliminated, or minimized to an acceptable level. As the former was probably impossible, in view of the fact that a foreign body had to be passed through the skin into the bloodstream and remain in place for prolonged periods of time, the latter was essential. Pharmaceutical and medical technology companies had to be convinced to develop, produce, and market the nutrient components and apparatus for safe formulation and administration of TPN within a reasonably affordable cost. Procedures and tests had to be established to assess and monitor the safety and efficacy of a TPN program. Continuous infusion of the solution at a constant rate throughout each 24 h period had to be maintained in order to ensure the administration of the maximally utilizable dosages of each of the nutrients in the mixture for the support of cellular metabolism. This concept was quite different from the usual infusion practices at that time. Furthermore, it was essential to overcome decades of written and verbal expressions by prominent physicians and scientists that long-term total parenteral nutrition was either impossible, improbable, impractical, unaffordable, or folly. Plausible fundamental evidence to the contrary had to be generated if skepticism and prejudices were to be neutralized or overcome, and if widespread clinical acceptance was eventually to occur. Accordingly, efforts were directed toward designing experiments in the laboratory to explore and verify the efficacy and safety of TPN with the ultimate

goal of applying clinically to patients, the basic knowledge, skills and techniques acquired, developed and mastered in animals [2].

The decision to abandon peripheral venous infusion in favor of central venous infusion as the preferred route for providing all required nutrients entirely by vein was a key factor leading to the successful development and clinical application of TPN. The quantity of high quality nutrients required to achieve and maintain positive nitrogen balance and its associated clinical benefits in a critically ill patient or preterm infant had to be concentrated in a volume of water which could be tolerated without untoward complications. The resulting hypertonic nutrient solutions exceeded the normal osmolality of the circulating blood approximately six-fold (1,800 mOsm/L) or more. The infusion of hypertonic solutions of this order of magnitude into peripheral veins caused an intolerable degree of pain, together with an inevitable and unjustifiable inflammation of the intima of the vein and damage to the formed elements of the blood, resulting in inordinately unacceptable phlebitis and thrombophlebitis, and associated adverse secondary consequences and complications. However, it was discovered and demonstrated in the animal laboratory, and subsequently confirmed in human subjects and patients, that hypertonic solutions, when infused at a constant rate over the 24 h of each day through a catheter with its tip in a large central vein, such as the superior vena cava, were rapidly diluted virtually to iso-osmolarity by the high blood flow in these major vessels. By titrating the nutrient and water administration precisely to the metabolic needs and tolerances of each patient, the nutrients and the water were removed or extracted from the circulation by the body cell mass at approximately the rate of infusion, thus avoiding problems of hyperosmolarity, overhydration, and/or nutrient losses in the urine. Accordingly, the successful development of TPN was eventually possible in large part because of the associated technical advances leading to safe, long-term, central venous access, infusion, and catheter maintenance [2, 15].

A plan evolved to concentrate the nutrients required for growth and development of Beagle puppies into the quantity of water the animals could tolerate per day and infuse the resultant 30 % hypertonic solution (1,800–2,400 mOsm/L) into a large diameter, high-blood-flow central vein where it could be diluted instantly to isotonicity [16–19]. Continuous infusion of the nutrient formulation at the maximum rates of utilization and tolerance, without exceeding renal threshold for the individual nutrients, was an additional goal of the protocol. An effective, practical infusion apparatus (which was counterbalanced and utilized a specially designed swivel in order to permit maximum mobility of the animal in the cage) was engineered, crafted, and tailored over a period of several months specifically for continuous intravenous infusions into active, unrestrained puppies [20–23].

During the academic year 1965–1966, six male pedigreed Beagle puppies were fed entirely by central venous infusion for 72–256 days and compared with their littermates fed orally [21]. After weaning at eight weeks of age, the puppies were paired according to their size and weight since birth, housed individually in metal cages and fed a standard oral diet for four weeks to determine their indigenous growth rates. At 12 weeks of age, a polyvinyl chloride (PVC) catheter was inserted into an external jugular vein and advanced into the mid-superior vena cava of one of the

puppies in each pair. The proximal end of the catheter was directed subcutaneously with a trocar and brought out through a puncture wound in the skin between the scapulae. A blunt needle was inserted into the catheter and secured to the back of the animal by a specially-designed stainless steel support apparatus, and an adjustable, tailored, canvas harness. A counterbalanced, swiveled infusion assembly was connected internally to delivery tubing attached to the catheter by a Luer™ fitting, and externally from the swivel to the support apparatus on the animal's back by a modified speedometer cable which protected the vinyl delivery tubing. A peristaltic pump anchored to the top of the cage propelled the solution dependably to the animal below at the desired rate through a 0.22 μ membrane filter attached to the swivel assembly [24]. This specifically engineered apparatus allowed the animal freedom of movement within the cage. During the continuous daily infusion over a 21–23 h period, the animals received the dosages of dextrose, protein hydrolysate, and all of the vitamins and minerals recommended for growth. In the dietary regimens, which included intravenous fat, the emulsion was infused separately over a 2–3 h period. The puppies were disconnected from the infusion apparatus for the remaining 0.5–1.0 h daily for exercise and recreation. The six puppies fed entirely intravenously outstripped their control, orally-fed littermates in weight gain and matched them in skeletal growth, development, and activity for the study periods of 72 days, 100 days (3 puppies), 235 days, and 256 days. Moreover, no significant differences could be discerned among the puppies receiving the three experimental diets which differed primarily in fat content [2, 15].

The two longest-term animals, which were fed for 235 and 256 days, more than tripled their body weights, and developed comparably to their control littermates. In both groups, the deciduous teeth were shed and replaced with permanent teeth at the same time. The intravenously fed animals were just as energetic as the controls, and demonstrated no obvious abnormalities of their skin, coats, or bony development. Having thus demonstrated beyond a doubt that it was possible and practical to feed animals entirely by vein for prolonged periods of time without excessive risks, or compromises of growth and development potential, attention was directed toward applying what had been learned in the laboratory to the treatment of human adults and infants [2, 15].

Subsequently, six severely malnourished patients with chronic, complicated gastrointestinal problems were nourished for 15–48 days entirely by vein with a modified puppy formula [21]. Central venous infusion catheters were maintained in place safely and effectively, sepsis-free for several weeks [24–26].

2 First TPN Treated Infant

In July 1967, a newborn female infant with near-total small bowel atresia underwent operative procedures at the Children's Hospital of Philadelphia [26–28]. Following massive intestinal resection, her duodenum had been anastomosed to the terminal 3 cm of ileum; her weight had declined from 5.1 lb at birth to 4.0 lb at 19 days of

age; she appeared catabolic, hypometabolic and moribund; and it was obvious that she was dying of starvation.

Accordingly, a PVC catheter was inserted via cut-down into her right external jugular vein, was advanced into her superior vena cava, and the other end was passed subcutaneously behind her right ear to emerge through the parietal scalp. It was anticipated theoretically, and based on the puppy experience, that the skin tunnel would reduce the risks of introducing microorganisms into the circulatory system. The infant was initially infused cautiously with a basic nutrient mixture containing hypertonic dextrose, fibrin hydrolysate, electrolytes, and vitamins. Each day or so, another nutrient was added to the mixture so that if the infant experienced an adverse reaction related to the formula change, the probable cause would likely be more apparent. The infusion was delivered continuously by a Harvard™ peristaltic pump though a closed intravenous administration system containing an in-line 0.22 μ membrane filter [21]. It was anticipated that the infection rate associated with an indwelling central venous foreign body inserted percutaneously would be 100 % if left in place indefinitely, thus, prevention of infection was foremost in this model. The initial proactive decision was to leave the catheter *in situ* until the least suggestion or clinical evidence of infection became manifest. If no other logical explanations were obvious and/or if signs or symptoms of infection were evident, the blood was cultured per protocol, the catheter was removed and cultured, and antimicrobial therapy was initiated. Another central venous catheter was inserted, and the intravenous feeding was continued. Following this plan, catheters were maintained sepsis-free clinically for 35–40 days by following meticulous, aseptic and antiseptic principles, and techniques in the insertion and long-term care of the catheters [2, 15].

Total 24 h metabolic balance studies were carried out daily to assist in the evaluation of nutrient utilization and determination of specific metabolic needs to the extent possible. A nylon net stretched over a specially constructed stainless steel metabolic bed cradled the child while allowing collection of all urine. Gastric drainage was easily obtained via the gastrostomy tube. A colostomy bag insured collection of distal gastrointestinal drainage which occurred as bowel function improved. Serum hemoglobin and electrolyte determinations, performed by microanalysis techniques, aided the day-to-day management of the infant using minimal blood volume samples. Body weight and size, and head size, served as the most important indices of growth. Bone age was evaluated at the start and conclusion of the study period with roentgenograms which showed increased bone density and the development of new epiphyseal areas. Muscular activity, coordination, and behavioral advancement were noted and recorded by the nursing staff, pediatricians, and a child development psychiatrist.

The baby weighed 5.1 lb at birth and 4 lb when the central venous catheter was inserted. Forty-five days later, she had gained 3.5 lb in weight and had increased 5.5 cm in length. Her head circumference increased by 6.5 cm, and she manifested normal activity and development for her age [26–28]. Eventually, she was fed for 22 months primarily by vein and achieved a maximum weight of 18.5 lb, during which she had undergone central venous catheterization via her jugular veins six times, her saphenous vein once, her cephalic vein once, and her subclavian veins

Table 10.4 Goals of TPN in the preterm infant [34, 35]

1.	To support optimal growth and development, particularly neurodevelopment at the rate of a fetus of the same gestational age, or along a growth curve consistent with the birth weight
2.	"Buying time" for the gastrointestinal tract to mature anatomically and functionally to allow enteral feedings to be used for optimal support with minimal or without complications
3.	Supporting the immature systems often associated with prematurity, including inadequately developed lung function requiring intubation and mechanical ventilation, and impaired immunocompetence manifested by infections, hypothermia, and hypotension
4.	Minimising both short-term and long-term early enteral feeding complications including necrotizing enterocolitis, failure to thrive, and feeding intolerances
5.	Maximising the nutritional and metabolic status of the neonate in preparation for surgical procedures indicated to correct or overcome major congenital anomalies

eight times. Although she eventually died, extraordinary experience was accrued metabolically and technologically during her management, and her legacy to neonatal parenteral nutrition is unparalleled [29–31]. From an historical point of view, it deserves mention that the standard technique for intravenous infusion of blood or fluid in neonates advocated at that time consisted of hand vein or scalp vein access by small gauge needles attached to rubber infusion tubing and connected proximally to a burette and syringe, sometimes fitted externally to a propelling device to maintain rate and volume control. Sedation was usually essential; the wrists, ankles, and arm-board were restrained; and the head was often immobilized with sand bags. In his textbook, "The Surgery of Infancy and Childhood," Robert Gross stated that, "This arrangement has been used for more than 25 years at the Children's Hospital (Boston), and it has been so satisfactory that we have no desire to change it in any way [32]." Central venous access for pump infusion was clearly a radical departure from this long-standing standard of care.

3 Principles and Practices of Neonatal TPN

3.1 Venous Access Sites

The obvious nutritional goal of TPN in the preterm infant, especially the very low birth weight (VLBW) neonate, less than 1,500 g, is at least five-fold (Table 10.4) [33–39]. In preterm neonates, TPN can be administered either by peripheral veins or by central veins, but peripheral infusions cannot provide nutrition substrates sufficient to support growth and development in ELBW infants weighing less than 1,000 g because the osmolarity of the solution given by peripheral veins is limited to a maximum of 900 milliosmoles per liter. This restriction does not allow delivery of adequate nutritional support, and coupled with the likelihood that the neonate will require TPN for more than two weeks, mandates the placement of a central venous

Table 10.5 Recommended daily neonatal TPN requirements [34, 37]

Day	1	2–3	4–7
Total volume (mL/kg/day)	60–100	80–120	120–150
Dextrose (mg/kg/min)	6–8	7–10	11–13
Amino Acids (g/kg/day)	3	3–3.5	3–4
Lipid emulsion (g/kg/day)	0.5–1	1	1–3
Electrolytes	None		
Potassium (mEq/100 mL TPN)		0.2	0.2
Sodium (mEq/100 mL TPN)		2.6	2.6
Calcium (mmol/100 mL TPN)	None	1.2	1.2
Phosphorus (mmol/100 mL TPN)	None	1.2	1.2
Magnesium (mg/100 mL TPN)	None	6.0	6.0
Zinc (mcg/kg)	None	400	400
Trace elements (MTE4)	None		
Manganese (mcg/kg)		1	1
Selenium (mcg/kg)		2	2
Copper (mcg/kg)		20	20
Chromium (mcg/kg)		0.2	0.2
Vitamins (MVI Pediatric) mL	None	None	2–5
Cysteine (mg/g Amino Acids)	None	40	40
Carnitine*	None		
Heparin (unit/mL TPN)	0.5–1.0	0.5–1.0	0.5–1.0

*Added to TPN at 2 weeks at 2–5 mg/kg if no enteral intake

access by established techniques that are available and are well known to neonatologists, perinatologists, and pediatric surgeons. The usual initial routes of central venous catheterization involve the external and internal jugular veins or the saphenofemoral vein approaches. Placement of "permanent" tunneled vascular access can be facilitated in neonates by ultrasound guidance [35]. Percutaneous, transhepatic, central venous catheters can be used in neonates or infants with multiple venous thromboses who require long-term access [36]. An umbilical artery can be used for access safely for up to 5 days, and the umbilical vein can be used for up to 14 days, if strict adherence to principles of asepsis and antisepsis are practiced. No data-backed recommendation can be made for the most preferred site of catheter insertion to minimize infection risk for a central venous catheter as long as it is tunneled. TPN catheters should never be used for infusion of other fluids or for blood withdrawal if maximum sepsis-free longevity of the catheter is to be expected.

TPN should be started as soon as possible after birth (within few hours) to minimize weight loss and endogenous protein catabolism, to maximize growth outcomes and neurodevelopment, and to minimize adverse outcomes and mortality related to prematurity (Table 10.5) [34, 37].

A standard pediatric TPN solution can be used cost-effectively, efficaciously, efficiently, and safely in about 80–90 % of neonates; can be formulated in the pharmacy, protected from light exposure, and refrigerated, preferably for 1–2 days, but maximally for up to one week prior to infusion; and can possibly improve patient care ultimately by being available on a timely basis, and meeting the neonates' needs more consistently sooner after birth. Some neonates (10–20 %) may require special

individualized TPN formulations, dictated by their metabolic maturity, congenital anomalies, other adverse concomitant conditions, early post-natal surgical procedures, etc. Significant cost reductions (up to 38 %) have been achieved by the use of standardized regimens. However, there are no randomized, controlled trials comparing standardized versus individualized neonatal TPN probably because logistics and patient safety make this unfeasible in the complex preterm population [38, 39]. On the other hand, ample evidence justifies the recommendation that amino acids should be administered optimally as promptly after birth as possible, preferably within 4 h.

A specially formulated pediatric amino acid solution such as TrophAmine™ should be used in preference to adult amino acid formulations. In addition to being neonate-specific, it has been shown to promote better nitrogen balance, has a better essential amino acid to nonessential amino acid ratio, enhances calcium and phosphate solubility, ameliorates development of cholestasis and hepatocyte pathology, and produces an aminogram in the neonate which mimics that of breast-fed infants. Cysteine is added to the TPN solution at a dose of 40 mg/g of total amino acids in the daily ration, as it is a semi-essential amino acid in the preterm neonate [37]. Other amino acids are also likely to be shown to be conditionally or temporarily essential in the preterm neonates as clinical data accrue in the future.

3.2 Monitoring TPN

Monitoring preterm neonates receiving TPN is essential to adjusting and fine-tuning the composition and rate of administration of the solution and to the avoidance of, early discovery of, and prompt, appropriate responses to, nutritional support-associated complications. Urine glucose should be monitored at least daily, or more frequently as indicated in some neonates, since glycosuria usually results from hyperglycemia of a sufficient level to exceed renal threshold for reabsorption. Together with daily blood glucose measurements, the infusion composition and/or rate can be adjusted immediately to maintain the blood sugar concentration in the 50 mg/dL to 100 mg/dL range. However, if this problem persists, the secondary decrease in calorie delivery may result in failure to thrive, or insufficient non-protein calories to allow endogenous and exogenous protein moieties to maintain synthetic and anabolic functions while being catabolized for energy and thermal production. In some neonates, it may be necessary to initiate a continuous simultaneous infusion of insulin (0.1–1.0 unit/mL) in half-strength saline at 0.01–0.1 unit/kg/h, after a loading dose of 0.1 unit/kg is infused over 15–20 min. Blood glucose levels should be determined frequently, and the TPN and insulin infusion rates should be adjusted to each other as required to maintain normoglycemia. The supplemental insulin infusion should be discontinued when the blood glucose is less than 100 mg/dL, and the blood glucose level can be expected to continue to drift downward (because the half-life of insulin is longer than the half-life of glucose), but should never be allowed to drop below 45 mg/dL.

Serum electrolytes, i.e., sodium, potassium, chloride, and bicarbonate are determined daily until stable, and as indicated thereafter, and their dosages in the TPN are adjusted accordingly. Blood urea nitrogen is determined initially, and weekly thereafter. It is a nonspecific marker of amino acid and protein metabolism and administration, which may be low as a result of inadequate provision of amino acids, or may be high as a result of amino acid infusion in excess of metabolic capacity for utilization for synthesis.

Calcium, phosphorus, magnesium, creatinine, bilirubin, alkaline phosphatase, and alanine and aspartate aminotransferases are obtained in one week and then at weekly or biweekly intervals as indicated or desired. Creatinine is primarily a measure of renal function and dictates modulation of TPN components that require renal excretion. The liver function tests may indicate cholestasis or other hepatic cellular dysfunction. The divalent cation levels will help adjust and fine-tune their infusion dosages. Serum triglyceride, prealbumin, albumin, and total protein levels, as well as erythrocyte, leukocyte, and lymphocyte counts may provide some specific additional aids in nutritional assessment, but are not ordinarily the highest priority in the evaluation of early nutritional support of the preterm neonate. Finally, many other additional factors may be monitored in the course of approved, rational, logical clinical studies, which may provide useful knowledge to the field of neonatal nutritional support in the future.

4 Prevention and Treatment of Complications Related to TPN

4.1 Catheter-Related Blood Stream Infection (CRBSI)

It is well recognized and respected that central venous catheter or line infection is the most serious, expensive, and life-threatening complication of TPN, and that the most constant, conscientious vigilance and persistent, meticulous care and attention to the established principles and practices of asepsis and antisepsis in the insertion and continuous, comprehensive maintenance of the catheter and the attached feeding "lifeline" are mandatory for the accomplishment of optimally safe and effective nutritional and metabolic management, and desired outcomes of these precarious, vulnerable patients [40]. The implication of increased morbidity and mortality associated with sepsis in this high risk population has led to recommendations including appropriate systemic antibiotic treatment as well as the use of various antibiotic or ethanol lock techniques [84–86]. However, no single or combination antibiotic regimen for the prevention or treatment of CRBSI has been uniformly successful in the management of this vexing problem. The most prudent and proven current practice recommendations are to follow the guidelines for the prevention and treatment of intravascular catheter-related infections established and updated regularly by the Center for Disease Control [40]. The catheter lock is a technique by which an

antimicrobial solution is used to fill a catheter lumen for a period of time while the catheter is idle. Various antimicrobial locks including vancomycin, ciprofloxacin, gentamicin, and amphotericin B, usually together with heparin, have been studied for prevention of Catheter-Related Blood Stream Infections (CRBSI) with variable efficacy reported between 30–100 % [87] (Table 10.6).

Recently, attention has concentrated on ethanol locks because it is rapidly bactericidal and fungicidal, and resistance is not a concern [88]. However, recently published guidelines by the Infectious Diseases Society of America state that there are still insufficient data to recommend ethanol lock for the treatment of CRBSI and continue to recommend the use of antibiotic locks instead, especially in patients with long-term catheters who have a history of multiple CRBSI despite maximal adherence to aseptic technique [40]. On the other hand, the incidence of line infection in neonates can be relatively low, secondary to the dedication, expertise, perseverance, professionalism, and character of the neonatology, perinatology, and pediatric health care teams. Nonetheless, the threat is, and will always be, present to challenge and test the talent, determination, and mettle of this special group of caregivers who choose to provide optimal nutrition support, especially TPN, to preterm neonates in order to give them not only the best chance for life, but also for the best possible quality of life.

4.2 The Risks and Benefits of Peripheral Infusion Catheters

The *sine qua non* for the successful development of TPN was the abandonment of peripheral venous infusion in favor of central venous infusion [41–49, 89]. All known attempts to meet nutritional requirements intravenously throughout multiple previous decades failed primarily because of the inadequacy of the peripheral venous system to tolerate the necessarily hyperosmolar nutrient substrates. Nonetheless, clinicians who do not follow the established principles of securing central venous access subject the patient to myriad complications related to inadequate catheter placement, position, safety and security. Some of the complications reported in the literature include thrombophlebitis [89], extravasation [41, 42], phrenic nerve injury [43], hemidiaphragmatic paralysis [44], chylothorax [45], liver erosion [46], hydrocoele and periorchitis [47], cardiac tamponade [48], and pleural effusion [49]. Peripheral parenteral nutrition (PPN) is often used in situations in which central venous access is deemed difficult or impossible; in patients with recurrent central venous catheter sepsis; in patients with thrombocytopenia; and for the short-term as a supplement until adequate enteral feeding goals can be achieved [89]. The most frequently perceived benefit of PPN is the relative ease in establishing peripheral venous access, which may prevent delays in establishing indicated nutrition support [89]. The conflicting recommendations from various organizations continue to complicate the literature and confuse practitioners [89]. Either more, controlled, rational studies in this area should be carried out, or this technique should be used only when absolutely no other possible choice of central venous access exists.

Table 10.6 Complications associated with TPN in the preterm neonate

1. Catheter related issues [40–49]	A. Catheter-related blood stream infection (CRBSI) [40] a. Migration of skin organisms into and along the catheter tract b. Direct contamination of the catheter by personnel, devices, fluids c. Hematogenous (metastatic) seeding from another infection source d. Direct contamination of the infusate in preparation and handling B. Others [41–49] a. Extravasation [41, 42] b. Phrenic nerve injury [43] c. Hemidiaphragmatic paralysis [44] d. Chylothorax [45] e. Liver erosion [46] f. Hydrocoele and periorchitis [47] g. Cardiac tamponade [48] h. Pleural effusion [49]
2. Hyperglycemia [50–53]	A. Usually occurs when dextrose infusion rate exceeds the glucose production rate (GPR) of healthy neonates (6–8 mg/kg/min) [51] B. Routine, early, continuous insulin infusion not recommended [52] C. Insulin infusion only when other methods of glucose control (reduction of dextrose infusion rate, elimination of medications predisposing neonates to hyperglycemia, correction of underlying causes of hyperglycemia, such as sepsis, etc) fail [53]
3. Parenteral Nutrition Associated Liver Disease (PNALD) [54–58]	A. Incidence increases with length of TPN therapy [54] B. Early enteral feeding remains the main factor for prevention [55] C. Fat emulsions providing omega-3 fatty acids from fish oil may prevent and/or reverse PNALD [56] D. Amino acid imbalance leading to hepatic dysfunction. Cysteine and/or taurine (semi-essential amino acids) deficiency, especially together with excessive methionine [57] E. Oxidative injury through the loss of antioxidant vitamins and the generation of oxidant products such as: hydrogen peroxide, lipid peroxides, ascorbyl peroxide, and aldehydes can all be generated in TPN solutions exposed to light which results in overproduction of free radicals [58] F. DHEP (di-2-ethylhexylphthalate) toxicity [59] DHEP is an industrially-added plasticizer found in polyvinylchloride (PVC) which increases oxidative stress, cholestasis, and toxicity, especially in preterm neonates. Changing to PVC-free infusion systems decreases these toxicities

Table 10.6 (continued)

4. Aluminum toxicity [60–64]	A. Preterm neonates receiving long-term TPN are at highest risk [61, 62] B. Aluminum is present in TPN components and can leach from some glass [63, 64] C. Toxicity includes encephalopathy, dementia, impaired neurologic development, bone pain, osteopenia, osteomalacia, microcytic anemia, and cholestasis D. Neonatal aluminum exposure may result in long-term (adolescent) impairment of mineralization of long bones, lumbar spine, hip bones, and impaired Bailey Mental Development Index [61, 62]
5. Trace element deficiencies [65–77]	A. Currently, no known or proven ideal trace element mix [65] B. Selenium is required for glutathione peroxidase synthesis [66] C. Selenium deficiency can result in myocardial disorders (Keshan Disease, skeletal muscle disorders, erythrocyte macrocytosis, finger-nail bed abnormalities, and pseudoalbinism [67–70] D. Zinc is required for protein synthesis and nucleic acid, carbonic anhydrase, and alkaline phosphatase metabolism; earliest detectable feature in neonates is decline in growth velocity [71] E. Zinc deficiency impairs innate and acquired immunity [72] F. Severe zinc deficiency causes bullous pustular dermatitis, alopecia, diarrhea, emotional disorder, weight loss, hypogonadism in males, neurosensory disorders, healing problems [73] G. Copper is an essential component of superoxide dismutase, cytochrome oxidase, lysyloxidase, and ceruloplasmin [74] H. Early copper deficiencies include neutropenia, osteoporosis, and microcytic anemia which is resistant to iron [75] I. Long-term copper deficiencies include neurological abnormalities, anorexia, failure to thrive [76] J. Chromium is a cofactor for insulin, facilitating its initial attachment to peripheral receptors K. Chromium deficiency is associated with carbohydrate intolerance, abnormally high insulin requirement, low respiratory quotient, weight loss, and peripheral neuropathy [77] L. Manganese is a cofactor for several enzyme systems M. Manganese deficiency can cause impaired skeletal muscle development and ataxia [77] N. Molybdenum is an enzyme cofactor for xanthine oxidase (involved in purine metabolism) and for sulfite oxidase O. Molybdenum deficiency is associated with tachycardia, tachypnea, vomiting, central scotomas, and coma [77]

Table 10.6 (continued)

6. Vitamin deficiencies are rare and are discussed in the text [77]	
7. Refeeding Syndrome [78]	A. A potentially lethal condition characterized by severe electrolyte and fluid shifts associated with metabolic abnormalities in severely malnourished infants undergoing overly aggressive nutritional support orally, enterally, or by TPN B. Can be prevented or reversed by reducing feeding quantity and rate, and/or by providing higher doses of phosphorus, potassium, and magnesium [78]
8. Choline Deficiency [79, 80]	A. Precursor for phospholipid (cell membrane) synthesis [79] B. Deficiency associated with hepatic morphologic (steatosis) and hepatic aminotransferase abnormalities in long-term TPN [80] C. Parenteral choline is not available in the United States, thus limiting its use in clinical neonatology practice
9. Glutamine [81–83]	A. The most abundant amino acid in plasma and human milk, however, is not included in TPN solutions because of its instability in solution [81] B. Can be given in TPN as a dipeptide, alanyl-glutamine, but is not available for intravenous use in the United States C. An essential fuel for enterocytes, leukocytes, cell division, acid-base balance, and for renal handling of ammonia [82] D. Double blind, randomized, controlled trial showed that glutamine added to TPN did not improve outcome in preterm neonates [83]

5 Extravasation Injury

In a series of 1,800 intravenous extravasations, only 2.2 % resulted in skin injury, and none required a skin graft [90]. Conservative management, which is the mainstay of treatment in 98 % of extravasation injuries, includes: first aspirating and then removing the catheter, not inserting a new catheter anywhere in the affected extremity, elevating the limb above the level of the heart, splinting the extremity in a position of function, and serial examinations over the first 48 h after injury. Ice or heat applications are controversial because they may exacerbate the injury, and their use is neither safe nor justified. The most important maneuver to eliminate the extravasated fluid is to elevate the affected extremity. The use of antidotes such as: hyaluronidase, phentolamine, and nitroglycerin after intravenous extravasation injury is also controversial, and their use is generally not recommended. Randomized, prospective studies on the efficacy of antidotes in extravasation injuries in neonates are not available [90]. *Elevate the limb and protect it from additional interventional injury!*

6 Photo-Oxidative Injury, Light Exposure and Light Protection

Preterm neonates are more vulnerable to oxidative stress because of their exposure to highly hyperoxic environments, and their immature antioxidant systems which can play a significant role in a number of morbid conditions including: chronic lung diseases, respiratory distress syndrome, retinopathy of prematurity, intracranial hemorrhage, necrotizing enterocolitis, and parenteral nutrition associated liver disease (PNALD) [91, 92]. Lipid peroxide and hydrogen peroxide are generated in light exposed TPN, and light sensitized riboflavin present in parenteral multivitamin (MVI) preparation catalyzes electron transfer between electron donors, such as vitamin C, dissolved lipids and oxygen, and amino acids in the TPN [92]. Other light sensitive vitamins in TPN include vitamins A and K. Compounded TPN solution to which MVI is added more than 24 h prior to administration and preserved in a refrigerator under 500 lux or stronger light strength potentially can lose about 10 % of their light-sensitive components. Reusable, opaque, light-protection covers are recommended to help preserve the integrity of the TPN solution. With use of triple or quadruple chamber TPN bags, together with the opaque light-protection cover, all light-sensitive components were maintained at 90 % or higher levels up to 7 days after squeezing the bag and activating the components. However, without the light protection cover, all light sensitive components could be maintained at 90 % or higher levels only for 24 h.

6.1 Changing TPN Solution Bags

The current acceptable standard of care mandates that all TPN solution bags are replaced every 24 h to minimize deterioration and untoward chemical interactions.

7 Parenteral Nutrition Associated Liver Disease (PNALD)

The cause of PNALD, also known as intestinal failure associated liver disease (IFALD), is complex, multifactorial and poorly understood [93]. Some evidence exists that certain components of TPN may be harmful, but bacterial endotoxins and the lack of enteral feeding are also believed to play significant roles in its development. Severe and progressive liver disease is more common in neonates than in adults, which suggests that the liver disease in neonates may have a pathophysiology different from that of adults, or that the neonatal liver may be more susceptible to injury. Established risk factors associated with PNALD include: prematurity, low birth weight, prolonged duration of TPN, intestinal stasis with bacterial overgrowth, early and/or recurrent catheter-related sepsis, and a diagnosis of gastroschisis or jejunal atresia. Preventative strategies include: early institution of enteral feedings, early weaning and cycling of TPN, and reduction in the dose of lipid emulsions

administered with TPN. Recent work also shows the efficacy of fish oil-based lipid emulsions containing omega-3 fatty acids in the possible prevention and/or treatment of PNALD (Table 10.6).

8 TPN Alternatives, Costs, and Future Use in Developing Nations

The use of a standardized TPN regimen instead of a specially, individually-formulated regimen has resulted in reduction in TPN costs by nearly 30 % [39, 94]. Moreover, it has been demonstrated that the costs of TPN differ among countries, but that a major portion of these expenditures can be attributed to reimbursements for staff services. In providing TPN for children less than 2 years of age, an analysis showed that the average cost per bag (25 % for nutrients, 18 % for supplies, 54 % for wages, 3 % for equipment) ranged between 60 and 90 € among the member nations in the European Union [94]. Specially prepackaged, sterile, multiple-compartment plastic bags designed specifically to contain and separate the required neonatal TPN components (in order to prevent or minimize possible chemical interactions of the nutrient substrates), can be readily transported, stored, and made available anywhere in the world. Immediately prior to infusion, the caregiver can simply squeeze the plastic bag sufficiently to cause the internal separating membranes of the compartments to rupture, allowing the nutrient components to be mixed within the bag and delivered intravenously to the infant. As with all medical innovations, the initial costs will likely be high (and probably prohibitively so, in under-developed countries), but will eventually become more affordable, cost-effective, and safer, while facilitating broader application of TPN by greatly simplifying the process at the bedside, and greatly reducing labor costs and the need for expensive special pharmacy formulation apparatus. However, the ultimate success of an ambitious, potentially life-saving endeavor of this magnitude will be dependent primarily upon the interest, generosity, ethics, morals, and humanity of the citizenry of the economically more advantaged nations. Finally, our future behavior in caring for our less fortunate neighbors in such matters will define and reveal our true character.

References

1. Dudrick SJ, Palesty JA (2011) Historical highlights of the development of total parenteral nutrition. Surg Clin North Am 91:693–717
2. Dudrick SJ (2005) Rhoads lecture: a 45 year obsession and passionate pursuit of optimal nutrition support: puppies, pediatrics, surgery, geriatrics, home TPN, A.S.P.E.N., et cetera. JPEN J Parenter Enteral Nutr 29:272–287
3. Dudrick SJ (1970) Intravenous feeding as an aid to nutrition in disease. CA Cancer J Clin 20:198–211
4. Dudrick SJ, Steiger E, Long JM et al (1970) Role of parenteral hyperalimentation in management of multiple catastrophic complications. Surg Clin North Am 50:1031–1038

5. Dudrick SJ, Wilmore DW, Steiger E et al (1969) Reversal of uremia and body wasting with intravenous essential amino acids. Fed Proc 28:808
6. Dudrick SJ, Wilmore DW, Steiger E et al (1970) Intravenous essential amino acids and hypertonic glucose in the treatment of renal failure. Medizin Ernahrung 11:111–117
7. Dudrick SJ, Wilmore DW, Steiger E et al (1970) Spontaneous closure of traumatic pancreatoduodenal fistulas with total intravenous nutrition. J Trauma 10:542–553
8. Wilmore D, Dudrick SJ (1969) Treatment of acute renal failure with intravenous essential L-amino acids. Arch Surg 99:669–673
9. Wilmore D, Dudrick SJ, Samuels GSA et al (1969) The role of nutrition in small bowel adaptation following massive intestinal resection. Fed Proc 28:305
10. Dudrick SJ, Rhoads JE (1971) New horizons for intravenous feedings. JAMA 215:939–949
11. Dudrick SJ, Steiger E, Long JM (1970) Renal failure in surgical patients: treatment with intravenous essential amino acids and hypertonic glucose. Surgery 68:180–186
12. Long JM, Steiger E, Dudrick SJ et al (1971) Total parenteral nutrition in the management of esophagocutaneous fistulas. Fed Proc 30:30
13. Steiger E, Wilmore DW, Dudrick SJ et al (1969) Total intravenous nutrition in the management of inflammatory disease of the intestinal tract. Fed Proc 28:808
14. Cuthbertson D (1980) Historical background to parenteral nutrition. Acta Chir Scand Suppl 498:1–11
15. Dudrick SJ (2003) Early developments and clinical applications of total parenteral nutrition. JPEN J Parenter Enteral Nutr 27:291–299
16. Elman R (1940) Parenteral replacement of protein with the amino-acids of hydrolyzed casein. Ann Surg 112:594–602
17. Allen JG, Head LR, Stemmer E (1956) Similar growth rates of litter mate puppies maintained on oral protein with those on the same quantity of protein as daily intravenous plasma for 99 days as only protein source. Ann Surg 144:349–355
18. Clark DE, Brunschwig A (1942) Intravenous nourishment with protein, carbohydrate and fat in man. Proc Soc Exp Biol Med 49:329–332
19. Helfrick FW, Abelson NM (1944) Intravenous feeding of a complete diet in a child: report of a case. J Pediatr 25:400–403
20. Rhode CM, Parkins W, Vars HM (1949) Method for continuous intravenous administration of nutritive solutions suitable for prolonged metabolic studies in dogs. Am J Physiol 159:409–414
21. Dudrick SJ, Wilmore DW, Vars HM, Rhoads JE (1968) Long-term total parenteral nutrition with growth, development, and positive nitrogen balance. Surgery 64:134–142
22. Dudrick SJ, Steiger E, Wilmore DW, Vars HM (1970) Continuous long-term intravenous infusion in unrestrained animals. Lab Anim Care 20:521–529
23. Dudrick SJ, Vars HM, Rhoads JE (1967) Growth of puppies receiving all nutritional requirements by vein. Fortschritte der Parenteralen Ernahrung 1–4
24. Dudrick SJ, Wilmore DW (1968) Long-term parenteral feeding. Hosp Pract 3:65–78
25. Wilmore DW, Dudrick SJ (1969) Safe long-term venous catheterization. Arch Surg 98:256–258
26. Dudrick SJ, Wilmore DW, Vars HM, Rhoads JE (1969) Can intravenous feeding as the sole means of nutrition support growth in the child and restore weight loss in an adult? An affirmative answer. Ann Surg 169:974–984
27. Wilmore DW, Dudrick SJ (1968) Growth and development of an infant receiving all nutrients exclusively by vein. JAMA 203:860–864
28. Dudrick SJ, Groff DB, Wilmore DW (1969) Long term venous catheterization in infants. Surg Gynecol Obstet 129:805–808
29. Wilmore DW, Dudrick SJ (1969) An in-line filter for intravenous solutions. Arch Surg 99: 462–463
30. Dudrick SJ, Rhoads JE (1972) Total intravenous feeding. Sci Am 226:73–80
31. Wilmore DW, Groff DB, Bishop HC, Dudrick SJ (1969) Total parenteral nutrition in infants with catastrophic gastrointestinal anomalies. J Pediatr Surg 4:181–189
32. Gross RE (1953) Preoperative and postoperative care. In: The surgery of infancy and childhood. W. B. Saunders, Philadelphia, pp 22–30

33. Thureen PJ (2007) The neonatologist's dilemma: catch-up growth or beneficial undernutrition in very low birth weight infants-what are optimal growth rates? J Pediatr Gastroenterol Nutr 45:S152–S154. Review
34. Schanler RJ (2012) Parenteral nutrition in premature infants. Official reprint from www.uptodate.com, pp 1–29
35. Arul GS, Livingstone H, Bromley P, Bennett J (2010) Ultrasound-guided percutaneous insertion of 2.7 Fr tunnelled Broviac lines in neonates and small infants. Pediatr Surg Int 26:815–818
36. Mortell A, Said H, Doodnath R, Walsh K, Corbally M (2008) Transhepatic central venous catheter for long-term access in paediatric patients. J Pediatr Surg 43:344–347
37. Valentine CJ, Puthoff TD (2007) Enhancing parenteral nutrition therapy for the neonate. Nutr Clin Pract 22:183–193. Review
38. Morgan C, Herwitker S, Badhawi I et al (2011) SCAMP: standardised, concentrated, additional macronutrients, parenteral nutrition in very preterm infants: a phase IV randomised, controlled exploratory study of macronutrient intake, growth and other aspects of neonatal care. BMC Pediatr 10:53
39. Yeung MY, Smyth JP, Maheshwari R, Shah S (2003) Evaluation of standardized versus individualized total parenteral nutrition regime for neonates less than 33 weeks gestation. J Paediatr Child Health 39:613–617
40. O'Grady NP, Alexander M, Bruns LA, Dellinger EP, Garland J, Heard SO (2011) Guidelines for the prevention of intravascular catheter-related infections, 2011. http://www.cdc.gov/hicpac/pdf/guidelines/bsi-guidelines-2011.pdf. Accessed Sept 2012
41. Belloni B, Andres C (2011) Images in clinical medicine: extravasation of peripherally administered parenteral nutrition. N Engl J Med 364:e20
42. Shareena I, Khu YS, Cheah FC (2008) Intraperitoneal extravasation of total parental nutrition infusate from an umbilical venous catheter. Singapore Med J 49:e35–e36
43. Ozdemir R, Oguz S, Uras N et al (2011) Phrenic nerve injury due to thoracentesis for TPN effusion in a preterm newborn: consecutive two unusual complications. Tuberk Toraks 59: 384–387
44. Tosello B, Michel F, Merrot T et al (2011) Hemidiaphragmatic paralysis in preterm neonates: a rare complication of peripherally inserted central catheter extravasation. J Pediatr Surg 46: E17–E21
45. Johnson TJ, Jamous FG, Kooistra A, Zawada ET (2010) Iatrogenic chylothorax due to pleural cavity extravasation of total parenteral nutrition in two adults receiving nutrition through a peripherally inserted central catheter. Hosp Pract (Minneap) 38:50–52
46. Coley BD, Seguin J, Cordero L, Hogan MJ, Rosenberg E, Reber K (1998) Neonatal total parenteral nutrition ascites from liver erosion by umbilical vein catheters. Pediatr Radiol 28:923–927
47. Krüse-Ruijter MF, Robben SG, Degraeuwe PL (2011) Hydrocoele and periorchitis after extravasation of parenteral nutrition solution. Arch Dis Child Fetal Neonat Ed 96:F359
48. Haass C, Sorrentino E, Tempera A et al (2009) Cardiac tamponade and bilateral pleural effusion in a very low birth weight infant. J Matern Fetal Neonat Med 22:137–139
49. Been JV, Degraeuwe PL (2008) Pleural effusion due to intra-abdominal extravasation of parenteral nutrition. Pediatr Pulmonol 43:1033–1035
50. Foote KD, MacKinnon MJ, Innis SM (1991) Effect of early introduction of formula vs fat-free parenteral nutrition on essential fatty acid status of preterm infants. Am J Clin Nutr 54:93–97
51. Sinclair JC, Bottino M, Cowett RM (2011) Interventions for prevention of neonatal hyperglycemia in very low birth weight infants. Cochrane Database Syst Rev 10:CD007615. Review
52. Bottino M, Cowett RM, Sinclair JC (2011) Interventions for treatment of neonatal hyperglycemia in very low birth weight infants. Cochrane Database Syst Rev 10:CD007453. Review
53. Arsenault D, Brenn M, Kim S et al (2012) American society for parenteral and enteral nutrition board of directors, A.S.P.E.N. Clinical guidelines: hyperglycemia and hypoglycemia in the neonate receiving parenteral nutrition. JPEN J Parenter Enteral Nutr 36:81–95

54. Sheard NF, Kleinman RE (1987) TPN cholestasis in premature infants: the role of parenteral nutrition solutions. Pediatr Ann 16:243. Review
55. Costa S, Maggio L, Sindico P, Cota F, De Carolis MP, Romagnoli C (2010) Preterm small for gestational age infants are not at higher risk for parenteral nutrition-associated cholestasis. J Pediatr 156:575–579
56. Gura KM, Lee S, Valim C et al (2008) Safety and efficacy of a fish-oil based fat emulsion in the treatment of parenteral nutrition-associated liver disease. Pediatrics 121:e678–e686
57. Cooper A, Betts JM, Pereira GR et al (1984) Taurine deficiency in the severe hepatic dysfunction complicating total parenteral nutrition. J Ped Surg 19:462–466
58. Lavoie PM, Lavoie JC, Watson C, Rouleau T, Chang BA, Chessex P (2010) Inflammatory response in preterm infants is induced early in life by oxygen and modulated by total parenteral nutrition. Pediatr Res 68:248–251
59. Sjoberg P, Bondesson U, Sedin G et al (1985) Dispositions of di- and mono- (2-ethylhexyl) phthalate in new born infants subjected to exchange transfusions. Eur J Clin Invest 15:430–436
60. Gura KM (2010) Aluminum contamination in products used in parenteral nutrition: has anything changed? Nutrition 26:585–594. Review
61. Bishop NJ, Morley R, Day JP, Lucas A (1997) Aluminum neurotoxicity in preterm infants receiving intravenous-feeding solutions. N Engl J Med 336:1557–1561
62. Fewtrell MS, Bishop NJ, Edmonds CJ, Isaacs EB, Lucas A (2009) Aluminum exposure from parenteral nutrition in preterm infants: bone health at 15-year follow-up. Pediatrics 124: 1372–1379
63. Bohrer D, Cicero do Nascimento P, Binotto R et al (2002) Contribution of the raw material to the aluminum contamination in parenterals. JPEN J Parenter Enteral Nutr 26:382–388
64. Bohrer D, Oliveira SM, Garcia SC, Nascimento PC, Carvalho LM (2010) Aluminum loading in preterm neonates revisited. J Pediatr Gastroenterol Nutr 51:237–241
65. Burjonrappa SC, Miller M (2012) Role of trace elements in parenteral nutrition support of the surgical neonate. J Pediatr Surg 47:760–771
66. Johnson RA, Baker SS, Fallon JT et al (1981) An accidental case of cardiomyopathy and selenium deficiency. N Engl J Med 304:1210–1212
67. Van Rij AM, Thomson CD, Mckenzie JM et al (1979) Selenium deficiency in total parenteral nutrition. Am J Clin Nutr 32:2076–2085
68. Chariot P, Bignani O (2003) Skeletal muscle disorders associated with selenium deficiency in humans. Muscle Nerve 27:662–668
69. Vinton NE, Dahistrom KA, Strobel CT et al (1987) Macrocytosis and pseudoalbinism: manifestations of selenium deficiency. J Pediatr 111:711–717
70. Yannicelli S, Hambridge KM, Picciano MF (1992) Decreased selenium intake and low plasma selenium concentrations leading to clinical symptoms in a child with propionic acidaemia. J Inherit Metab Dis 15:261–268
71. Taneja S, Bhandari N, Rongsen-Chandola T et al (2009) Effect of zinc supplementation on morbidity and growth in hospital-borm, low-birth-weight infants. Am J Clin Nutr 90:385–389
72. Prasad AS (2007) Zinc: mechanism of host defense. J Nutr 137:1345–1349
73. Prasad A (2008) Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Exper Gerontol 43:370–377
74. Solomons NW (1985) Biochemical, metabolic, and clinical role of copper in human nutrition. J Am Col Nutr 4:83
75. Fuhrman MP, Hermann V, Masidonski P et al (2000) Pancytopenia after removal of copper from total parenteral nutrition. JPEN J Parenter Enteral Nutr 24:361–366
76. Hurwitz M, Garcia MG, Poole RL et al (2004) Copper deficiency during parenteral nutrition: a report of four pediatric cases. Nutr Clin Pract 19:305–308
77. Greene HL, Hambidge KM, Schanler R, Tsang RC (1988) Guidelines for the use of vitamins, trace elements, calcium, magnesium, and phosphorus in infants and children receiving total parenteral nutrition: report of the Subcommittee on Pediatric Parenteral Nutrient Requirements from the Committee on Clinical Practice Issues of the American Society for Clinical Nutrition. Am J Clin Nutr 48:1324–1342

78. Shulman RJ, Phillips S (2003) Parenteral nutrition in infants and children. J Pediatr Gastroenterol Nutr 36:587–607. Review
79. Gaull G, Sturman JA, Raiha NCR (1972) Development of mammalian sulfur metabolism: absence of cystathionase in human fetal tissues. Pediatr Res 6:538
80. Buchman AL, Dubin MD, Moukarzel AA et al (1995) Choline deficiency: a cause of hepatic steatosis during parenteral nutrition that can be reversed with intravenous choline supplementation. Hepatology 22:1399
81. Poindexter BB, Ehrenkranz RA, Stoll BJ et al (2004) Parenteral glutamine supplementation does not reduce the risk of mortality or late-onset sepsis in extremely low birth weight infants. Pediatrics 113:1209–1215
82. Duggan C, Stark AR, Auestad N et al (2004) Glutamine supplementation in infants with gastrointestinal disease: a randomized, placebo-controlled pilot trial. App Nutr Investig 20:752–756
83. Mohamad Ikram I, Quah BS, Noraida R, Djokomuljanto S, Faris Irfan CY, Van Rostenberghe H (2011) A randomised controlled trial of glutamine-enriched neonatal parenteral nutrition in Malaysia. Singapore Med J 52:356–360
84. Mermel LA, Farr BM, Sherertz RJ et al (2001) Guidelines for the management of intravascular catheter-related infections. Clin Infect Dis 32:1249–1272
85. Messing B, Peitra-Cohen S, Debure A et al (1988) Antibiotic-lock technique:a new approach to optimal therapy for catheter related sepsis in home-parenteral nutrition patients. JPEN J Parenter Enteral Nutr 12:185–189
86. Dannenberg C, Bierbach U, Rothe A et al (2003) Ethanol-lock technique in the treatment of blood stream infections in pediatric patients oncology patients with Broviac catheter. J Pediatr Hem Onc 25:616–621
87. Segarra-Newnham M, Martin-Cooper EM (2005) Antibiotic lock technique: a review of the literature. Ann Pharmacother 39:311–318
88. Metcalf SCL, Chambers ST, Pithie AD (2004) Use of ethanol locks to prevent recurrent central line sepsis. J Infect 49:20–22
89. Gura KM (2009) Is there still a role for peripheral parenteral nutrition? Nutr Clin Pract 24: 709–717. Review
90. Greene AK, Hergrueter CA (2009) Intravenous extravasation injury. In: Hansen AR, Puder M (eds) Manual of neonatal surgical intensive Care, 2nd edn. People's Medical Publishing House, Connecticut, pp 481–488
91. Skouroliakou M, Konstantinou D, Koutri K et al (2010) A double-blind, randomized clinical trial of the effect of omega-3 fatty acids on the oxidative stress of preterm neonates fed through parenteral nutrition. Eur J Clin Nutr 64:940–947
92. Laborie S, Lavoie JC, Chessex P (1998) Paradoxical role of ascorbic acid and riboflavin in solutions of total parenteral nutrition: implication in photoinduced peroxide generation. Pediatr Res 43:601–606
93. Nehra D, Fallon EM, Puder M (2011) The prevention and treatment of intestinal failure-associated liver disease in neonates and children. Surg Clin North Am 91:543–563
94. Walter E, Liu FX, Maton P et al (2012) Cost analysis of neonatal and pediatric parenteral nutrition in Europe: a multi-country study. Eur J Clin Nutr 18

Chapter 11
Intravenous Lipids in Neonates

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Abstract Postnatal growth restriction remains a major issue in high risk preterm neonates and term surgical neonates. Compared to other fields of medicine, development of intravenous lipid emulsion (LE) is quite recent. The first stable LE was introduced in 1960s. Currently typical soybean oil based LE remains the most commonly used intravenous LE in neonatal units. However there are still concerns about the adverse effects of soybean oil LE on immune system, liver metabolism and pulmonary physiology. The risk of infections may also be higher. Newer intravenous LEs are developed from various sources including olive oil, fish oil and combinations of various oils. Although, the newer LEs have potential short-term benefits with regards to laboratory markers such as peroxidation and cytokines as compared to typical soybean oil based LE, there is a lack of data on clinical outcomes and long term effects. This chapter reviews the history and chemistry of and the current evidence for typical soybean oil based LEs. The newer LEs and the data supporting their use in preterm and term neonates are also reviewed.

LEs	Lipid Emulsions
OO	Olive oil
FO	Fish oil
SO	Soy oil
EFA	Essential fatty acids
PUFA	Polyunsaturated fatty acids
LA	linoleic acid
ALA	alpha linolenic acid
LC-PUFAs	Long chain polyunsaturated fatty acids
MCT	Medium chain triglycerides
DHA	Docosahexaenoic acid
AA	Aarachidonic acid
EPA	Eicosapentaenoic acid

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_11, © Springer Science+Business Media Dordrecht 2013

Key points

- Intravenous lipid emulsions (LEs) have an established role in promoting growth and preventing essential fatty acid deficiency in neonates.
- Almost 50 years ago intravenous LEs were introduced to the medical field. Till date, most of the research has involved typical soybean oil based LEs
- The development of new LEs is focused on reducing the proportion of omega-6 PUFA rich soybean oil in the emulsion, and replace it with olive oil or fish oil.
- Newer LEs are known to have short-term benefits such as reduced lipid peroxidation and inflammation. Fish oil based LEs have been shown to be effective in the prevention and treatment of intestinal failure associated liver disease.
- There is a paucity of data on clinical and long-term neurodevelopmental outcomes from studies involving newer LEs. Further research is required to address these gaps in knowledge.
- Based on the current data, we are unable to recommend a specific type of LE.

1 Introduction

The survival of high-risk neonates has improved significantly since the introduction of antenatal glucocorticoids and postnatal surfactant therapy. It is well recognized that early postnatal nutrition plays a critical role in later neurocognitive development [1, 2]. Feed intolerance though, is a common issue in extremely preterm neonates. In this population, early enteral feeding is often limited by the immaturity of gastrointestinal motor function; manifested principally as delayed gastric emptying, gastro-oesophageal reflux, abdominal distension, and infrequent stooling. In addition, it takes a long time to establish enteral feeding in neonates with surgical conditions such as gastroschisis, necrotizing enterocolitis (NEC), intestinal atresias and short-bowel syndrome. Therefore, long-term support with parenteral nutrition (PN) is crucial for this population of neonates to provide optimal nutrition at a critical stage of somatic and cerebral development.

Intravenous lipid emulsions (LEs) have been in use for almost 50 years and are an integral component of parenteral nutrition regimes [3]. The principal roles of lipids include energy provision, and supply of essential fatty acids (EFA), long chain polyunsaturated fatty acids (LC-PUFA) and fat-soluble vitamins.

2 History of Intravenous Lipids

After many earlier attempts by scientists in the United States and Japan at making a safe LE, Schuberth and Wretlind were the first to develop a nontoxic and readily available LE. They introduced Intralipid in 1961 [4]. After many years of experiments, they found that an emulsion prepared from soybean oil and egg yolk phospholipids used as an emulsifier, could be safely infused [5]. Commercialization was possible by working cooperatively with the Vitrum Company in Stockholm, a

family-owned enterprise committed to the concept of total parenteral nutrition (TPN). One of the earliest papers describing use of PN in a neonate was published in 1968 by Wilmore and Dudrick in which a neonate with short bowel syndrome was supported with PN for 44 days [6]. Their solution however did not contain any lipids. In 1972, Coran published his experience of using PN including intralipid in 32 post-operative neonates, 26 of whom survived [7]. Cashore et al. [8] described their experience of using PN with intralipid in 23 very low birth weight (VLBW) infants, 19 of whom survived. Since these earlier studies, intravenous LEs have been used worldwide for neonatal population.

3 Intravenous Lipids as Source of Energy

As excessive fluid administration is associated with adverse outcomes, the high energy density (~9 Kcal per gm) of lipids makes it feasible to provide calories with lesser volumes [9]. Intravenous lipid emulsion is a good source of energy, and in fact, provision of some of the energy in the form of lipid is preferable over carbohydrate as the sole energy substrate [10]. In stable infants aged 1.5–6 months, Bresson et al. [11] found that amino acid oxidation and protein breakdown were significantly lower when lipids provided 50 % of non-protein calories than when glucose alone served as energy substrate. Lipids are also isotonic with plasma and suitable for administration through a peripheral vein as opposed to a concentrated glucose solutions [12]. There is some evidence that in cases of peripheral PN, using lipids improves duration of venous patency [13].

4 Intravenous Lipids as Source of EFAs

Intravenous LEs are the only source of EFAs like Linoleic Acid (LA) and alpha-linoleic acid (ALA) for the parentally fed neonates. Friedman et al. [14] were the first to report rapid onset of EFA deficiency in preterm neonates maintained on lipid-free PN. Neonates developing EFA deficiency the earliest were also the smallest and did so by second day. Many other studies have also reported evidence of biochemical deficiency of EFA in preterm infants given fat free PN [15–17]. It is well known that EFA deficiency may lead to dermatitis, growth failure, coagulopathy and increased susceptibility to infections [18]. Tomsits et al. [19] have reported increased auto-oxidative susceptibility of erythrocytes in preterm VLBW neonates with EFA deficiency. EFA deficiencies can be easily prevented with introduction of as little as 0.5–1 g/kg/day lipid infusion as part of PN [19, 20]. The importance of LC-PUFA for the development of the brain and retina has been well recognized, particularly in preterm neonates [21]. Neonates are not capable of forming sufficient quantities of LC-PUFA from the respective precursor fatty acids (LA and ALA) and thus depend on an exogenous source of LC-PUFA. Intravenous LEs contain small amounts of these fatty acids as part of the egg phospholipid used as a stabilizer [22].

Table 11.1 Oil combinations used to formulate currently available lipid emulsions

	Intralipid	Lipofundin	Lipoplus	SMOFlipid	Clinoleic	Omegaven
Soybean oil	100	50	40	30	20	0
Olive oil	0	0	0	25	80	0
MCT	0	50	50	30	0	0
Fish oil	0	0	10	15	0	100

MCT Medium chain triglycerides, Data expressed as percentage of lipid

5 Chemistry of Intravenous Lipids

Intravenous LEs are oil-in-water emulsions consisting of one or more triglyceride-containing oils, a phospholipid emulsifier and glycerine to adjust tonicity. The types of oil(s) currently used vary both in terms of the source (plant, marine) and carbon chain length [3]. Examples of plant oil include soybean oil and olive oil while marine oil includes fish oil. Table 11.1 gives details of the oil combinations used to formulate currently available lipid emulsions [23].

The fatty acid (FA) component of LEs can be classified according to the size of their carbon chain (Short: up to 4 carbon atoms; Medium: 6–12 carbon atoms; Long :> 12 carbon atoms); their degree of unsaturation (no double bonds: saturated; 1 double bond: monounsaturated; 2 or more double bonds: polyunsaturated); and the location of the first double bond, counted starting from their methyl end (first double bond in the 9th carbon atom: ω-9; in the 6th carbon atom: ω-6; and in the 3rd carbon atom: ω-3) [24]. The traditional LE like Intralipid is derived from soybean oil and is rich in long chain triglycerides (LCT). It is also rich in ω-6 PUFA with a ω-6/ω-3 ratio of 7:1.

Phospholipid emulsifiers such as egg phosphatide used in intravenous LEs produce a barrier to prevent coalescence of oil droplets dispersed in the internal phase of the emulsion. The purpose of the emulsifying agent is to keep the mean particle size $< 0.5\mu$, with a large diameter ($> 5\mu$) tail of $< 0.05\,\%$ [25].

6 Metabolism and Clearance of Intravenous Lipids

Intravenous LEs are designed to be similar to endogenous chylomicrons [26]. Their plasma clearance is dependent on the activity of lipoprotein lipase in the capillary endothelial cells. This enzyme hydrolyses triglycerides releasing free fatty acids, glycerine and phospholipids. Other factors which affect the plasma clearance of intravenous LEs include the phospholipid content (10 vs 20 % LEs), particle size and infusion rate. The phospholipid content of the 10 and 20 % formulations is the same; therefore, there is proportionally more free phospholipid available in the 10 % formulation. Free phospholipids interfere with lipoprotein lipase activity thereby decreasing lipid clearance and increasing the potential for adverse effects. Clearance of 20 % LEs is faster than that of 10 % solution due to its relatively lower concentration of

free phospholipids and its larger particle size [27]. This reduces the risk of dyslipoproteinemia characterized by elevation of serum cholesterol and phospholipids and hence most of the modern lipids are prepared as 20 %.

The infusion rate is the other factor determining plasma clearance of LEs. An early study cautioned against exceeding an infusion rate of 0.15 g/kg/h in appropriate for gestational age (AGA) neonates [28]. A slower infusion may be required for small for gestational age (SGA) neonates.

After plasma clearance, metabolic fate of the free fatty acids depends on the source oil. Soybean oil consists predominantly of long-chain triglycerides (LCT). LCTs require carnitine dependent co-transport system to enter mitochondria for beta oxidation. Medium chain triglycerides (MCT) on the other hand get oxidized independent of the carnitine system.

Whilst 'all in one' (AIO) solutions are popular for adult PN, the intravenous lipids must be infused separately in preterm infants who have extra requirements for calcium and phosphate for skeletal mineralization. In order to achieve maximal solubility of calcium and phosphate, an acidic pH of the PN solution is necessary. Addition of the LE raises the pH and carries the risk of precipitation of calcium and phosphate. This may result in catheter occlusion and pneumonitis [29].

7 Intravenous Lipid Administration and Monitoring

Lipids should contribute 30–40 % of total non-protein calories but should not exceed 60 % to avoid ketogenesis. Although LEs are available in 10 %, 20 % and 30 % strengths, the recommended concentration is 20 % rather than 10 % solution to avoid dyslipoproteinemia as mentioned earlier. The 30 % emulsion is administered as an AIO solution and cannot be given alone via a peripheral vein. As AIO solution is not recommended for preterm infants, 30 % solution is not used in the neonatal population. Currently there is no clear consensus for initial lipid dose and increments in the first week of life [30]. Drenckpohl et al. [31] compared high- versus low- dose intravenous lipids in 110 preterm neonates (birth weight 750–1,500 g). Neonates in the high-dose group received 2 g/kg/day of lipid emulsions as a starting dose compared with 0.5 g/kg/day in the control group. Intravenous lipid emulsions were increased by 0.5 g/kg/day increments up to 3 g/kg/day in both groups. Infants in the experimental group had better energy intake, higher serum triglyceride levels, decreased initial weight loss, and better clinical outcomes in terms of reduction in NEC and retinopathy of prematurity (ROP) compared with infants in the control group. Another randomised study compared early aggressive initiation of PN (starting amino acids at 3.5 g/kg/day and 20 % intralipid at 3 g/kg/day within 1 h of birth in ventilated preterm infants) with later initiation (at 48 h of age) [32]. The study results showed that aggressive intake of protein and lipids could be tolerated immediately after birth by VLBW infants. Earlier initiation also significantly increased positive nitrogen balance and caloric intake, without increasing the risk of metabolic acidosis, hypercholesterolemia, or hypertriglyceridemia. Current evidence-based guidelines

for VLBW neonates recommend starting lipid emulsion within the first 24–30 h of birth at 0.5–1 g/kg/day (to avoid EFA deficiency) and advancing to 3–3.5 g/kg/day in a stepwise manner [33].

Intravenous lipid should be infused over 24 h whenever possible. Continuous intravenous lipid infusions (24 h/day) are better tolerated than are intermittent infusions (8–16 h/day) with less fluctuation and lower concentrations of plasma lipids, especially at higher rates of infusion [34, 35].

Intravenous lipid emulsions such as Intralipid are compatible with vitamin solutions (both fat and water soluble vitamins e.g., Vitalipid N Infant and Soluvit N). No other medications should be added to lipid emulsions as their compatibility is not documented.

Tolerance of lipid emulsions could be reduced in extremely preterm neonates with birth weight less than 1,000 g [36]. Hyperlipidemia can cause pulmonary dysfunction including haemorrhage, liver damage and coagulopathy. Nephelometry for measuring lipid level and measurement of triglyceride level have been used as monitoring techniques in an attempt to prevent hyperlipidemia [37]. However earlier studies suggested that the neonates receiving intravenous fat emulsions cannot be monitored by nephelometry alone and adequate monitoring requires measurement of specific lipid fractions [37]. The American Society for Parenteral and Enteral Nutrition (ASPEN) recommends discontinuing lipid emulsion if triglyceride level rises above 200 mg/dl and then restarting at 0.5–1 g/kg/day [38]. The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends reduction of the lipid emulsion dose if serum triglyceride level rises above 250 mg/dl [39]. There is no clear consensus about the frequency of triglyceride monitoring and many neonatal units do not routinely monitor these levels in neonates. However considering the poor tolerance of intravenous LEs in extremely preterm neonates (< 1,000 g birth weight) and critically ill neonates, it may be helpful to monitor the triglyceride levels in there selected group of neonates [36].

8 Clinical Effects of Intravenous Lipid Emulsions

8.1 Desirable Effects

8.1.1 Growth

Use of intravenous LEs as part of PN in neonates has helped in supplying the required energy to mimic fetal growth. Earlier studies using intravenous lipids combined with PN showed good early postnatal growth in VLBW neonates [8]. As the parenteral energy requirements for VLBW neonates' growth is 90–95 Kcal/kg/day, addition of lipids is mandatory to achieve this figure as there are limits to protein and carbohydrate intakes to avoid azotemia and hyperglycaemia respectively. Coran et al. [7] have documented positive nitrogen balance and good growth in a group of post-surgical neonates with their use of PN including lipids. Also, a prospective study of

peripheral PN use in 45 neonatal and paediatric surgical patients (where lipids provided a major portion of the daily calories) showed that all neonates gained weight during the study period.

8.1.2 Provision of EFAs

After documenting that EFA deficiency can develop rapidly in preterm neonates on fat-free PN [40], the same group of researchers have also documented that safflower oil based LE administration providing linoleic acid at 2–4 % of the estimated calorie requirement or 5–10 % of the actual caloric intake prevented any significant changes in EFA status [20]. Earlier experiments of using soybean oil emulsion at 0.5–2.0 g/kg/day in neonates documented the normal level of essential fatty acid status [41]. Similarly in preterm neonates, two prospective studies showed that EFA deficiency could be prevented by 0.5–1 g/kg/day of intravenous lipids [16–17]. All these studies prove beyond doubt that EFA deficiency could be easily prevented by a very small dose of intravenous LEs at 0.5–1 g/kg/day.

8.1.3 Provision of LC-PUFA

Role of LC-PUFA (e.g., docosahexaenoic acid-DHA) is recognized in the development of neurological tissues including brain and retina. In humans, DHA is synthesized from its precursor alpha linolenic acid (ALA). For preterm neonates, DHA is a semi-essential nutrient as the efficiency of DHA synthesis from ALA is reduced. Unfortunately, standard soybean oil based LEs do not contain LC-PUFA except in small amounts as part of the egg phospholipid used as a stabilizer. As 10 % intralipid contains more phospholipids as compared to 20 % solution, Morris et al. [42] conducted a piglet study to see if 10 % intralipid would improve DHA delivery. Whilst the plasma levels of the DHA were improved in the group receiving 10 vs 20 % intralipid, there was no difference in the DHA levels in RBCs, liver or cerebral cortex. Hence, they concluded that infusion of additional phospholipid is an ineffective strategy for increasing DHA delivery to piglet tissues. Recent evidence suggests the possibility of improved levels of DHA with fish oil based LEs in VLBW neonates [43]. Similar results were also documented in high risk PN dependent children by Le et al. [44].

8.2 Undesirable Effects

A multitude of adverse effects have been described with the use of intravenous LEs. As soybean oil based LEs have been the oldest and most studied preparation, these effects have mainly been described with the use of soybean oil based LEs. These are summarised below.

8.2.1 Pulmonary Effects

In a study by Periera et al. [45], the risk of hyperlipidemia and reduced oxygenation in small preterm neonates receiving intravenous LEs was significantly more compared to term or near term neonates in the first week of life. They have also shown that there were no changes in other lung function parameters and the capacity to tolerate intravenous fats was enhanced after the first week of life. However it is important to note that the lipid emulsion was infused over relatively short (4-h) period of time in this study.

There are two mechanisms put forward to explain the hypoxemia. One of these relates to the presence of lipid microemboli in pulmonary capillaries causing ventilation-perfusion imbalance. Many studies have shown presence of fat embolism in pulmonary capillaries in autopsy specimens from infants who received lipid infusions [46–48]. However, some scientists have questioned whether it is a post-mortem artefact [49]. The other mechanism relates to fat metabolism leading to production of prostaglandins which may have dilating or constricting effect on pulmonary vascular tone leading to ventilation-perfusion mismatch. Hageman et al. [50] showed that intralipid infusion in lung-damaged rabbits increased pulmonary production of vasodilating prostaglandins and associated hypoxemia, presumably caused by an unblocking of hypoxic vasoconstriction and resultant increase in intrapulmonary right-to-left shunt. In an echocardiographic study, Lloyd et al. [51] showed increase in pulmonary vascular resistance with intralipid infusion in preterm infants. Significance of this was unclear as no adverse clinical effects were observed. However, all infants were relatively stable to begin with and the authors suggest caution in using lipids in infants with pulmonary hypertension and perinatal asphyxia. On the other hand, Brans et al. [52] found no deleterious effect on blood pH and alveolar-arterial oxygen difference in VLBW neonates when they infused up to 4 g/kg/day of lipids over a longer period of time ranging from 16 to 24 h. This is one of the basis of current recommendation from ASPEN and ESPGHAN to infuse lipid over 24 h to avoid overloading the clearance mechanisms.

Some studies from pre-surfactant era have associated early introduction of parenteral lipids with increased incidence of chronic lung disease (CLD) [53, 54]. In a randomised study, early vs. late introduction of lipids found no significant difference in the incidence of CLD between ‘early’ (intralipid started at < 12 h of age) and ‘late’ (no lipid in first week of life) lipid groups [55]. Alarmingly, they found increased incidence of pulmonary haemorrhage and mortality in the subgroup weighing 600–800 g allocated to early lipid administration. However, the number of mothers who received antenatal steroids was significantly lower in this group introducing a potential bias. A Cochrane review including two studies involving a total of 193 preterm infants found no significant difference in incidence of CLD between ‘early’ (lipid started at ≤ 5 days of age) and ‘no early’ (lipid started at 6–14 days of age) lipid groups [30, 56, 57].

8.2.2 Effect on Free Bilirubin

There has been a theoretical concern that the use of intravenous LEs may be hazardous in jaundiced neonates as the free fatty acids (FFA) released during lipid metabolism can displace bilirubin from albumin-binding sites, producing free bilirubin and thus increasing the risk of encephalopathy [58]. Studies have shown that FFA do not begin to displace bilirubin from albumin until the FFA/albumin molar ratio is > 6 in vivo (60) and > 4 in vitro [58–60]. Hence, in a neonate with severe unconjugated jaundice receiving intravenous lipids, it is advisable to follow FFA/albumin molar ratio and to adjust the dose of lipids to keep it < 6 [58].

8.2.3 Hepatic Effects

Prolonged use of PN in neonates can lead to parenteral nutrition associated liver disease (PNALD) manifested by elevated direct bilirubin levels which has the potential to progress to hepatic failure. There is evidence that PNALD may be in part due to the phytosterols derived from soy oils in the lipid emulsions [61]. This has led to interest in study of fish oil based lipid emulsions in an attempt to reduce PNALD. The evidence regarding this will be summarised in the next section.

8.2.4 Effect on Platelet Counts

There are some reports of thrombocytopenia in association with the use of intravenous LEs. In a letter to the editor, Lipson et al. [62] reported the occurrence of thrombocytopenia with a temporal relationship to intralipid administration in a neonate with gastroschisis. The authors had excluded sepsis, disseminated intravascular coagulation and bone marrow aplasia. However a large study ($n = 180$) in paediatric population receiving intralipid infusion did not find any evidence of thrombocytopenia [63]. A small prospective study by Goulet et al. [64] did find the evidence of reduction in platelet life span in 7 children on long term (3–18 mo) PN (including intralipid). All these children had recurrent thrombocytopenia. They hypothesized that long term administration of intravenous fat leads to hyperactivation of monocyte-macrophage system. Thus it seems that there may be some adverse effect of infusion of typical soybean oil based LEs (e.g., Intralipid) on platelet counts when employed for long periods.

8.2.5 Peroxide Formation

The omega-6 fatty acids in the soybean based LEs are highly susceptible to peroxidation due to presence of excess carbon double bonds and lead to peroxide formation. These can alter arachidonic acid metabolism or react to form organic free radicals which can lead to damage to plasma membranes. These free radicals have been linked

to various morbidities of prematurity e.g., CLD, retinopathy of prematurity (ROP) and NEC [23]. Earlier studies in preterm neonates using exclusive soybean oil based LEs have shown high levels of peroxide levels [65]. Neuzil et al. [66] have studied the formation of hydroperoxides with the exposure of intralipid to ambient and phototherapy lights. They have concluded that intralipid is highly susceptible to oxidation and that elevated levels of oxidized lipids can be formed during its clinical use, especially when intralipid infusion is combined with phototherapy. Silvers et al. [67] were able to show reduction in lipid peroxidation with the use of multivitamin preparation when intralipid was delivered via dark delivery tubing and recommended this method for routine use. While photoprotection is universally considered to be beneficial in reducing lipid peroxidation, there is some literature suggesting that multivitamin preparations are contributory to the oxidative stress [68, 69]. In summary, protection of intravenous lipid solutions from ambient and phototherapy lights by using dark delivery tubing is recommended to reduce lipid peroxidation.

8.2.6 Effect on Immune Function

LEs influence immune cell functions at various levels including cell membrane properties, phagocytosis and production of bioactive substances such as prostaglandins and leukotrienes [25, 70]. Fatty acids with multiple double bonds (omega-6 fatty acids) serve as precursors to eicosanoids which include prostaglandins, leukotrienes, thromboxanes and prostacyclins. These substances have powerful effects on platelet aggregation, vascular function, smooth muscle activity and inflammatory cascade.

Typical soybean oil based LEs have been reported to lead to impaired bacterial clearance in mice and inhibition of chemotaxis of human neutrophils in vitro [71]. There is evidence that the bactericidal activity against coagulase negative staphylococci is impaired in neonates receiving long-term PN [72]. One of the mechanisms put forward is the administration of lipid emulsions containing LCTs which results in the depletion of host defense mechanisms, particularly in neutrophilic bactericidal activity and migration. Waitzberg et al. [73] have reported moderate decrease in neutrophilic bactericidal activity with the use of lipid emulsions. Powell et al. [74] have reported M. furfur sepsis in 5 infants receiving intravenous fats. The authors' hypothesized that the origin of the infection appeared to have been through colonization of the Broviac catheters in the presence of lipid infusions. On the other hand, it has been reported that LEs can influence immunostimulatory properties with augmentation in peripheral T cell subsets, antibody dependent cellular cytotoxicity and interleukin 2 production [75]. Similarly, Palmblad et al. [76] also could not detect any impairment of neutrophilic migration or bactericidal function with the use of intralipid.

LEs can also influence immune function through the incorporation of their fatty acids in the membrane phospholipids of the immunologic cells [24]. In addition; ω-3 and ω-6 PUFA participate directly in the inflammatory immune responses, serving as a substrate for eicosanoid synthesis. Arachidonic acid (ω-6 PUFA) is a substrate for

pro-inflammatory prostaglandins, leukotrienes and thromboxanes. The ability of ω-3 PUFA to compete with ω-6 PUFA for eicosanoid synthesis constitutes a key point in its anti-inflammatory properties which are well established [24]. There is still a controversy about whether ω-3 fatty acids can impair immunologic functions such as phagocytosis, chemotaxis and respiratory burst, thereby increasing susceptibility to infections. Virella et al. [77] have reported inhibitory effects of fish oil extracts and eicosapentanoic acid on phagocytic and humoral responses. However, in a more recent study, ω-3 PUFA did not alter the functional activity of neutrophils, monocytes or lymphocytes [78].

It is recognized that excess of either ω-6 or ω-3 PUFA in parenteral LE could be immunosuppressive, whereas immune response can be maintained by LE infusion with an appropriate ω-3/ω-6 ratio. Grimm et al. [79] have reported no immunosuppressive effect at a ω-3/ω-6 ratio of 1:2.1 in their study. Soybean oil based LE (e.g., intralipid) has ω-3/ω-6 ratio of 1:7 and this could be one reason for its inhibitory effects on lymphocyte, macrophage and neutrophils function.

There is also evidence regarding impaired fat utilization in parenterally fed low-birth-weight (LBW) infants suffering from sepsis [80]. Triglycerides and free fatty acid levels rose sharply in septic LBW infants once the dose of lipids was increased from 2 to 3 g/kg/day. Hence, the authors advised to use lipid dosages not exceeding 2 g/kg/day in septic LBW infants. These observations have fuelled the research into newer lipid emulsions whereby proportion of soybean oil is reduced curtailing ω-6 PUFA and providing other types of fatty acids with increased amount of ω-3 PUFA or monounsaturated fatty acids.

8.3 Newer Lipid Emulsions

8.3.1 Olive Oil Based LEs (OO)

In order to reduce the content of omega-6 rich soybean oil (SO), predominantly olive oil based LEs have been developed [(Clinoleic® Baxter Pharmaceuticals contains predominantly OO (OO:SO ratio of 4:1)]. OO based LEs contain higher levels of monounsaturated fatty acids (MUFA) which are potentially more resistant to free radical attack and have additional anti-inflammatory properties which could be beneficial [24]. It has been proposed that use of an OO lipid emulsion may be as efficient as standard SO lipid emulsion in supplying EFA and LC-PUFAs including DHA and arachidonic acid (AA), but at the same time provide the added benefit of attenuating potential oxidation injury [81, 82].

Recently published two RCTs in preterm and near term neonates have established the safety of OO based LEs [83, 84]. As compared to traditional soybean oil based LEs, theoretically OO emulsions have the potential to reduce the oxidative stress while enhancing the anti-inflammatory effects due to their high MUFA content. The short term results in the 3 RCTs in preterm and term neonates did not show any significant difference in oxidative stress compared to standard SO emulsion [83–85].

OO based LEs are rich in MUFAs which are potentially immune-neutral. In a RCT in preterm neonates (< 32 weeks, < 1500 g) Gawecka et al. [86] have compared the inflammatory effects between SO and OO based LEs by evaluating markers including tumor necrosis factor-alpha (TNF-α) and interleukins (IL-6, IL-10). Baseline cytokine levels were comparable in the two groups; however pro-inflammatory cytokine levels (IL-6) were significantly higher in the SO group. Other secondary outcomes including the incidence of CLD, NEC, and ROP were similar in both groups. Currently there are no studies documenting long term immunological and neurodevelopmental outcomes of using OO based LEs in preterm and term neonates.

8.3.2 Fish Oil Based LEs (FO)

FO based LEs are rich in omega-3 fatty acids that are known for their anti-inflammatory properties [87]. Currently FO based LEs are available in various preparations (Table 11.1). Safety of various types of FO based LEs has been well established [18, 19].

Considering FO based LE have predominant ω-3 fatty acids, there is a concern about EFA deficiency in neonates fed 100 % FO based LE like Omegaven. It is usually recommended to be used along with SO based LEs which are rich in ω-6 fatty acids rich in LA. A prospective cohort study by de Meijer et al. [18] has assessed EFA and growth status of 10 preterm and near term neonates who were exclusively given 100 % FO based LE (Omegaven) for treatment PN induced cholestasis. Their results showed no evidence of EFA deficiency in preterm or near term neonates.

Newer FO based LEs like SMOFLipid® have potential to reduce lipid peroxidation due to the presence of MCTs, and an appropriate amount of antioxidant alpha-tocopherol and MUFAs [88]. In a study by Skouroliakou et al. [89], 38 preterm neonates (Gestation < 32 weeks, Birth weight < 1500 g) were randomized to receive SMOFlipid emulsion or pure SO based LE (Intralipid) for at least 7 days. Significant reduction in oxidative stress in the SMOFlipid group was documented by a significant rise in alpha-tocopherol and total anti-oxidant potential levels compared with standard LE.

FO based LEs are rich source of ω-3 LC-PUFAs including DHA and eicospentaenoic acid (EPA) with associated anti-inflammatory properties [25]. The eicosanoids produced from ω-3 LC-PUFAs are less inflammatory compared to those originating from ω-6 LCPUFAs [90]. Although none of the neonatal studies have documented specific immunological effects of FO based LEs, several adult and animal studies have reported benefits in terms of anti-inflammatory effects in critically ill/septic patients [91–93].

Role of FO based LEs in neonates and paediatric patients with intestinal failure associated liver disease has been well established [94]. In a recent cohort study, investigators have compared the safety and efficacy of a 100 % FO based LE (Omegaven) in 18 ex preterm neonates with short-bowel syndrome who developed cholestasis (serum conjugated bilirubin > 2 mg/dL) while receiving SO LE with those from a historical cohort of 21 infants with short-bowel syndrome who also developed

cholestasis while receiving SO LE [94]. Participants who received FO–based LE experienced reversal of cholestasis 4.8 times faster than those who received SO LE. Similar results were reported by two other recent studies [95, 96].

9 Summary

Since introduction of the first stable intravenous LE 50 years ago, soybean oil based LE remains most studied intravenous LE. Newer LEs prepared from olive oil and fish oil or other combinations may have short-term benefits in terms of reducing lipid peroxidation and inflammation. Additionally, newer FO based LEs have shown benefits in the treatment of intestinal failure associated liver disease. However, at this stage it is not clear if there are any long term benefits of introducing costly newer LEs in preterm neonates and other high risk populations such as neonates with surgical conditions. Further research is needed in terms of large RCTs comparing the short as well as long term benefits and risks of different preparations of newer LEs.

Conflict of interests None

References

1. Franz AR, Pohlandt F, Bode H, Mihatsch WA, Sander S, Kron M et al (2009) Intrauterine, early neonatal, and postdischarge growth and neurodevelopmental outcome at 5.4 years in extremely preterm infants after intensive neonatal nutritional support. Pediatrics 123:e101–e109
2. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK (2006) Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 117:1253–1261
3. Driscoll DF (2006) Lipid injectable emulsions. Nutr Clin Pract 21:381–386
4. Schuberth O, Wretlind A (1961) Intravenous infusion of fat emulsions, phosphatides and emulsifying agents. Acta Chir Scand 278 (Suppl):S1–S21
5. Vinnars E, Wilmore D (2003) History of parenteral nutrition. J Parenter Enteral Nutr 27:225–231
6. Wilmore DW, Dudrick SJ (1968) Growth and development of an infant receiving all nutrients exclusively by vein. JAMA 203:860–864
7. Coran AG (1972) The intravenous use of fat for the total parenteral nutrition of the infant. Lipids 7:455–458
8. Cashore WJ, Sedaghatian MR, Usher RH (1975) Nutritional supplements with intravenously administered lipids, protein hydrolysate, and glucose in small premature infants. Pediatrics 56:8–16
9. Bell EF, Acarregui MJ (2008) Restricted versus liberal water intake for preventing morbidity and mortality in preterm infants. Cochrane Database Syst Rev Issue 1:CD000503
10. Flatt JP, Ravussin E, Acheson KJ, Jequier E (1985) Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J Clin Invest 76:1019–1024
11. Bresson JL, Bader B, Rocchiccioli F, Mariotti A, Ricour C, Sachs C et al (1991) Protein-metabolism kinetics and energy-substrate utilization in infants fed parenteral solutions with different glucose-fat ratios. Am J Clin Nutr 54:370–376

12. Skeie B, Askanazi J, Rothkopf MM, Rosenbaum SH, Kvetan V, Thomashow B (1988) Intravenous fat emulsions and lung function: a review. Crit Care Med 16:183–194
13. Pineault M, Chessex P, Piedboeuf B, Bisaillon S (1989) Beneficial effect of co-infusing a lipid emulsion on venous patency. J Parenter Enteral Nutr 13:637–640
14. Friedman Z, Danon A, Stahlman MT, Oates JA (1976) Rapid onset of essential fatty acid deficiency in the newborn. Pediatrics 58:640–649
15. Foote KD, MacKinnon MJ, Innis SM (1991) Effect of early introduction of formula vs fat-free parenteral nutrition on essential fatty acid status of preterm infants. Am J Clin Nutr 54:93–97
16. Gutcher GR, Farrell PM (1991) Intravenous infusion of lipid for the prevention of essential fatty acid deficiency in premature infants. Am J Clin Nutr 54:1024–1028
17. Lee EJ, Simmer K, Gibson RA (1993) Essential fatty acid deficiency in parenterally fed preterm infants. J Paediatr Child Health 29:51–55
18. de Meijr VE, Le HD, Meisel JA, Gura KM, Puder M (2010) Parenteral fish oil as monotherapy prevents essential fatty acid deficiency in parenteral nutrition-dependent patients. J Pediatr Gastroenterol Nutr 50:212–218
19. Tomsits E, Rischak K, Szollar L (2000) Effects of early nutrition on free radical formation in VLBW infants with respiratory distress. J Am Coll Nutr 19:237–241
20. Cooke RJ, Zee P, Yeh YY (1985) Safflower oil emulsion administration during parenteral nutrition in the preterm infant. 1. Effect on essential fatty acid status. J Pediatr Gastroenterol Nutr 4:799–803
21. Uauy R, Hoffman DR, Peirano P, Birch DG, Birch EE (2001) Essential fatty acids in visual and brain development. Lipids 36:885–895
22. Ziegler EE, Thureen PJ, Carlson SJ (2002) Aggressive nutrition of the very low birthweight infant. Clin Perinatol 29:225–244
23. Deshpande G, Simmer K (2011) Lipids for parenteral nutrition in neonates. Curr Opin Clin Nutr Metab Care 14:145–150
24. Waitzberg DL, Torrinhas RS, Jacintho TM (2006) New parenteral lipid emulsions for clinical use. J Parenter Enteral Nutr 30:351–367
25. Mirtallo JM, Dasta JF, Kleinschmidt KC, Varon J (2010) State of the art review: Intravenous fat emulsions: Current applications, safety profile, and clinical implications. Ann Pharmacother 44:688–700
26. Carpentier YA, Thonnart N (1987) Parameters for evaluation of lipid metabolism. J Parenter Enteral Nutr 11(5 Suppl):104S–108S
27. Lutz O, Meraihi Z, Mura JL, Frey A, Riess GH, Bach AC (1989) Fat emulsion particle size: influence on the clearance rate and the tissue lipolytic activity. Am J Clin Nutr 50:1370–1381
28. Gustafson A, Kjellmer I, Olegard R, Victorin LH (1974) Nutrition in low-birth-weight infants. II. Repeated intravenous injections of fat emulsion. Acta Paediatr Scand 63:177–182
29. Kerner JA, Poole RL (2006) The use of IV fat in neonates. Nutr Clin Pract 21:374–380
30. Simmer K, Rao SC (2005) Early introduction of lipids to parenterally-fed preterm infants. Cochrane Database Syst Rev (2):CD005256
31. Drenckpohl D, McConnell C, Gaffney S, Niehaus M, Macwan K (2008) Randomized trial of very low birth weight infants receiving higher rates of infusion of intravenous fat emulsions during the first week of life. Pediatrics 122:743–751
32. Ibrahim HM, Jeroudi MA, Baier RJ, Dhanireddy R, Krouskop RW (2004) Aggressive early total parenteral nutrition in low-birth-weight infants. J Perinatol 24:482–486
33. Ehrenkranz RA (2007) Early, aggressive nutritional management for very low birth weight infants: what is the evidence? Semin Perinatol 31:48–55
34. Kao LC, Cheng MH, Warburton D (1984) Triglycerides, free fatty acids, free fatty acids/albumin molar ratio, and cholesterol levels in serum of neonates receiving long-term lipid infusions: controlled trial of continuous and intermittent regimens. J Pediatr 104:429–435
35. Brans YW, Andrew DS, Carrillo DW, Dutton E, Menchaca EM, Puelo-Scheppke BA (1988) Tolerance of fat emulsions in very-low-birth-weight neonates. Am J Dis Child 142:145–152
36. Brans YW, Andrew DS, Carrillo DW, Dutton E, Menchaca EM, Puelo-Scheppke BA (1990) Tolerance of fat emulsions in very low birthweight neonates: effect of birthweight on plasma lipid concentrations. Am J Perinatol 7:114–117

37. D'Harlingue A, Hopper AO, Stevenson DK, Shahin SM, Kerner JA Jr (1983) Limited value of nephelometry in monitoring the administration of intravenous fat in neonates. J Parenter Enteral Nutr 7:55–58
38. ASPEN Board of Directors and the clinical guideline task force (2002) Guidelines for the use of parenteral and enteral nutrition in adult and paediatric patients. J Parenter Enteral Nutr 26(Suppl 1):106SA–107SA
39. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R (2005) Parenteral nutrition guidelines working group; european society for clinical nutrition and metabolism; european society of paediatric gastroenterology, hepatology and nutrition (ESPGHAN); european society of paediatric research (ESPR). 1. guidelines on paediatric parenteral nutrition of the european society of paediatric gastroenterology, hepatology and nutrition (ESPGHAN) and the european society for clinical nutrition and metabolism (ESPEN), supported by the european society of paediatric research (ESPR). J Pediatr Gastroenterol Nutr 41(Suppl 2):S1–S87
40. Cooke RJ, Zee P, Yeh YY (1984) Essential fatty acid status of the premature infant during short-term fat-free parenteral nutrition. J Pediatr Gastroenterol Nutr 3:446–449
41. Cooke RJ, Yeh YY, Gibson D, Debo D, Bell GL (1987) Soybean oil emulsion administration during parenteral nutrition in the preterm infant: effect on essential fatty acid, lipid, and glucose metabolism. J Pediatr 111:767–773
42. Morris S, Simmer K, Gibson R (2000) Utilization of docosahexaenoic acid from intravenous egg yolk phospholipid. Lipids 35:383–388
43. Pawlik D, Lauterbach R, Walczak M, Hurkala J (2011) Docosahexaenoic acid (DHA) concentration in very low birth weight newborns receiving a fish-oil based fat emulsion from the first day of life. Preliminary clinical observation. Med Wieku Rozwoj 15:312–317
44. Le HD, de Meijer VE, Robinson EM, Zurakowski D, Potemkin AK, Arsenault DA et al (2011) Parenteral fish-oil-based lipid emulsion improves fatty acid profiles and lipids in parenteral nutrition-dependent children. Am J Clin Nutr 94:749–758
45. Periera GR, Fox WW, Stanley CA, Baker L, Schwartz JG (1980) Decreased oxygenation and hyperlipemia during intravenous fat infusions in premature infants. Pediatrics 66:26–30
46. Barson AJ, Chistwick ML, Doig CM (1978) Fat embolism in infancy after intravenous fat infusions. Arch Dis Child 53:218–223
47. Shulman RJ, Langston C, Schanler RJ (1987) Pulmonary vascular lipid deposition after administration of intravenous fat to infants. Pediatrics 79:99–102
48. Puntis JWL, Rushton DI (1991) Pulmonary intravascular lipid in neonatal necropsy specimens. Arch Dis Child 66:26–28
49. Schroder H, Paust H, Schmidt R (1984) Pulmonary fat embolism after intralipid therapy-a post-mortem artefact? Light and electron microscopic investigations in low-birth-weight infants Acta Paediatr Scand 73:461–464
50. Hageman JR, McCulloch K, Gora P, Olsen EK, Pachman L, Hunt CE (1983) Intralipid alterations in pulmonary prostaglandin metabolism and gas exchange. Crit Care Med 11:794–798
51. Lloyd TR, Boucek MM (1986) Effect of intralipid on the neonatal pulmonary bed: An echographic study. J Pediatr 108:130–133
52. Brans YW, Dutton EB, Andrew DS, Menchaca EM, West DL (1986) Fat emulsion tolerance in very low birth weight neonates: effect on diffusion of oxygen in the lungs and on blood pH. Pediatrics 78:79–84
53. Hammerman C, Aramburo MJ (1988) Decreased lipid intake reduces morbidity in sick premature neonates. J Pediatr 113:1083–1088
54. Cooke RW (1991) Factors associated with chronic lung disease in preterm infants. Arch Dis Child 66:776–779
55. Sosenko IR, Rodriguez-Pierce M, Bancalari E (1993) Effect of early initiation of intravenous lipid administration on the incidence and severity of chronic lung disease in premature infants. J Pediatr 123:975–982
56. Alwaidh MH, Bowden L, Shaw B, Ryan SW (1996) Randomised trial of effect of delayed intravenous lipid administration on chronic lung disease in preterm neonates. J Pediatr Gastroenterol Nutr 22:303–306

57. Brownlee KG, Kelly EJ, Ng PC, Kendall-Smith SC, Dear PR (1993) Early or late parenteral nutrition for the sick preterm infant? Arch Dis Child 69:281–283
58. Andrew G, Chan G, Schiff D (1976) Lipid metabolism in the neonate. II. The effect of intralipid on bilirubin binding in vitro and in vivo. J Pediatr 88:279–284
59. Starinsky R, Shafrir E (1970) Displacement of albumin-bound bilirubin by free fatty acids: implications for neonatal hyperbilirubinemia. Clin Chim Acta 24:311–318
60. Thiessen H, Jacobssen J, Brodersen R (1972) Displacement of albumin-bound bilirubin by fatty acids. Acta Paediatr Scand 61:285–288
61. Clayton PT, Whitfield P, Iyer K (1998) The role of phytosterols in the pathogenesis of liver complications of pediatric parenteral nutrition. Nutrition 14:158–164
62. Lipson AH, Pritchard J, Thomas G (1974) Thrombocytopenia after intralipid infusion in a neonate. Lancet 304:1462–1463
63. Cohen IT, Dahms B, Hays DM (1977) Peripheral total parenteral nutrition employing a lipid emulsion (Intralipid): complications encountered in pediatric patients. J Pediatr Surg 12:837–845
64. Goulet O, Girot R, Maier-Redeisperger M, Bougle D, Virelizier JL, Ricour C (1986) Hematologic disorders following prolonged use of intravenous fat emulsions in children. J Parenter Enteral Nutr 10:284–288
65. Helbock HJ, Motchnik PA, Ames BN (1993) Toxic hydroperoxides in intravenous lipid emulsions used in preterm infants. Pediatrics 91:83–87
66. Neuzil J, Darlow BA, Inder TE, Sluis KB, Winterbourn CC, Stocker R (1995) Oxidation of parenteral lipid emulsion by ambient and phototherapy lights: potential toxicity of routine parenteral feeding. J Pediatr 126:785–790
67. Silvers KM, Sluis KB, Darlow BA, McGill F, Stocker R, Winterbourn CC (2001) Limiting light-induced lipid peroxidation and vitamin loss in infant parenteral nutrition by adding multivitamin preparations to Intralipid. Acta Paediatr 90:242–249
68. Bassiouny MR, Almarsafawy H, Abdel-Hady H, Nasef N, Hammad TA, Aly H (2009) A randomized controlled trial on parenteral nutrition, oxidative stress, and chronic lung disease in preterm infants. J Pediatr Gastroenterol Nutr 48:363–369
69. Chessex P, Laborie S, Lavoie JC, Rouleau T (2001) Photoprotection of solutions of parenteral nutrition decreases the infused load as well as the urinary excretion of peroxides in premature infants. Semin Perinatol 25:55–59
70. Yaqoob P (2004) Fatty acids and the immune system: from basic science to clinical applications. Proc Nutr Soc 63:89–104
71. Fischer GW, Hunter KW, Wilson SR, Mease AD (1980) Diminished bacterial defences with intralipid. Lancet 2:819–820
72. Okada Y, Klein NJ, van Saene HKF, Webb G, Holzel H, Pierro A (2000) Bactericidal activity against coagulase-negative Staphylococci is impaired in infants receiving long-term parenteral nutrition. Ann Surg 231:276–281
73. Waitzberg DL, Bellinati-Pires R, Salgado MM, Hypolito IP, Colleto GM, Yagi O et al (1997) Effect of total parenteral nutrition with different lipid emulsions on human monocyte and neutrophils functions. Nutrition 13:128–132
74. Powell DA, Aungst J, Snedden S, Hansen N, Brady M (1984) Broviac catheter-related Malassezia furfur sepsis in five infants receiving intravenous fat emulsions. J Pediatr 105:987–990
75. Monson JR, Ramsden CW, MacFie J, Brennan TG, Guillou PJ (1986) Immunorestorative effect of lipid emulsions during total parenteral nutrition. Br J Surg 73:843–846
76. Palmblad J, Brostrom O, Lahnborg G, Uden AM, Venizelos N (1982) Neutrophil functions during total parenteral nutrition and intralipid infusion. Am J Clin Nutr 35:1430–1436
77. Virella G, Kilpatrick JM, Rugeles MT, Hyman B, Russell R (1989) Depression of humoral responses and phagocytic functions in vivo and in vitro by fish oil and eicosapentanoic acid. Clin Immunol Immunopathol 52:257–270
78. Kew S, Banerjee T, Minihane AM, Finnegan YE, Muggli R, Albers R et al (2003) Lack of effect of foods enriched with plant- or marine-derived n-3 fatty acids on human immune function. Am J Clin Nutr 77:1287–1295

79. Grimm H, Tibell A, Norrlind B, Blecher C, Wilker S, Schwemmle K (1994) Immunoregulation by parenteral lipids: impact of the n-3 to n-6 fatty acid ratio. J Parenter Enteral Nutr 18:417–421
80. Park W, Paust H, Brosicke H, Knoblach G, Helge H (1986) Impaired fat utilization in parenterally fed low-birth-weight infants suffering from sepsis. J Parenter Enteral Nutr 10:627–630
81. Sala-Vila A, Barbosa VM, Calder PC (2007) Olive oil in Parenteral nutrition. Curr Opinion Clin Nutr and Metab Care 10:165–174
82. Goulet O, de Potter S, Antébi H et al (1999) Long-term efficacy and safety of a new olive oil-based intravenous fat emulsion in pediatric patients: a double-blind randomized study. Am J Clin Nutr 70:338–345
83. Deshpande GC, Simmer K, Mori T, Croft K (2009) Parenteral lipid emulsions based on olive oil compared with soybean oil in preterm (< 28 weeks' gestation) neonates: a randomised controlled trial. J Pediatr Gastroenterol Nutr 49:619–625
84. Webb AN, Hardy P, Peterkin M et al (2008) Tolerability and safety of olive oil-based lipid emulsion in critically ill neonates: a blinded randomized trial. Nutrition 24:1057–1064
85. Roggero P, Mosca F, Giannì ML et al (2010) F2-isoprostanes and total radical-trapping antioxidant potential in preterm infants receiving parenteral lipid emulsions. Nutrition 26:551–555
86. Gawecka A, Michalkiewicz J, Kornacka MK et al (2008) Immunologic properties differ in preterm infants fed olive oil vs soy-based lipid emulsions during parenteral nutrition. J Parenter Enteral Nutr 32:448–453
87. Grimm H, Mertes N, Goeters C et al (2006) Improved fatty acid and leukotriene pattern with a novel lipid emulsion in surgical patients. Eur J Nutr 45:55–60
88. Varsila E, Hallman M, Andersson S (1994) Free-radical-induced lipid peroxidation during the early neonatal period. Acta Paediatr 83:692–695
89. Skouroliakou M, Konstantinou D, Koutri K et al (2010) A double-blind, randomized clinical trial of the effect of omega-3 fatty acids on the oxidative stress of preterm neonates fed through parenteral nutrition. Eur J Clin Nutr 64:940–947
90. Wanten GJ, Calder PC (2007) Immune modulation by parenteral lipid emulsions. Am J Clin Nutr 85:1171–1184
91. De Nardi L, Bellinati-Pires R, Torrinhas RS et al (2008) Effect of fish oil containing parenteral lipid emulsions on neutrophil chemotaxis and resident-macrophages' phagocytosis in rats. Clin Nutr 27:283–288
92. Antébi H, Mansoor O, Ferrier C et al (2004) Liver function and plasma antioxidant status in intensive care unit patients requiring total parenteral nutrition: comparison of 2 fat emulsions. J Parenter Enteral Nutr 28:142–148
93. Jacintho TM, Gotho H, Gidlund M et al (2009) Anti-inflammatory effect of parenteral fish oil lipid emulsion on human activated mononuclear leukocytes. Nutr Hosp 24:288–296
94. Gura KM, Lee S, Valim C et al (2008) Safety and efficacy of a fish-oil-based fat emulsion in the treatment of parenteral nutrition-associated liver disease. Pediatrics 121:678–686
95. Diamond IR, Sterescu A, Pencharz PB et al (2009) Changing the paradigm: omegaven for the treatment of liver failure in pediatric short bowel syndrome. J Pediatr Gastroenterol Nutr 48:209–215
96. Rollins MD, Scaife ER, Jackson WD et al (2010) Elimination of soybean lipid emulsion in parenteral nutrition and supplementation with enteral fish oil improve cholestasis in infants with short bowel syndrome. Nutr Clin Pract 25:199–204

Chapter 12
Amino Acids

Hester Vlaardingerbroek and Johannes B. van Goudoever

Abstract Neonatologists' ultimate goal is to achieve a functional outcome in preterm infants that is comparable to outcomes in healthy term-born infants. Current guidelines recommend the initiation of amino acid administration as soon as possible in the first postnatal day at a dose of 2–3 g/kg/day. Within the next few days, amino acid intake should be increased to a maximum amount of 4 g/kg/day. However, actual intakes are still lower than target intakes in many neonatal intensive care units worldwide. Subsequently, many preterm infants fail to grow well, which is associated with long-term consequences with regard to growth and neurodevelopment. While most studies on early amino acid administration show beneficial effects predominantly on short-term outcomes, some studies also warn of adverse effects, especially in extremely immature and extremely low birth weight infants. Future studies should be directed towards elucidating the long-term anthropometric and neurodevelopmental outcomes of early (high dose) amino acid administration.

Key Points

- Functional outcomes in preterm infants are related to protein quantity and quality during the first few weeks of life.
- In most neonatal intensive care units, target intakes of amino acids are not reached within the first days of life, resulting in suboptimal growth that may subsequently affect neurodevelopmental outcome.
- Several studies provide strong evidence of the beneficial effects of administering 2–3 g amino acids/kg/day from birth onwards on nitrogen balance and growth in the average preterm infant.
- The supplementation of high doses of amino acids of approximately 4 g/kg/day should be prescribed with caution in the most immature group of infants (< 26 weeks gestational age) and in those who were severely growth-restricted in utero.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_12,

Malnutrition during the critical stages of the development of prematurely born infants is associated with long-lasting negative effects on growth [1] and neurodevelopment [2], at least through school age and possibly also into adulthood [3]. Postnatal growth retardation is mainly caused by the insufficient administration of protein and calories that do not meet the requirements for achieving a growth velocity similar to fetal rates [4]. Clinical problems that preterm infants experience, especially during the first several days of life, prevent the meeting of nutritional needs; these needs are also not given the highest priority. As a result, postnatal growth failure is one of the most commonly observed morbidities in very-low-birth-weight (VLBW) infants [5–7]. Regrettably, the incidence of postnatal growth failure has not declined much during the last decades [6, 8]. When energy intake is not limited, proteins are pivotal to achieving adequate growth [9, 10]. The first week resting energy expenditure is approximately 30–35 kcal/kg/day, so protein is frequently the only limiting nutritional factor [11].

Proteins are polymers of 20 different amino acids, which are nitrogen-containing molecules. These 20 different amino acids are called α-amino acids. The order of the various amino acids in a protein is defined after translation from the DNA. The β-amino acids (e.g., taurine) and γ-amino acids (e.g., hydroxyl-proline) cannot be incorporated into proteins, but have important intracellular functions as individual acting amino acids. The α-amino acids can be divided into essential and non-essential amino acids, depending on whether they are completely derived from the diet, or whether they can be produced endogenously from other substrates in sufficient amounts. Classically, isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, tryptophan, and histidine are considered essential amino acids for adults. However, several metabolic processes are not fully developed in preterm and term infants. Therefore, for the infant, the following amino acids are conditionally essential: arginine, glutamine, glycine, proline, taurine, and tyrosine. Cysteine was historically defined as conditionally essential, but recent studies have demonstrated that this is not the case for enterally fed infants [12, 13]. Amino acids can also be divided by their chemical characteristics: leucine, isoleucine, and valine are branched-chain amino acids; phenylalanine, tyrosine, and tryptophan are aromatic amino acids; methionine and cysteine are sulphur-containing amino acids; serine and threonine contain a hydroxyl (or alcohol) group; glutamine and asparagines are acidic; and lysine, histidine, and arginine are basic amino acids.

Almost all proteins undergo a continuous process of synthesis and breakdown to guarantee their optimal function. Protein breakdown releases amino acids during fasting, removes defective proteins after erroneous translation of after (oxidative) damage. Protein breakdown can also occur to release certain indispensible amino acids that are highly needed but not available otherwise, to provide these amino acids for the synthesis of other proteins. Excess amounts of glucose and fatty acids can be stored in the body as glycogen and fat, respectively. However, no storage pool exists for individual amino acids. Therefore, an excess amount of individual amino acids that are not needed for protein synthesis cannot be stored separately. Similarly, specific amino acids that are insufficiently available for synthesis of certain proteins cannot be released selectively from some other protein. To avoid aminoacidemia, the

only metabolic fate of an amino acid that is available in excess amounts is, therefore, degradation into ammonia (later converted into urea) and a carbon skeleton that can either be oxidized to yield energy or used for glucose synthesis.

1 Approaches to Determine Amino Acid Requirements for Preterm Infants

Different approaches can be used to determine the adequate requirements for amino acids in preterm infants. First, the intake of the fetus of a similar gestational age can be regarded as suitable. However, following preterm birth, the continuous nutritional supply ceases abruptly, and the infant is immediately challenged by the sudden change from a usually well-fed state in utero to the extra-uterine environment, which has very different physical and physiological properties. At birth, most preterm infants are ill, requiring ventilatory support, antibiotic therapy and, sometimes, cardiac support. It is likely that amino acid requirements are influenced by these conditions. Second, the requirement can be based on the factorial approach. The factorial approach combines the estimated growth rate of a fetus of a certain gestational age with the composition of newly formed tissue. However, the data on the composition of fetal tissue were derived a very long time ago (early twentieth century onwards) from carcasses of deceased fetuses, with little or no knowledge of the condition of the mothers or their fetuses. Third, the requirement can be based on the composition of human milk. Human milk is highly variable in quantity and composition, not only between feeds but also within a feeding. Thus, the latter two approaches are not likely to provide preterm infants' real requirements for all nutrients.

From a biochemical standpoint, amino acid requirements are mainly determined by the rate of net protein synthesis, which depends on the availability of rate-limiting amino acids [14] and on the obligatory oxidation rate. The utilization of the amino acid supply for protein synthesis depends on sufficient energy intake, and often, an energy supply of 30–40 kcal per 1 g amino acids is recommended [14]. Methods to assess the adequacy of amino acid intake include anthropometry (weight and length gain), nitrogen balance, metabolic indices (e.g., amino acid concentration, albumin, pre-albumin, total protein concentrations, plasma urea concentration, and metabolic acidosis), whole-body nitrogen kinetics, specific amino acid kinetics and the indicator amino acid method [14]. To determine the requirements for individual amino acids, the indicator amino acid method is an accurate and fast method. For estimations of the total amino acid requirement, the most widely used method is the amount needed to achieve a positive nitrogen balance. As summarized in the ESPGHAN guidelines, a minimum amino acid intake of 1.5 g/kg/day is necessary to prevent a negative nitrogen balance [14]. Higher intakes are needed to achieve physiological protein accretion.

2 Fetal Requirement and Metabolism

Information regarding fetal protein requirements and metabolism is limited, and most of it originates from animal studies, particularly studies of fetal sheep. Under physiological conditions in pregnant ewes, the fetal amino acid uptake exceeds the amount required for protein synthesis. Excess amino acids are oxidized and contribute considerably to fetal energy generation [15, 16]. These quantitative balance studies require blood sampling from both the venous and arterial umbilical vessels and the measurement of flow rates. In humans, these activities can only be performed safely around birth. Chien et al. [17] and Van den Akker et al. [18, 19] undertook human studies on fetal leucine, valine, phenylalanine and tyrosine kinetics at the time of elective cesarean section. These authors reported that the amino acid uptake exceeded the amount that would be necessary for net protein accretion, indicating that the human fetus also oxidizes amino acids to generate energy. In addition, a portion of the surplus of phenylalanine was hydroxylated to tyrosine, indicating that tyrosine can be considered a non-essential amino acid under normal conditions [19]. Thus, similarities were found in the way the ovine and the human fetus utilize the available amino acids for ongoing metabolism and growth. The protein requirements for fetal growth have been estimated at 3.5–4 g/kg/day [20]. However, these requirements should be interpreted with caution as they rely only on phenylalanine, tyrosine, valine, and leucine kinetics.

3 Amino Acids for the Preterm Infant, a Historical Perspective

The immaturity of the gastrointestinal tract prevents its use; therefore, preterm infants are mainly dependent on parenteral nutrition during the first week of life. When the infant receives only glucose after birth, obligatory nitrogen losses are not compensated for, which results in a catabolic state. In that situation, the estimated protein loss amounts to 1 % of the endogenous body protein per day [21, 22]. The resulting protein deficit may be difficult if not impossible to recoup, resulting in suboptimal growth and development.

The first report on parenteral amino acid administration dates from 1939. Positive nitrogen balances were obtained by parenteral administration of hydrolyzed casein. However, many complications were described, most prominently a marked elevation of body temperature [23]. More successful was the report of Helfrick and Abelson in 1944 where a marasmic infant received total parenteral nutrition for five consecutive days as the only nutrition source [24]. In the early 1970s, the routine use of parenteral nutrition in the neonatal intensive care unit (NICU) was implemented. Soon after the implementation of these solutions, various metabolic disturbances, such as hyperammonemia and acidosis, were reported [25, 26]. Preterm infants were not given amino acid solutions during the first postnatal days under the assumption that they could not tolerate these solutions. Later studies demonstrated that these complications were

likely the result of the manufacturing method and the composition of the amino acid solutions [27]. From then on, crystalline amino acid solutions have been modified to reduce the risk of complications [28]. Nevertheless, fear of metabolic derangements is still firmly rooted in clinical practice.

4 Timing and Amount of Amino Acid Administration

In early studies of parenteral amino acid administration to preterm infants, amino acid administration was initiated several days after birth [29, 30]. Over the last decades, multiple studies have demonstrated that earlier parenteral amino acid administration (with starting doses of 1.0–2.5 g/kg/day) can reverse a negative nitrogen or stable isotope balance, which is indicative of protein accretion and thus growth, even at a low caloric intake [28, 30–34]. This treatment also increases plasma amino acid concentrations [31, 33] and has been associated with improved neurodevelopmental outcomes compared to infants who received no amino acids during the first postnatal days [2].

Recent studies have focused on the initiation of amino acid infusion at high doses within 24 h after birth in conjunction with the infusion of glucose and sometimes lipids. In agreement with previous studies, using high dose amino acids on day one of life reverses the negative nitrogen balance to a positive balance and thus induces anabolism (Table 12.1, [35–37]).

In the study of Ibrahim et al. [35] 32 ventilator-dependent preterm infants were prospectively randomized to 3.5 g amino acids/kg/day plus 3 g lipid/kg/day, starting within 1 h after birth, or to only glucose during the first 48 h of life, followed by 2 g amino acids/kg/day and 0.5 g lipid/kg/day. In the latter group amino acids and lipids were each increased by 0.5 g/kg/day to a maximum of 3.5 and 3 g/kg/day, respectively. Nitrogen balances were significantly higher in the early amino acid and lipid group compared to the late initiation group (385 ± 20 mg/kg/day vs 203 ± 21 mg/kg/day, $P < 0.001$). Weight gain and plasma biochemistry concentrations during the first seven days of life were similar between both groups. Te Braake et al. [36] randomly assigned 135 VLBW infants to 2.4 g amino acids/kg/day from birth onwards or to only glucose during the first two days, followed by 1.2 g amino acids/kg/day and increased to 2.4 g/kg/day on day 3. Nitrogen balances were significantly higher in the early amino acid group compared to the late initiation group (145 ± 104 vs -84 ± 70, $P < 0.05$). Days to regain birth weight were not different between groups. Plasma urea concentrations on day 2 were higher in the early amino acid group, while blood gas base excess and bicarbonate concentrations were lower. However, these biochemical differences had no clinical influence. In the study of Thureen et al. [37] 28 infants with a birth weight below 1,300 g were randomly assigned to 3 g amino acids/kg/day vs 1 g/kg/day at a mean age 52 ± 3 h of life. After a minimum of 12 h of parenteral nutrition, nitrogen balances were significantly higher in the high amino acid group (186 ± 93 vs -42 ± 63, $P < 0.00005$).

Table 12.1 Summary of most recent randomized controlled trials and cohort studies on the effect of early and higher dose amino acid administration on nitrogen balance and growth until discharge home

First author, year, reference	Design	Population	*n*	Study nutrition	Nitrogen balance (mg/kg/day)	Regain birth weight (day)	Weight gain in first 28 days (g/kg/day)	Weight at discharge home
Blanco [39, 56]	RCT	Birth weight <1,000 g	32	0.5–3 g AA/kg/day vs 2–4 g AA/kg/day	ND	ND	10.8 ± 4.2 vs 12.2 ± 4.6[a], $P = 0.4$	Weight *z*-scores: -1.0 ± 0.4 vs -0.8 ± 0.4, $P > 0.05$
Clark [38]	RCT	Gestational age 23–29 6/7 weeks	122	1.5–3.5 g AA/kg/day vs 1.0–2.5 AA g/kg/day	ND	ND	12.9 (9.4–14.9) vs 11.4 (7.2–14.9)[c], $P = 0.6$	ND
Kashyap [40]	RCT	Birth weight <1,250 g		4 g AA/kg/day vs 3 g AA/kg/day	ND	10 vs 12.3[b], $P < 0.05$	ND	ND
Te Braake [36]	RCT	Birth weight <1,500 g	135	2.4 g AA/kg/day < 2 h after birth vs only glucose during first 48 h	145 ± 104 vs -84 ± 70, $P < 0.05$ (day 2)	8 (2–25) vs 10 (2–26)[d], $P = 0.286$	ND	ND
Ibrahim [35]	RCT	Gestational age 24–32 weeks, birth weight 501–1,250 g	32	3.5 g AA/kg/day + 3 g lipid/kg/day starting < 1 h after birth vs only glucose during first 48 h	384 ± 78 vs -203 ± 78, $P < 0.05$ (day 1)	ND	ND	ND
Thureen [37]	RCT	Birth weight <1,300 g	28	3 g AA/kg/day vs 1 g AA/kg/day	186 ± 93 vs -42 ± 63, $P < 0.00005$ (day 2)	ND	ND	ND
Wilson [55]	RCT	Birth weight <1,500 g	125	Start amino acids < 12 h vs on day 3, start lipid on day 2 vs day 5, early minimal enteral feeding	ND	9 (6–11) vs 12 (9–17)[d], $P < 0.001$		Percentage of infants with weight < 10th percentile: 59 % vs 82 %, $P < 0.05$

Table 12.1 (continued)

First author, year, reference	Design	Population	*n*	Study nutrition	Nitrogen balance (mg/kg/day)	Regain birth weight (day)	Weight gain in first 28 days (g/kg/day)	Weight at discharge home
Geary [54]	Cohort	Birth weight ≤1,000 g	163	AA at 3 g/kg/day on day 2 in combination with surfactant at delivery followed by CPAP, and decreased oxygen exposure vs slow stepwise increase of AA	ND	12 ± 5 vs 16 ± 6, $P < 0.0001$	ND	Percentage of infants with postnatal growth failure: 18 % vs 48 %, $P < 0.003$
Dinerstein [4]	Cohort	Birth weight 750–1,500 g	182	AA immediately after birth at 1.5–4 g/kg/day vs AA from day 3 onwards at 0.5–3 g/kg/day	ND	10 (1–21) vs 16 (1–29)[d], $P < 0.001$	ND	Percentage of infants with postnatal growth failure: 53 % vs 77 %, $P = 0.005$
Kotsopoulos [45]	Cohort	Gestational age <28 weeks	108	AA immediately after stabilization 1.5–4 g/kg/day vs after 12–30 h (1–3.5 g AA/kg/day)	ND	11 ± 4 vs 13 ± 4, $P = 0.050$	1.6 ± 2.0 vs 1.7 ± 1.7, $P = 0.834$	Percentage of infants with weight < 10th percentile: 43 % vs 52 %, $P = 0.680$

AA amino acid, *ND* no data
[a]Mean ± SD (all such values)
[b]SD was not presented and could not be calculated from available data
[c]Median (IQR)
[d]Median (range)

Biochemistry was not different between groups. In addition, the plasma concentrations of all essential amino acids and of most non-essential amino acids increase with early amino acid administration and are more in accordance with reference ranges from healthy fetuses or breast-fed term infants [36, 37]. The side effects observed with early high dose amino acid infusion, such as increased mean peak serum indirect bilirubin, lower base excess, lower concentrations of bicarbonate, and increased blood urea nitrogen, do not have short term clinical implications [35, 36]. Some remarks can be made about the studies presented. The target dose of amino acid administration (2.4–4.0 g/kg/day) was usually not reached before the third day of life because most of these studies started at a lower dose (1.5–2.0 g/kg/day) with a daily stepwise increase [37–39]. In addition to the amounts infused, the amino acid solutions used differed between the studies as well; the studies performed in the USA used TrophAmine (plus cysteine) or Aminosyn PF [35, 37–40], whereas in Europe, Primene (not available in the USA) was used [36]. The difference between the amino acid solutions is described in more detail in the protein quality section. Overall, the studies with early high-dose amino acid administration show good efficacy during short-term follow-up without major side effects.

5 Composition of Amino Acid Solutions

As stated above, amino acids can be divided into essential and non-essential amino acids, depending on whether they are completely derived from the diet or whether they can be produced endogenously from other substrates in sufficient amounts. All proteins have a fixed sequence of amino acid residues after DNA translation. The rate of protein synthesis is determined by the first limiting amino acid in the cytoplasmic compartment. An insufficient availability of a (conditionally) essential amino acid may result in increased protein breakdown to provide sufficient amounts of the (conditionally) essential amino acids needed for protein synthesis. The capacity of infants to regulate amino acid concentrations is probably limited because of their immature kidney and liver function. Therefore, not only the quantity but also the quality (i.e., the composition) of the amino acid supply is crucial for achieving optimal growth and development. The overall quality of available parenteral amino acid mixtures is probably not adequate because individual requirements for parenterally fed preterm infants are not known, except for tyrosine [41]. The lack of knowledge regarding the "optimal" amino acid pattern in parenteral solutions is clearly demonstrated by the diversity in the composition of current pediatric amino acid solutions (Table 12.2). Trophamine was originally formulated to match the plasma amino acid concentrations of healthy, term, breast-fed infants. The composition of Primene is derived from fetal and neonatal cord blood concentrations. Due to insolubility or instability in solution, these mixtures contain low amounts of or lack the conditionally essential amino acids tyrosine, glutamine, and often cysteine, although cysteine is often supplemented separately [3]. As stated above, the availability of amino acids below the requirement may result in increased proteolysis of endogenous proteins and/or in increased irreversible oxidation of all other amino acids.

Table 12.2 Amino acid concentrations of commercially available parenteral amino acid solutions (g/100 g amino acids)

	Product (% amino acids) (manufacturer)								
	Aminosyn (10 %) (Hospira)	Aminosyn-PF (10 %) (Hospira)	Aminoven (10 %) (Fresenius Kabi)	FreAmine III (10 %) (B. Braun)	Novamine (10 %) (Hospira)	Primene (10 %) (Baxter)	Travasol (10 %) (Baxter)	TrophAmine (10 %) (B. Braun)	Vaminolact (6.5 %) (Fresenius Kabi)
Essential									
Ile	7.3	7.6	5.0	6.9	5.0	6.7	6.0	8.2	5.5
Leu	9.5	11.9	7.4	9.1	6.9	9.9	7.3	14.0	10.8
Val	8.1	6.6	6.2	6.6	6.7	7.6	5.8	7.8	5.5
Lys	7.3	6.8	9.3	7.3	7.9	10.9	5.8	8.2	8.6
Met	4.0	1.8	4.3	5.3	5.0	2.4	4.0	3.4	2.0
Phe	4.7	4.3	5.1	5.6	6.9	4.2	5.6	4.8	4.2
Thr	5.2	5.1	4.4	4.0	5.0	3.7	4.2	4.2	5.5
Trp	1.6	1.8	2.0	1.5	1.7	2.0	1.8	2.0	2.2
His	3.0	3.1	3.0	2.8	6.0	3.8	4.8	4.8	3.2
Conditionally essential									
Cys	0	0	0	0	0	1.9	0	0.1	1.5
Tyr	0.9	0.6	0.4	0	0.3	0.9	0.4	2.3[a]	0.8
Arg	9.9	12.3	12.0	9.5	9.8	8.4	11.2	12.2	6.3
Glu	0	8.2	0	0	5.0	9.9	0	5.0	10.9
Gly	12.9	3.9	11.0	14.0	6.9	4.0	10.3	3.6	3.2
Pro	8.7	8.1	11.2	11.2	6.0	3.0	6.8	6.8	8.6
Tau	0	0.7	1.0	0	0	0.6	0	0.2	0.5
Non-essential									
Ala	12.9	7.0	14.0	7.1	14.5	7.9	20.7	5.4	9.7
Asp	0	5.3	0	0	0	6.0	0	3.2	6.3
Ser	4.2	5.0	6.5	5.9	3.9	4.0	5.0	3.8	5.8

[a]Supplied as L-tyrosine (0.7 g/100 g amino acids) and N-acetyl-tyrosine (1.6 g/100 g amino acids)

6 Safety of Early Amino Acid Administration

The safety of amino acid administration is generally based on biochemical parameters such as pH, urea and ammonia concentrations and concentrations of potentially neurotoxic amino acids. However, none of these parameters are specific to amino acid intolerance [22, 42], and they are also influenced by the general clinical status of the neonate [43]. Elevated urea concentration in the extremely low birth weight infant may reflect an appropriate amount of amino acid oxidation and not protein intolerance [37]. Cohort studies with 92–188 infants per study did not find an association between plasma urea nitrogen and protein intake [44–48]. Conflicting results were obtained with regard to urea concentrations and the amount of amino acids infused [30, 35–40]. In the study of Blanco et al., high-dose amino acid infusion (up to 4 g/kg/day on day 3 of life) was discontinued in six out of 30 preterm infants because they showed elevated urea concentrations peaking at 101 mg N/dL (~ 36 mmol/L) [39]. Four of these infants, born at 23 weeks gestation, were extremely ill and died within 7 days. No causal relationship between high urea concentrations and death was demonstrated. In addition, the mean peak ammonia concentration in the deceased infants (103 μmol/L) was in the upper range of the reference values [49, 50].

Overall, the evidence suggests that elevations in the urea concentration per se should not lead to postponing the initiation of amino acids or decreasing the amounts of amino acids in preterm infants. In addition, none of the studies with early amino acid infusion have reported metabolic acidosis, hyperaminoacidemia, or, when measured, ammonemia concentrations, above the reference values [28–34].

7 Benefits of Early Amino Acid Administration

After birth the transplacental supply stops abruptly while metabolic demands increase significantly. Therefore, preterm birth has to be acknowledged as a nutritional emergency in which minutes and not days matter when it comes to providing nutrition immediately after birth.

For neonatologists, the ultimate goal of feeding preterm infants is to improve the outcomes for these infants to a level that is comparable to the outcomes for healthy term-born infants. That is, the goal is a postnatal growth rate that duplicates the fetal growth rate and a functional outcome similar to that of healthy term-born infants, as stated by the American Academy of Pediatrics Committee on Nutrition and the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition [3, 51, 52]. Studies on early amino acid administration have mainly investigated its effect in the direct postnatal phase; only a few studies have investigated medium- or long-term outcome parameters. In general, outcomes can be based on several criteria, such as postnatal growth, the incidence of neonatal morbidities, the duration of the hospital stay, and neurodevelopmental parameters. Long-term metabolic and endocrine outcome parameters following intervention parenteral nutrition trials have rarely been in the scope of these investigations.

8 Short Term Outcomes and Benefits

Observational studies and a few randomized clinical trials overwhelmingly support the short-term efficacy of early amino acids in reversing protein loss; Denne and Poindexter [22] and Kashyap provide excellent reviews of these studies [43]. Most observational studies [45, 53, 54] and randomized clinical trials using high doses of parenterally administered amino acids [40] or combined parenteral and enteral administration [4, 55] demonstrate improved growth at hospital discharge or 36 weeks postmenstrual age, while others do not (Table 12.1) [38, 39].

In the study of Kasyap et al. [40] appropriate for gestational age infants with a birth weight below 1,250 g were randomized to 4 g amino acids/kg/day or to 3 g amino acids/kg/day. Higher amino acid administration resulted in earlier regain of birth weight (10 vs 12.3 days, $P < 0.05$). Plasma urea concentrations were not different between groups. Wilson et al. [55] randomly allocated 125 VLBW infants to 0.5 g amino acids/kg/day within 12 h after birth, with a stepwise increase to 3.5 g/kg/day, together with initiation of lipids on day 2, and early minimal enteral feeding or to 1 g amino acids/kg/day (target 2.5 g/kg/day) on day 3 together with initiation of lipids on day 5. Infants in the early amino acid group regained birth weight sooner (9 (6–11) vs 12 (9–17) day, $P < 0.001$) and had improved growth at discharge home. Survival and the incidence of common neonatal morbidities were similar between groups. Clark et al. [38] randomly assigned 122 preterm infants (23–29 6/7 week gestational age) to 1.5 g amino acids/kg/day (target 3.5 g/kg/day) or to 1.0 g amino acids/kg/day (target 2.5 g/kg/day). Weight gain until day 28 of birth and incidence of secondary morbidities were not different between groups. On day seven concentrations of several amino acids and plasma urea nitrogen were higher in the higher amino acid group. In the study of Blanco et al. [39] 62 extremely low birth weight infants (birth weight < 1,000 g) were allocated to 2 g amino acids/kg/day and a target intake of 4 g/kg/day or to 0.5 g/kg/day with a target of 3 g/kg/day. Weight gain at day 28 of life or at discharge home were not different between groups [56]. In the historical cohort study of Geary et al. [54] the era before and after three early management practice changes—early parenteral amino acids at 3 g/kg/day, surfactant at delivery followed by immediate extubation to nasal continuous positive airway pressure, and decreased oxygen exposure—were compared. Infants in the era after these changes regained birth weight sooner, maintained appropriate size for weight at 36 weeks and had less morbidity associated with poor long-term outcome. Dinerstein et al. [4] compared a cohort of 117 VLBW infants receiving amino acids at 1.5 g/kg/day (target 4 g/kg/day), 0.5 g lipids/kg/day (target 3.5 g/kg/day), 5.6 mg glucose/kg/min (target 13 mg/kg/day), and enteral feeding on day 1, with a historical cohort of 65 VLBW infants conservatively fed. In the more aggressively fed cohort infants regained birth weight sooner (10 (1–21) vs 16 (1–29), $P < 0.001$) and there was a 66 % reduction in the risk of postnatal malnutrition at 40 weeks corrected age (OR 0.34; 95 % CI 0.17–0.67). Kotsopoulos et al. [45] compared a cohort of early amino acid administration (1.5 g/kg/day immediately after stabilization, target of 4 g/kg/day) to a historical cohort where amino acids were initiated after 12–30 h at

1 g/kg/day (target 3.5 g/kg/day). Infants in the early amino acid cohort regained birth weight more quickly; growth until day 28 or discharge home and neonatal outcomes were not different between groups.

In addition to these effects, early amino acid administration also has beneficial effects on the synthesis of specific proteins. For example, Van den Akker et al. [57] reported an up-regulation of albumin synthesis with the infusion of 2.4 g amino acids/kg/day from birth onwards. However, and despite the increased albumin synthesis rates (median of 225 mg/kg/day vs 170 mg/kg/day), albumin concentrations remained low, and the albumin synthesis rates in preterm infants were still lower than those measured in utero in fetuses between 28 and 35 weeks gestation (median 290 mg/kg/day) [58]. Early amino acid administration is also beneficial in that it raises the concentration and absolute synthesis rates of glutathione, the major intracellular anti-oxidant (10 ± 3 mg/kg/day with 2.4 g amino acids/kg/day from birth onwards vs 6.5 ± 1.8 mg/kg/day without amino acid administration during the first two days) [59]. The postnatal depletion of glutathione can partially be reversed with early amino administration.

9 Long Term Outcomes and Benefits

Beneficial long-term effects on neurodevelopment have been difficult to prove because nutrition is only one of the many variables determining neurodevelopment. Disease itself might also negatively affect nutritional intake, and on average, the time on total parenteral nutrition is usually limited to periods of less than a week.

Studies have shown that proteins are critical to the development of neurological functions and that malnutrition can alter neuronal density [60–62]. The relationship between postnatal growth and neurodevelopment has been illustrated in several studies [63–66]. In a large observational study of 613 infants born at < 30 weeks gestation, weight and body mass index gains to term age were associated with better psychomotor developmental outcomes at 18 months corrected age [67]. In that study, the results were adjusted for various confounding factors such as neonatal morbidities, the administration postnatal steroids and parental education. In another cohort study of 219 VLBW infants ($< 1{,}250$ g), the postnatal growth pattern during the first nine months, rather than weight status at birth, was significantly associated with neurological outcomes at two years of age, also after adjusting for several morbidities [64]. In addition, a preterm infant's growth rate in the NICU (the first few weeks of life) was correlated with neurodevelopment and growth outcome as well, as was demonstrated in a cohort of 500 ELBW infants [63]. Neurocognitive outcomes at ages five to eight were also shown to be associated with in-hospital weight gain and post-discharge head circumference growth in VLBW infants [65, 66].

Longer and more precise follow-up is available for trials using enteral nutrient interventions. The trial by Lucas et al., in which four weeks of an enriched formula instead of a standard formula supplemented to preterm infants led to an improved neurodevelopmental outcome at 18 months corrected age, is well-known [68]. At

7.5 years of age, the cognitive development was still better in this group [69]. A subset of the original cohort was re-evaluated between 15 and 19 years of age. Those who received the enriched formula during the first month of life had higher verbal IQ scores as well as larger caudate nucleus volumes on magnetic resonance imaging (the latter in boys only) [70, 71]. Recent randomized trials of a higher protein plus energy intake during the first month [72] or year [73] of life resulted in improved developmental scores at three months but not at nine months corrected age [72] and increased occipitofrontal circumference and axonal diameters in the corticospinal tract [73]. The value of developmental tests at such a young age is, however, doubtful.

To date, studies investigating the effect of high dose parenteral amino acid administration to preterm infants have not exceeded two years' follow-up. Poindexter et al. retrospectively investigated the effects of having received ≥ 3 g amino acid/kg/day within the first five days of life in infants with birth weights below 1,000 g. At 36 weeks postmenstrual age, those who had received ≥ 3 g amino acid/kg/day showed better weight, length and head circumference compared to those who had not. However, at 18 months corrected age, the effects on length and weight disappeared, whereas the effects on head circumference only remained in boys; there were no effects on neurodevelopment (Table 12.3) [74]. Potentially harmful effects of early high-dose amino acid administration are reported in the follow-up study by Blanco et al. [39] That study showed that extremely preterm infants who received a high-dose infusion of amino acids (up to 4.0 g amino acids/kg/day on day 3 ($n = 32$)) had lower long-term anthropometric measurements and lower cognitive development at 18 months corrected gestational age compared to those who had not. However, at two years corrected age, cognitive development was no longer significantly different between the groups. In addition, plasma amino acid concentrations were negatively correlated with neurodevelopmental scores. Although only 63 % of surviving infants were studied at follow-up, and although the study was clearly underpowered for neurodevelopmental or growth outcomes ($n = 32$ infants), it indicates that neonatologists should be cautious regarding the early administration of high doses of amino acids, especially to the most immature (< 25 weeks GA) and extremely low birth weight (ELBW, BW $< 1{,}000$ g) infants. In contrast to the study by Blanco et al., Stephens et al. showed that the Mental Development Index scores were associated with increases of 8.2 and 4.6 points, respectively, for every extra single g protein/kg/day or every extra 10 kcal/kg/day they received during the first week of life in a retrospective study in ELBW infants [2]. Nutritional intakes during the following weeks of life were not associated with neurocognitive outcome, indicating a very short window of opportunity to improve outcome.

In summary, most of the studies on long-term development indicate that the first few days of life might provide a critical window and that nutrition should be part of immediate care in the preterm born infant.

Table 12.3 Summary of most recent randomized controlled trials on the effect of early and higher dose amino acid administration on weight gain and mental development at 18–24 months corrected age

First author, year, reference	Design	Population	*n*	Intervention	Age at follow-up	Growth	MDI
Blanco [56]	RCT	Gestational age > 24 weeks, birth weight < 1,000 g	32	0.5–3 g AA/kg/day vs 2–4 g AA/kg/day	18 mo CA 24 mo CA	Weight *z*-score: -0.5 ± 0.5 vs -1.5 ± 0.5[a], $P < 0.01$ Weight *z*-score: -0.5 ± 0.5 vs -1.5 ± 0.5, $P < 0.01$	73 ± 15 vs 84 ± 11, $P = 0.03$ 57 ± 11 vs 63 ± 13, $P = 0.2$
Poindexter [74]	RCT, subgroup analysis	Birth weight 401–1,000 g	1,018	≥ 3 g AA/kg/day within the first five days of life vs < 3 g/kg/day within first five days	18 mo CA	Weight < 5th percentile: Adjusted odds ratio 1.1 (0.7–1.6)	78.1 ± 16.1 vs 79.0 ± 18.02, $P = 0.39$

mo CA months corrected age, *MDI* mental developmental index
[a]Mean ± SD (all such values)

Table 12.4 Recommendations for parenteral amino acid intake in preterm infants

Reference	Initiation of amino acids	Starting dose in g/kg/day	Target dose in g/kg/day
Simmer [76]	First postnatal day	2	–
Ehrenkranz [75]	Within hours after birth	3	4
ESPGHAN committee, Koletzko [14]	First postnatal day	Minimum of 1.5	Maximum of 4

10 Current Guidelines and Current Practices

The current guidelines for amino acid intake in preterm infants, which are mainly based on small studies and expert opinion, are summarized in Table 12.4. According to these guidelines, amino acids should be started at the first postnatal day and preferably within hours after birth. Starting doses of 2–3 g/kg/day are recommended, with increases to a maximum dose of 4 g/kg/day over the next few days [14, 75, 76].

Despite the current recommendations, the administration of parenteral nutrition still varies widely between NICUs and countries. Most NICUs start amino acid infusion in preterm infants between 0 and 36 h postnatally [77, 78] Starting doses vary widely, from as low as 0.5–1.0 g/kg/day up to 3.5 g/kg/day. Some NICUs apply a stepwise procedure to reach the target dose of amino acids (3.0–4.0 g/kg/day) [78, 79], However, the preference for a stepwise procedure is purely anecdotal and is based on fluid limitations, worries about intolerance, and fear of hyperglycemia in case of mixed glucose/amino acid solutions. In addition, several audit studies show that the actual intake of amino acids is not in accordance with international guidelines [77–82]. As a result, most preterm infants still have a significant protein deficit during early postnatal life.

11 Conclusions and Future Directions

The present data provide strong evidence for the beneficial effects of the administration of 2–3 g amino acids/kg/day from birth onwards in the average preterm infants on nitrogen balances, growth, and neurodevelopment. However, we should be cautious with the most immature group of infants (< 26 weeks GA) and those that are severely growth-restricted in utero when administering high doses of protein (approximately 4 g/kg/day). Improvements in the composition of amino acids to make the formulas more in accordance with nutritional needs for individual amino acids might overcome potential problems, at least partly.

Future research should be directed towards elucidating the long-term anthropometric metabolic, endocrine and neurodevelopmental outcomes of early (high dose) amino acid administration. Strategies to increase weight gain, from preterm birth to term age and beyond, have been shown to be associated with improved neurodevelopment. In contrast to this period until term age, clinicians should be alert for

excess weight for length gain in early infancy (beyond term age), as this has been associated with later overweight [83] and higher blood pressure [84], and it has not been associated with improved neurodevelopment [67].

Little is known about the metabolic impact and special nutritional needs in cases of particular diseases and conditions, such as chronic lung disease or being born small for gestational age. Future studies in these infants may lead to more individualized, finely tuned nutritional protocols designed for preterm infants.

References

1. Berry MA, Abrahamowicz M, Usher RH (1997) Factors associated with growth of extremely premature infants during initial hospitalization. Pediatrics 100:640–646
2. Stephens BE, Walden RV, Gargus RA et al (2009) First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123:1337–1343
3. Hay WW Jr, Lucas A, Heird WC et al (1999) Workshop summary: nutrition of the extremely low birth weight infant. Pediatrics 104:1360–1368.
4. Dinerstein A, Nieto RM, Solana CL, Perez GP, Otheguy LE, Larguia AM (2006) Early and aggressive nutritional strategy (parenteral and enteral) decreases postnatal growth failure in very low birth weight infants. J Perinatol 26:436–442
5. Thureen P, Heird WC (2005) Protein and energy requirements of the preterm/low birthweight (LBW) infant. Pediatr Res 57:95R–98R
6. Hulst JM, van Goudoever JB, Zimmermann LJ et al (2004) The effect of cumulative energy and protein deficiency on anthropometric parameters in a pediatric ICU population. Clin Nutr 23:1381–1389
7. Martin CR, Brown YF, Ehrenkranz RA et al (2009) Nutritional practices and growth velocity in the first month of life in extremely premature infants. Pediatrics 124:649–657
8. Ehrenkranz RA (2000) Growth outcomes of very low-birth weight infants in the newborn intensive care unit. Clin Perinatol 27:325–345
9. van Goudoever JB, Sulkers EJ, Lafeber HN, Sauer PJ (2000) Short-term growth and substrate use in very-low-birth-weight infants fed formulas with different energy contents. Am J Clin Nutr 71:816–821
10. Kashyap S, Schulze KF, Ramakrishnan R, Dell RB, Heird WC (1994) Evaluation of a mathematical model for predicting the relationship between protein and energy intakes of low-birth-weight infants and the rate and composition of weight gain. Pediatr Res 35:704–712
11. Bauer J, Werner C, Gerss J (2009) Metabolic rate analysis of healthy preterm and full-term infants during the first weeks of life. Am J Clin Nutr 90:1517–1524
12. Riedijk MA, Voortman G, van Beek RH, Baartmans MG, Wafelman LS, van Goudoever JB (2008) Cyst(e)ine requirements in enterally fed very low birth weight preterm infants. Pediatrics 121:e561–567
13. Thomas B, Gruca LL, Bennett C, Parimi PS, Hanson RW, Kalhan SC (2008) Metabolism of methionine in the newborn infant: response to the parenteral and enteral administration of nutrients. Pediatr Res 64:381–386
14. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R (2005) 3. Amino acids Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), supported by the European Society of Paediatric Research (ESPR). J Pediatr Gastroenterol Nutr 41(Suppl 2):12–18
15. Lemons JA, Adcock EW, 3rd, Jones MD Jr, Naughton MA, Meschia G, Battaglia FC (1976) Umbilical uptake of amino acids in the unstressed fetal lamb. J clin invest 58:1428–1434

16. van Veen LC, Teng C, Hay WW Jr, Meschia G, Battaglia FC (1987) Leucine disposal and oxidation rates in the fetal lamb. Metabolism 36:48–53
17. Chien PF, Smith K, Watt PW, Scrimgeour CM, Taylor DJ, Rennie MJ (1993) Protein turnover in the human fetus studied at term using stable isotope tracer amino acids. Am J Physiol 265:E31–35
18. van den Akker CH, Schierbeek H, Minderman G et al (2011) Amino acid metabolism in the human fetus at term: leucine, valine, and methionine kinetics. Pediatr Res 70:566–571
19. van den Akker CH, Schierbeek H, Dorst KY et al (2009) Human fetal amino acid metabolism at term gestation. Am J Clin Nutr 89:153–160
20. Ziegler EE, O'Donnell AM, Nelson SE, Fomon SJ (1976) Body composition of the reference fetus. Growth 40:329–341
21. Dusick AM, Poindexter BB, Ehrenkranz RA, Lemons JA (2003) Growth failure in the preterm infant: can we catch up? Semin Perinatol 27:302–310
22. Denne SC, Poindexter BB (2007) Evidence supporting early nutritional support with parenteral amino acid infusion. Semin Perinatol 31:56–60
23. Shohl AT, Butler AM, Blackfan KD, MacLachlan E (1939) Nitrogen metabolism during the oral and parenteral administration of the amino acids of hydrolyzed casein. J Pediatr 15:469
24. Helfrick FW, Abelson NM (1944) Intravenous feeding of a complete diet in a child: report of a case. J Pediatr 25:400–403
25. Johnson JD, Albritton WL, Sunshine P (1972) Hyperammonemia accompanying parenteral nutrition in newborn infants. J Pediatr 81:154–161
26. Heird WC, Dell RB, Driscoll JM Jr, Grebin B, Winters RW (1972) Metabolic acidosis resulting from intravenous alimentation mixtures containing synthetic amino acids. New Engl J Med 287:943–948
27. Hay WW Jr (2008) Strategies for feeding the preterm infant. Neonatology 94:245–254
28. Saini J, MacMahon P, Morgan JB, Kovar IZ (1989) Early parenteral feeding of amino acids. Arch Dis Child 64:1362–1366
29. Yu VY, James B, Hendry P, MacMahon RA (1979) Total parenteral nutrition in very low birthweight infants: a controlled trial. Arch Dis Child 54:653–661
30. van Lingen RA, van Goudoever JB, Luijendijk IH, Wattimena JL, Sauer PJ (1992) Effects of early amino acid administration during total parenteral nutrition on protein metabolism in pre-term infants. Clin Sci (Lond) 82:199–203
31. Anderson TL, Muttart CR, Bieber MA, Nicholson JF, Heird WC (1979) A controlled trial of glucose versus glucose and amino acids in premature infants. J Pediatr 94:947–951
32. Rivera A Jr, Bell EF, Bier DM (1993) Effect of intravenous amino acids on protein metabolism of preterm infants during the first three days of life. Pediatr Res 33:106–111
33. Van Goudoever JB, Colen T, Wattimena JL, Huijmans JG, Carnielli VP, Sauer PJ (1995) Immediate commencement of amino acid supplementation in preterm infants:effect on serum amino acid concentrations and protein kinetics on the first day of life. J Pediatr 127:458–465
34. Thureen PJ, Anderson AH, Baron KA, Melara DL, Hay WW Jr, Fennessey PV (1998) Protein balance in the first week of life in ventilated neonates receiving parenteral nutrition. Am J Clin Nutr 68:1128–1135
35. Ibrahim HM, Jeroudi MA, Baier RJ, Dhanireddy R, Krouskop RW (2004) Aggressive early total parental nutrition in low-birth-weight infants. J Perinat 24:482–486
36. te Braake FW, van den Akker CH, Wattimena DJ, Huijmans JG, van Goudoever JB (2005) Amino acid administration to premature infants directly after birth. J Pediatr 147:457–461
37. Thureen PJ, Melara D, Fennessey PV, Hay WW Jr (2003) Effect of low versus high intravenous amino acid intake on very low birth weight infants in the early neonatal period. Pediatr Res 53:24–32
38. Clark RH, Chace DH, Spitzer AR (2007) Effects of two different doses of amino acid supplementation on growth and blood amino acid levels in premature neonates admitted to the neonatal intensive care unit: a randomized, controlled trial. Pediatrics 120:1286–1296
39. Blanco CL, Falck A, Green BK, Cornell JE, Gong AK (2008) Metabolic responses to early and high protein supplementation in a randomized trial evaluating the prevention of hyperkalemia in extremely low birth weight infants. J Pediatr 153:535–540

40. Kashyap S, Abildskov K, Holleran SF, Ramakrishnan R, Towers HM, Sahni R (2007) Effects of early aggressive nutrition in infants with birth weight (BW) < 1250 g: a randomized controlled trial. PAS (abstract) E-PAS2007:5912.2002
41. Roberts SA, Ball RO, Moore AM, Filler RM, Pencharz PB (2001) The effect of graded intake of glycyl-L-tyrosine on phenylalanine and tyrosine metabolism in parenterally fed neonates with an estimation of tyrosine requirement. Pediatr Res 49:111–119
42. Thureen PJ, Hay WW Jr (2001) Early aggressive nutrition in preterm infants. Semin Neonatol 6:403–415
43. Kashyap S (2008) Is the early and aggressive administration of protein to very low birth weight infants safe and efficacious? Curr Opin Pediatr 20:132–136
44. Ridout E, Melara D, Rottinghaus S, Thureen PJ (2005) Blood urea nitrogen concentration as a marker of amino-acid intolerance in neonates with birthweight less than 1,250 g. J Perinatol 25:130–133
45. Kotsopoulos K, Benadiba-Torch A, Cuddy A, Shah PS (2006) Safety and efficacy of early amino acids in preterm preterm < 28 weeks gestation: prospective observational comparison. J Perinatol 26:749–754
46. Radmacher PG, Lewis SL, Adamkin DH (2009) Early amino acids and the metabolic response of ELBW infants (≤ 1,000 g) in three time periods. J Perinatol 29:433–437
47. Balakrishnan M, Tucker R, Stephens BE, Bliss JM (2011) Blood urea nitrogen and serum bicarbonate in extremely low birth weight infants receiving higher protein intake in the first week after birth. J Perinatol 31:535–539
48. Roggero P, Gianni ML, Morlacchi L et al (2010) Blood urea nitrogen concentrations in low-birth-weight preterm infants during parenteral and enteral nutrition. J Pediatr Gastroenterol Nutr 51:213–215
49. Rosenthal P (1997) Assessing liver function and hyperbilirubinemia in the newborn. National Academy of Clinical Biochemistry. Clin Chem 43:228–234
50. Usmani SS, Cavaliere T, Casatelli J, Harper RG (1993) Plasma ammonia levels in very low birth weight preterm infants. J Pediatr 123:797–800
51. American Academy of Pediatrics Committee on Nutrition (1985) Nutritional needs of low-birth-weight infants. Pediatrics 75:976–986
52. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R (2005) 1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), supported by the European Society of Paediatric Research (ESPR). J Pediatr Gastroenterol Nutr 41(Suppl 2):S1–S87
53. Vogt RA, Gargus RA, Tucker R, McKinley L, Vohr BR (2004) Impact of early postnatal nutrition on growth in extremely low birth weight infants born small for gestational age. PAS PAS2004:2525
54. Geary CA, Fonseca RA, Caskey MA, Malloy MH (2008) Improved growth and decreased morbidities in < 1,000 g neonates after early management changes. J Perinatol 28:347–353
55. Wilson DC, Cairns P, Halliday HL, Reid M, McClure G, Dodge JA (1987) Randomised controlled trial of an aggressive nutritional regimen in sick very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 77:F4–F11
56. Blanco CL, Gong AK, Schoolfield J et al (2012) Impact of early and high amino acid supplementation on ELBW infants at 2 years. J Pediatr Gastroenterol Nutr 54:601–607
57. van den Akker CH, te Braake FW, Schierbeek H et al (2007) Albumin synthesis in premature neonates is stimulated by parenterally administered amino acids during the first days of life. Am J Clin Nutr 86:1003–1008
58. van den Akker CH, Schierbeek H, Rietveld T et al (2008) Human fetal albumin synthesis rates during different periods of gestation. Am J Clin Nutr 88:997–1003
59. Te Braake FW, Schierbeek H, de Groof K et al (2008) Glutathione synthesis rates after amino acid administration directly after birth in preterm infants. Am J Clin Nutr 88:333–339
60. Soto-Moyano R, Fernandez V, Sanhueza M et al (1999) Effects of mild protein prenatal malnutrition and subsequent postnatal nutritional rehabilitation on noradrenaline release and neuronal density in the rat occipital cortex. Brain Res Dev Brain Res 116:51–58

61. Winick M, Rosso P (1969) The effect of severe early malnutrition on cellular growth of human brain. Pediatr Res 3:181–184
62. Morgane PJ, Mokler DJ, Galler JR (2002) Effects of prenatal protein malnutrition on the hippocampal formation. Neurosci Biobehav Rev 26:471–483
63. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK (2006) Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 117:1253–1261
64. Latal-Hajnal B, von Siebenthal K, Kovari H, Bucher HU, Largo RH (2003) Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. J Pediatr 143:163–170
65. Franz AR, Pohlandt F, Bode H et al (2009) Intrauterine, early neonatal, and postdischarge growth and neurodevelopmental outcome at 5.4 years in extremely preterm infants after intensive neonatal nutritional support. Pediatrics 123:e101–109
66. Kan E, Roberts G, Anderson PJ, Doyle LW (2008) The association of growth impairment with neurodevelopmental outcome at eight years of age in very preterm children. Early Hum Dev 84:409–416
67. Belfort MB, Rifas-Shiman SL, Sullivan T et al (2011) Infant growth before and after term: effects on neurodevelopment in preterm infants. Pediatrics 128:e899–906
68. Lucas A, Morley R, Cole TJ et al (1990) Early diet in preterm babies and developmental status at 18 months. Lancet 335:1477–1481
69. Lucas A, Morley R, Cole TJ (1998) Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ 317:1481–1487
70. Isaacs EB, Gadian DG, Sabatini S et al (2008) The effect of early human diet on caudate volumes and IQ. Pediatr Res 63:308–314
71. Isaacs EB, Morley R, Lucas A (2009) Early diet and general cognitive outcome at adolescence in children born at or below 30 weeks gestation. J Pediatr 155:229–234
72. Tan M, Abernethy L, Cooke R (2008) Improving head growth in preterm infants—a randomised controlled trial II: MRI and developmental outcomes in the first year. Arch Dis Child Fetal Neonatal Ed 93:F342–F346
73. Dabydeen L, Thomas JE, Aston TJ, Hartley H, Sinha SK, Eyre JA (2008) High-energy and -protein diet increases brain and corticospinal tract growth in term and preterm infants after perinatal brain injury. Pediatrics 121:148–156
74. Poindexter BB, Langer JC, Dusick AM, Ehrenkranz RA (2006) Early provision of parenteral amino acids in extremely low birth weight infants: relation to growth and neurodevelopmental outcome. J Pediatr 148:300–305
75. Ehrenkranz RA (2007) Early, aggressive nutritional management for very low birth weight infants: what is the evidence? Semin Perinatol 31:48–55
76. Simmer K (2007) Aggressive nutrition for preterm infants—benefits and risks. Early Hum Dev 83:631–634
77. Hans DM, Pylipow M, Long JD, Thureen PJ, Georgieff MK (2009) Nutritional practices in the neonatal intensive care unit: analysis of a 2006 neonatal nutrition survey. Pediatrics 123:51–57
78. Collins CT, Chua MC, Rajadurai VS et al (2010) Higher protein and energy intake is associated with increased weight gain in pre-term infants. J Paediatr Child Health 46:96–102
79. Lapillonne A, Fellous L, Mokthari M, Kermorvant-Duchemin E (2009) Parenteral nutrition objectives for very low birth weight infants: results of a national survey. J Pediatr Gastr Nutr 48:618–626
80. Grover A, Khashu M, Mukherjee A, Kairamkonda V (2008) Iatrogenic malnutrition in neonatal intensive care units: urgent need to modify practice. JPEN J Parenter Enteral Nutr 32:140–144
81. Hopewell J, Miletin J (2012) Parenteral nutrition in very low birth weight infants in the United Kingdom and Ireland. Ir Med J 105:42–45
82. Kirk EL (2009) Audit to determine whether current parenteral nutrition regimens for pre-term infants on the neonatal unit are in accordance with international guidelines. Arch Dis Child 94:e2

83. Taveras EM, Rifas-Shiman SL, Belfort MB, Kleinman KP, Oken E, Gillman MW (2009) Weight status in the first 6 months of life and obesity at 3 years of age. Pediatrics 123:1177–1183
84. Belfort MB, Rifas-Shiman SL, Rich-Edwards J, Kleinman KP, Gillman MW (2007) Size at birth, infant growth, and blood pressure at three years of age. J Pediatr 151:670–674

Chapter 13
Aggressive Parenteral Nutrition

Karen Simmer

Abstract Aggressive parenteral nutrition (PN) after preterm birth is commenced on day one with the aim of matching fetal growth rate and body composition. The aim of aggressive PN is to reduce the incidence and severity of ex-utero growth retardation (EUGR). EUGR is currently common in extremely low birth weight (ELBW) infants and is associated with co-morbidities including developmental delay and adult coronary artery disease.

Parenteral amino acids at 1.5 g/kg/day is required to prevent negative nitrogen balance and 3.5 g/kg/day for positive nitrogen balance similar to that in-utero. Randomised controlled trials provide further evidence that an aggressive nutritional regime can minimise the large protein deficit most ELBW babies incur, and reduce the rate of EUGR. Preventing EUGR avoids the risk of catch-up growth and associated long-term health sequelae. Improving energy and protein intakes in week 1 is associated with better developmental outcomes of ELBW infants.

For preterm infants, it is recommended that parenteral amino acids solutions with profiles to mimic fetal aminogram, be started on day 1. Provision of adequate non-protein calories is necessary to prevent oxidation and facilitate protein synthesis. Early intravenous lipid is safe and an optimal fat blend should be chosen to improve long-chain polyunsaturated fatty acid (LCPUFA) status, reduce lipid peroxidation and maintain immune function. Nutritional intake by day 5 should be 3–4 g/kg/day protein and $\geq$ 100 kcal/kg/day. Growth and body composition should be analysed with long-term health and developmental outcomes to guide future nutritional interventions within the preterm period.

Key points

- Extra-uterine growth retardation (EUGR) is common after very preterm birth and is associated with adverse neurological and metabolic outcomes.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_13,

- Aggressive parenteral nutrition (PN) is defined as PN commenced within hours of birth and graded up early to meet recommended intakes with the aim of meeting fetal accretion rates, growth and body composition.
- Aggressive PN will reduce the incidence and severity of EUGR.
- The amino acid solution and lipid emulsion need be chosen carefully to avoid toxicity and inflammation. Randomised controlled trials need to assess refinements of aggressive PN aimed at improving short and long-term growth health and development.
- Standardised PN using pre-prepared bags provides high early nutrient intakes compared with individually prescribed PN. Neonates can tolerate some variation in intake and standardized PN appears safe.
- Long-term follow up of very preterm infants should include measures of cardiovascular health and insulin resistance. Optimal growth targets are likely to vary depending on clinical outcome of interest.

1 Preterm Birth and Optimal Nutrition

The rate of preterm birth has increased over the last few years with over 8 % of births in Australia and almost 13 % in the US occurring prematurely. Survival of very preterm infants continues to improve and is currently > 95 % from 28 weeks gestation and > 80 % from 24 weeks gestation in Western Australia [1]. Approximately10 % of preterm infants have some long-term developmental needs and a significant proportion is at increased risk of adult respiratory and cardiovascular disease. Adverse outcomes include abnormalities associated with insulin sensitivity, lipid metabolism, blood pressure and fat distribution. Ex-preterm infants have reduced insulin sensitivity in childhood after being exposed to adverse nutritional environment while in the neonatal intensive care unit (NICU) [2–4]. Preterm infants have increased percent body fat and reduced lean muscle mass secondary to protein deficits in early life after preterm birth. Their distribution of fatty tissues is different with increased abdominal fat and increased intrahepatic fat (Fig. 13.1) [5, 6].

Poor early postnatal growth is associated with adverse neurodevelopmental outcomes. Ehrenkraz et al. (2006) followed 495 infants with BW 501–1,000 g to 18–22 months as part of the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network. Infants were divided into weight gain quartiles for in-hospital growth velocity rates with the lowest being 12 g/kg/week and the highest 21 g/kg/week. Bivariate analysis demonstrated that as the rate of weight gain increased, the incidence of cerebral palsy, abnormal neurological examination, neurodevelopmental impairment and need for rehospitalization, fell significantly [7].

The early weeks of life present an opportunity to reduce the co-morbidities associated with preterm birth by the provision of optimal nutrition. Preventing EUGR may reduce the risk of many of these adverse outcomes. The risk of developing non communicable diseases such as type 2 diabetes appears to be modified by early nutrition especially protein and this is referred to as epigenetic programming.

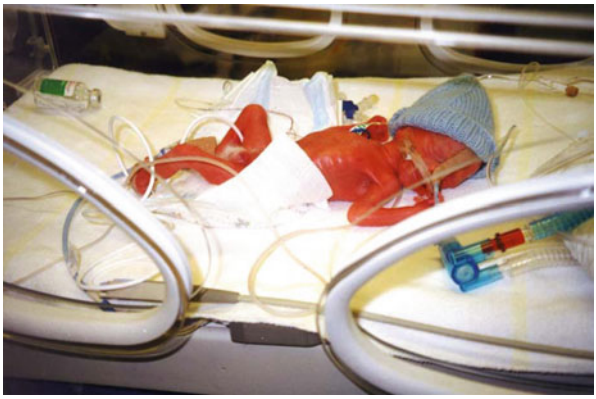

Fig. 13.1 Extremely preterm (gestation 24 weeks) infant on ventilatory support in an incubator

Nutritional strategies, once infants become protein deficient and growth retarded, may differ depending on the target outcome with the possibility that catch up growth may lead to better neurodevelopmental outcomes but more metabolic disease. For example, Regan et al. (2006) found that ex-preterm who were protein deficient in the neonatal period had decreased insulin sensitivity compared with term controls and those with the greatest catch-up weight gains had the lowest insulin sensitivities [8]. Animal models suggest that if you are born lean, better health outcomes may be achieved if you remain lean. Mice fed nutrient-enriched diets to achieve catch-up growth in early life have reduced longevity [9]. Possible mechanisms suggested by the investigators include accelerated shortening of chromosomal telomeres.

2 Aggressive Parenteral Nutrition (PN)

Aggressive PN implies that amino acids are started earlier and at higher doses than was routine clinical practice. The rationale is based on fetal accretion rates and evidence of improved nitrogen balance and safety. The aim is to reduce the incidence and severity of extra-uterine growth retardation (EUGR).

Early aggressive nutrition for extremely low birth weight (ELBW) infants is usually defined as PN from the first day of life increasing to provide full nutrient requirements before day 5. The definition often includes minimal enteral feeding, and early grading to full enteral feeds with fortification of human milk. Early aggressive nutrition has become a priority in the NICU with recognition of the high incidence of EUGR and the increasing evidence that EUGR is associated with long-term growth and developmental problems [10–12].

3 EUGR

EUGR is defined as a discharge weight of less than the 10th percentile. Until recently, the incidence of EUGR in ELBW infants has been very high and inversely correlated with gestational age. Clark et al. (2003) demonstrated for very preterm infants in

the U.S, the difference between weight gain in the first 28 days and estimated fetal growth was large with fetal weight gain nearly double that ex-utero [10]. Dusick et al. (2003) reported, using NICHD data in the U.S, that 97 % of very low birth weight (VLBW) infants have EUGR at discharge and 40 % of these infants still weigh less than the 10th percentile at one and a half years of age [11]. In WA, for infants born at < 28 weeks gestation in 2009–2010, the incidence of EUGR was 50 %.

Embleton et al. (2001) demonstrated cumulative deficits in enteral energy and protein intakes in infants born ≤ 30 weeks' gestation, amounting to a mean energy deficit of 813 kcal/kg and a protein deficit of 23 g/kg by the end of the 5th postnatal week. By an equivalent postnatal age, older infants also accrued an energy and protein deficit of around 382 kcal/kg and 13 g/kg, respectively. Feeding at 120 kcal/kg/day and 3.0 g protein/kg/day after the first few weeks of life did not correct these deficits by discharge [13].

Preventing nutritional deficits is the focus of contemporary neonatal practice. Senterre and Rigo (2011) in a prospective non-randomised consecutive observational study in 102 infants with birthweight < 1250 g demonstrated that postnatal growth reduction can be dramatically reduced if nutritional protocols were optimized to meet recent RDI. Mean intake in week one was 3.2 g/kg/day amino acids and 80 kcal/kg/day. They used a standardised PN solution prepared by their hospital pharmacy 2.7 g amino acids and 12 g dextrose/100 ml with electrolytes and minerals. They report a similar proportion of EUGR in their population as was growth-retarded at birth (20 %) [14].

The amount and ratio of macronutrients given to preterm infants often differ from that in-utero which might explain some of the observed differences in body composition between term and preterm infants at term. During the later half of pregnancy, it is estimated that the placenta transfers to the fetus 3.6–4.8 g/kg/day protein and 12–14 g/kg/day glucose. In-utero uptake by the fetus exceeds the amount needed for protein accretion and the excess is oxidized to produce energy. The total fetal requirement for fatty acids at mid-gestation is 1 g/kg/day suggesting that oxidation of fatty acids for energy is relatively unimportant in fetal life [15]. Conversely, the infant born preterm is initially fed glucose intravenously often at concentrations higher than can be metabolized. Amino acid solutions are infused but often at low initial rates and initiation of lipid emulsion may be delayed with final infusion rates at 3.0–3.5 g/kg/day exceed in utero rates of fatty acid uptake.

4 Provision of Protein and Energy—Evidence and Guidelines

It has long been known that PN in the first 24 h improves the early nutrition of sick preterm infants [16]. However, the incidence, severity and consequences of inadequate nutrition have been increasingly recognized especially with the improved survival of very immature infants.

5 Protein

At least 1.5 g/kg/day parenterally is required for the newborn to prevent negative nitrogen balance. If a 26 weeks gestational newborn receives only 10 % dextrose in the first week of life, he will develop a 25 % body protein deficit by the end of the first week [17]. This deficit is difficult to compensate and will likely influence future health.

Provision of parenteral amino acids at 3 g/kg/day by day 5 of life will reduce the incidence of EUGR and of sub-optimal head growth at 18 months. This relatively high protein intake is well tolerated with plasma amino acids within the reference range [18, 19].

Others have demonstrated that for every additional 1 g/kg/day protein intake in week one, there is an associated 8 point increase in mental developmental index on the Bayley Scale of Infant Development [20].

Thureen et al. (2003) using isotope infusions and indirect calorimetry measurements demonstrated that very preterm infants tolerate infusions of 3 g/kg/day early in life with plasma amino acid levels similar to the fetus and with improved protein accretion and nitrogen balance (185.6 vs. − 41.6 mg N/kg/day day 2) compared with those receiving 1 g/kg/day [21]. The outcomes were primarily achieved by increased protein synthesis rather than proteolysis and appeared well tolerated in the first days of life.

Ibrahim et al. (2004) infused 16 infants with glucose and 3.5 g amino acids and 3.0 g lipid/kg/day within 2 h of birth and compared nitrogen balance and related metabolic indices with infants who only received glucose for the first 48-h of life. Nitrogen balance was positive with the high amino acid infusion without relevant clinical implications on day one of life, compared with control infants, who were in negative balance (400 vs. − 180 mg N/kg/day) [22].

Te Braake et al. (2005) randomised 135 infants to receive either glucose and 2.4 g AA/kg/day from birth onward ($n = 66$) or solely glucose during the first day with a stepwise increase in AA intake to 2.4 g AA/kg/day on day 3 ($n = 69$). Lipid was introduced on day two to both groups. Age to regain birth weight was not statistically different, but nitrogen balance on day two was improved in the infants receiving early amino acids (145 vs. − 84 mg N/kg/day) [23].

Clark et al. (2007) compared high and low dose of early PN in 122 infants with gestational ages between 23 and 29 weeks. The low dose group started 1 g/kg of amino acids on day 1 and increased to 2.5 g/kg/day by day 4, the high dose groups started at 1.5 g/kg and increased to 3.5 g/kg/day by day 3. Both doses were found to be safe but their difference in growth over the first 4 weeks of life was not significant. They concluded that increasing the dose of protein without additional energy did not significantly increase growth and that the quality and quantity of amino acids for the very preterm infants required further research [24]. The paper received some criticism included that from van den Akker et al. [25]. This latter group has performed detailed studies using stable isotopes techniques to determine that albumin is synthesized at very high rates in the preterm fetuses compared with

matured fetuses and they believe protein intakes higher than 3 g/kg/day should be more beneficial for preterm infants [26].

Provision of non protein calories is important, aiming at protein to energy ratios at around 3 g/100 kcal, but the relationship is curvilinear with most effect at about 60 kcal/kg/day. Protein intake by contrast is likely to be associated with increased protein accretion at every level of protein intake. Embleton (2007) reviewed randomised clinical trials comparing high versus low dose of parenteral amino acids and demonstrated a linear relationship with a plateau yet to be reached. He concluded that 3.5 g/kg/day in the first week was safe but further trials are needed to determine if more is safe and of benefit [27].

In summary, the evidence to date suggests that introducing amino acids on day 1 is beneficial in the short-term [21–24, 28, 29]. However, there is still a delay in implementation to clinical practice with some neonatologists remaining cautious about high early protein intake. An editorial in the Journal of Perinatology comments on this practice of iatrogenic malnutrition with the title "Is it time to stop starving premature infants?" [30]. Cohort studies suggest better neurodevelopment and head growth [19, 20]. High doses, although increasing nitrogen retention, have not been demonstrated to improve growth but data is limited and there have been few long term studies.

6 Carbohydrate

ELBW infants have limited glucose tolerance, although providing amino acids will increase endogenous insulin secretion. Maximum glucose oxidation in preterm infants is 8.3 mg/kg/min or 12 g/kg/day [28]. The upper rate of glucose administration is determined by glucose oxidative capacity for energy production and glycogen deposition and is influenced by gestational age and clinical condition, and may range from 7–12 mg/kg/min. Hyperglycaemia is common after preterm birth, possibly related to surges in catecholamines, decrease in insulin production and insulin resistance. Hyperglycaemia is associated with death, IVH, sepsis and death. Excessive glucose intakes may increase carbon dioxide production and exacerbate chronic lung disease. Treatment with insulin is not recommended as it offers no clinical benefit and insulin infusions are associated with risk of hypoglycaemia and associated morbidity [31].

7 Lipid

Lipid is a useful source of energy and the Cochrane Review concludes that early lipid is safe [32]. Lipid can be started on day 1 or 2 at 1 g/kg/day and the recommended upper limits for preterm and term infants are 3 and 4 g/kg/day, respectively [28].

8 Guidelines

Given the wide recognition of the high incidence and risk of EUGR, nutritional guidelines for ELBW infants have been revised with consideration of the fetal reference related to lean body mass and protein gain. Recommendations include protein 1.5–2 g/kg/day grading to 3.5–4.4 g/kg/day; energy as tolerated to 120 kcal/kg/day (resting metabolic rate 40–60 kcal and 45–65 kcal for growth with variable additional requirements based on activity, thermogenesis); glucose 4–6 mg/kg/min grading to 12 mg/kg/min; fat 1–2 g/kg/day grading to 3–3.5 g/kg/day; Na 3.5 mmol/kg/day; Ca 1.3–3 and P 1–2.3 mmol/kg/day; vitamin and mineral supplementation including vitamin A 700–1,500 IU/kg/day, vitamin D40–160 IU/kg/day, and Se 2–4 μg/kg/day, Zn 400–500 μg/kg/day and Iodine 1 μg/kg/day. Lipid should be started early as no harm has been demonstrated [32] and no later than day 3 of PN [28].

9 Monitoring of PN Includes

1. Na, K, Cl, HCO2 and PGL at least daily. Definition of hypoglycaemia is < 2.6 mmol/l and hyperglycaemia, > 10 mmo/l or lower if glycosuria. Ca, P and Mg twice a week until stable;
2. Urea daily to twice weekly (amino acids can serve as a significant energy source beyond the requirements for protein accretion in the ELBW infant, an elevated urea concentration may reflect an acceptable metabolic by-product rather than protein intolerance);
3. Plasma TG aiming weekly at 150–250 mg/dl (2.8 mmol/l) but this is not routine practice in many NICU where plasma TG are ordered only when lipaemic serum noted. PN lipid clearance is determined by lipoprotein lipase, hepatic lipase and lecithin cholesterol acyltransferase. When lipid infusion rate exceeds hydrolysis rate, concentrations of plasma triglycerides and free fatty acids increase;
4. If PN is required for more than a few weeks, weekly liver function tests and monthly bone bloods (Alk Phos, Ca, P) should be measured;
5. Catheter-related sepsis is a constant concern during PN and routine CRP measurements are used in some units to detect late-onset sepsis early;
6. Daily weight; and weekly length and head circumference measurements. Ideally some measurement of body composition should be documented prior to discharge (peapod air displacement plethysmography or electrical impedence, or skin fold thickness by calipers or ultrasound).

10 Choice of Amino Acid Solutions

Historically the use of amino acid preparations not designed for preterm infants had resulted in metabolic acidosis and hyperammonemia, and this history is associated with the reluctance of some clinicians to use parenteral protein early.

Amino acids delivered parenterally do not undergo the same extent of enteric and hepatic metabolism as amino acids delivered enterally, including conversion to other amino acids (e.g., glutamate to arginine; phenylalanine to tyrosine; methionine to cysteine) and thus, higher amounts of these constituents are required in parenteral solutions. TrophAmine (B Braun) is designed to achieve plasma amino acid levels of healthy 30 day old breastfed infants [33]. Other amino acid preparations attempt to mimic amino acid concentrations in either the mid-trimester fetus or cord blood of preterm or term infants. These relatively new preparations avoid the previous reported high levels of phenylalanine and tyrosine and improve clinical outcomes.

Currently, in Western Australia, we use Primene which was designed by Rigo et al. [34] and contains less phenylalanine and methionine than solutions formulated for children and adults. Primene also contains taurine and ornithine. McIntosh et al. (1990) initially demonstrated that this new amino acids solution was well tolerated by very preterm infants with plasma amino acid levels within the fetal reference range and higher protein intakes improving nitrogen balance [35]. McIntosh and Mitchell (1990) conducted a randomised controlled trial of Primene compared with the standard amino acid solution in the UK and Australia at the time (Vamin, *Fresenius Kabi)* and confirmed the fetal aminogram with Primene but reported increased mortality in the Vamin group. They believed that the poor outcome in the Vamin group may be related to the toxic levels of phenylalanine and tyrosine which are known to be neuro-toxic and hepatotoxic [36].

11 Choice of Lipid Emulsion

Choice of intravenous lipid is important as the composition influences peroxidation and fatty acid metabolism. Fatty acids are biologically active and influence gene expression, signal transduction pathways and cellular responses, and are precursors of important long chain fatty acids such as docosahexaenoic acid (DHA) which may be important for brain development. Lipid emulsions currently available for neonates have been recently reviewed by Deshpande and Simmer [37].

The optimal blend of lipid for PN needs to provide essential fatty acids, maintain LC PUFA and immune function and reduce lipid peroxidation. Historically, the only intravenous lipid available in most countries has been based on soybean oil (omega 6 PUFAs rich in linoleic acid (LA) and double bonds which are particularly susceptible to peroxidation and the production of toxic hyper-peroxides). More recently, lipid emulsions have become available containing blends of soy and olive oils (Clinoleic, Baxter) and blends of soy, medium chain triglycerides, olive and fish oils (SMOF, Fresenius Kabi) (Table 13.1). Omega 6 fatty acids (soy oil) are generally thought of as inflammatory, omega 9 (olive oil) as immune-neutral and omega 3 (fish oil) as anti-inflammatory. From studies in older children, we also know that the infusion of fish oil may reverse the cholestasis associated with parenteral nutrition [38].

We conducted two randomised controlled trials (RCT) of lipid emulsions in very preterm infants (Gestation < 30 weeks). In the first, soil oil (SO Intralipid) v olive

Table 13.1 Content of lipid emulsions

	SO	OL	SMOF
Soybean oil	100 %	20 %	30 %
Coconut oil (MCT)	–	–	30 %
Olive oil	–	80 %	25 %
Fish oil	–	–	15 %
Egg. phospholipid g %	1.2	1.2	1.2
α-tocopherol μg/ml	14.5	30.3	200
Linoleic (C18:2n −6) % fatty acids	50	17.2	37.2
Arachadonic (C20:4n −6) % fatty acids	0.3	0.5	1.0
Alpha-linoleic (C18:3n −3) % fatty acids	7.0	2.3	4.7
Eccosapentanoic EPA (C20:5n −3) % fatty acids	–	–	4.7
Docosahexaenoic DHA (C22:6n −3) % fatty acids	0.34	0.5	4.4

SO (Soy oil, Intralipid), OL (Olive oil, Clinoleic) and SMOF (Soy/MCT/Olive/Fish oil)

oil (OL Clinoleic) RCT, we hypothesised that infants in the OL group would have improved DHA status due to lower LA intake reducing inhibition of metabolism of n-3 LCPUFA. We also hypothesized that infants in the OL group would have reduced peroxidation as measured by F2 isoprostanes due to replacing some PUFA with mono-unsaturated fatty acids (MUFA). However, we found that both groups had similar and significant decreases in plasma and red blood cell (RBC) DHA and in plasma F2 isoprostane levels. Patients in the OL group had a fatty acid profile more comparable to human milk-fed preterm infants and tolerated a higher glucose load [39]. The lower glucose tolerance in the intralipid group is partly explained by the fact that soy oil lipid emulsions are thought to enhance glucose production via gluconeogenesis and glycogenolysis [40].

Our second RCT in infant < 30 weeks' gestation was of OL v SMOF. SMOF contains a good source of essential fatty acids, energy, MUFAs, omega 3 fatty acids including eicosapentanoic acid (EPA) and DHA, and is supplemented with an antioxidant, alpha tocopherol. We found increased RBC EPA, reduced peroxidation (plasma F2 isoprostanes) and increased plasma vitamin E levels in the SMOF versus OL group and the end of the seven day intervention. Surprisingly, there was no difference in RBC DHA levels between the groups (Deshpande G et al., in press).

RBC fatty acid levels of very preterm infants after a week of lipid emulsions in these two neonatal RCTs are summarized in Table 13.2.

We conducted a similar RCT (SMOF v OL) in infants > 34 weeks gestation and demonstrated increased RBC DHA and EPA levels and reduced F2 isoprostanes with SMOF v OL (Deshpande G et al., in press).

The lack of increase in RBC DHA with SMOF in very preterm infants (< 30 weeks) is difficult to explain. The same protocol in the more mature infants resulted in the expected result of increased DHA levels. One interpretation of the different results in different gestational age groups using the same protocol is that very immature infants may need even more DHA and this is consistent with previous clinical trials of DHA supplements [41].

In summary, OL may be preferable to SO in preterm infants because its use avoids impairment of immune function and depletion of long chain omega-6 PUFA

Table 13.2 RBC % fatty acids after one week of lipid emulsion in infants < 30 weeks gestation

	OL ($n = 24$)	SO ($n = 21$)	
LA C18:2n −6	8.84 (3.27)	13.40 (5.54)	$P = 0.001$
AA C20:4n −6	8.12 (4.09)	7.70 (4.40)	
DHA C22:6n −3	2.62 (1.26)	2.14 (1.39)	
	OL (n = 15)	*SMOF (n = 15)*	
LA C18:2n −6	10.19 (2.74)	11.53 (2.57)	
AA C20:4n −6	13.59 (3.44)	12.77 (2.33)	
EPA C20:5n −3	1.07 (0.38)	2.29 (0.81)	$P = 0.001$
DHA C22:6n −3	3.91 (1.18)	4.39 (0.73)	

OL: Olive oil, SO: Soy oil, SMOF: Soy/MCT/Olive/Fish oil

derivatives, decreases the possibility of oxidative stress, has advantages for glucose metabolism and is safe and well tolerated [42]. SMOF provides a fast source of energy comparatively, less immunological influence and may reduce cholestasis although data from very preterm infants remains limited.

12 Preparations and Cost: Standardized v Individually Prescribed PN

Traditionally in NICU, individual prescriptions for neonatal PN were written every 24 h. In recent years, the safety of hanging bags for 48 h has been demonstrated. One problem with individually prescribed PN (IPN) is the relative unavailability on the first day and opportunity for errors and under nutrition. The process encourages trivial adjustments and often results in inadequate protein intake. Standardised parenteral nutrition (SPN) using pre-prepared bags is used commonly in adult medicine and recently also in neonatal units. Large units prepare standardised packs in-house, smaller units order from commercial sources. With the recognition that neonates can tolerate some variation in intake and require relatively high amounts of protein and energy, it is acknowledged that optimal standardised solutions, or at least a few options, may be sufficient. Specific standardised packs can be ordered as in many Australian States, commercial company or industry can manufacture ideal solutions based on reaching recommended intakes.

Lenclen et al. (2006) performed a retrospective case controlled study in France when changing from IPN to SPN (20 per group < 32 weeks gestational age). SPN provided higher earlier intakes of amino acids and glucose and better calcium phosphate ratio [43]. Similarly Iacobelli et al. (2010) in France prospectively evaluated SPN and IPN ($n = 67$ and 40 < 33 weeks' gestation) and found higher intakes of amino acids and energy in SPN group associated with reduced early weight loss [44]. Conversely, Smolkin et al. (2009) is a case-controlled study (70 infants per group VLBW infants) in Israel found that infants received IPN, not SPN, was optimal for growth [45].

The immediate benefit of SPN is that it can be commenced within an hour of birth. "Starter packs" containing dextrose and amino acids have a shelf life of 40 days and

formulated to provide up to 2 g/kg/day. SPN as compared with IPN is increasingly preferred from a cost and safety view. Whether SPN is prepared in-house or ordered commercially depends on the quantity required. Collaboration between neonatal units in the same region, increasing the orders for specific SPN bags, may lead to greater efficiencies.

In the NICU in Western Australia, starter packs of PN which contain 2 g of amino acids per 100 ml of 5 and 7.5 % dextrose are immediately available (as bags have a shelf life of 40 days). Patients < 32 weeks gestation receive this solution within the first hour of birth. Patients generally receive only 1–1.5 g/kg/day over the first day as multiple other infusions contribute to daily fluid intake. Decision not to increase further the amino acid concentration in starter bags was influenced by the RCT of Clark et al. (2007) [24]. From day 2, standardized bags are used with 2 or 3 g amino acid/100 ml 4–14 % dextrose with 4 mmol Na, 1 mmol K, 0.75 mmol Ca, 0.25 mmol Mg, 0.75 mmol P, 3.23 mmol Cl and 3 mmol acetate/100 ml. Ca and P solubility issues are related to pH, temperature, amino acids and other components in PN. Aluminium contamination of some forms of electrolytes is a concern as aluminium can accumulate in the preterm infants and is associated with anaemia, neurotoxicity and bone disease. Part of the chloride is replaced with acetate to avoid hyperchloraemic metabolic acidosis [46]. Trace element solution (Biomed) at 1 ml/kg/day and heparin at 0.5 U/ml are added. Clinoleic or SMOF emulsions are started on day 2 at 1 g/kg/day and increased to 3–3.5 g/kg/day. Fat and water soluble vitamins are added to the lipid emulsions to meet RNI (Vitalipid N and Soluvit N). PN bags are hung for 48 h rather than 24 h bags to save costs with no increased risk of infection, Lipid is prepared in amber-coloured 50 ml syringes which provide 36 ml 20 % Clinoleic, Vitalipid N 10 % 11.2 ml and Soluvit N 2.8 ml) with shelf life of 8 days, which provide 3 g/kg/day of fat at 15 ml/kg/day.

In other states in Australia, many NICUs collaborate to order a limited number of PN bags from one commercial supplier. With collaboration, the composition of PN bags ordered changed from over 60 formulae to three, thus reducing cost. In large NICUs (80–100 bed), standardized bags are prepared “in-house” which further reduces the cost. An alternate approach used in the NICU in Auckland, New Zealand, ensure full nutrient requirements are met, is to deliver total PN in a small volume (30 ml) and the remaining fluid intake adjusted as required (www.adhb.govt.nz/newborn/Guidelines/Nutrition/Nutrition.htm).

Recently, Rigo et al. (2012) assessed a multi-chamber ready-to-use solution in 113 preterm infants enrolled in a multicentre prospective non-comparative study in Belgium [47]. The pack contained three chambers with the third lipid chamber being optional to activate. The solution contained 3.1 g amino acids, 13.3 g dextrose and 2.5 g lipid/100 ml. The benefits are thought to be sterility, longer shelf life and increased likelihood of delivering required nutrition early. To improve stability and reduce peroxidation, vitamins and trace elements were added by the hospital pharmacy, and frequently electrolyte supplementation was requested by physicians (43 %). However, the PN was well tolerated and increased nutritional intakes (> 2.5 g/kg/day amino acids and > 75 kcal/kg/day in the first week. This preparation is available commercially in some countries (Baxter Healthcare, Switzerland).

13 Conclusion and Future Directions

Preterm infants require more aggressive nutrition than paediatric patients and specialized specific programs for their PN should exist in all perinatal and paediatric tertiary centres. Standardised PN is likely to deliver nutrient safely, effectively and efficiently, often with cost savings, and should be considered in all units.

RCT evaluation of high parenteral protein intakes aimed at preventing EUGR are required with long-term follow-up of neurodevelopment and metabolic health. Increasing lean mass and reducing adiposity should be achievable. Further research into understanding the mechanism behind programming of adverse metabolic outcomes should lead to a real improvement of long-term health of adults born preterm without compromising neurological outcomes.

Getting the macronutrient right is a good start. Future research need to address the optimal quality of nutrition, including influence on outcomes directly and indirectly through epigenetic regulation of the genome. Future studies of preterm infants need to consider nutritional interventions and epigenetic changes with longer term outcomes.

Recent research has focused on LCPUFA as inflammation is core to many outcomes. Similarly, the effect of vitamin A on the incidence and severity of chronic lung disease needs more work and sourcing appropriate preparation for clinical practice is an ongoing important area, as barriers remain to translate current evidence into practice in preterm patients requiring prolonged PN. Future research into osteopenia of prematurity (metabolic bone disease) with fractures needs to evaluate components of PN that are soluble at high doses, safe and effective in preventing bone disease in this high risk population.

Very preterm patients are surviving to live long and productive lives. They may suffer the adverse events of relative malnutrition in the preterm period if action is not taken now. The optimal postnatal growth trajectory may differ for different target outcomes, sometimes referred to as the neuro-metabolic trade-off. The balance between the short and long term benefits and risks of rapid catch up growth has also been referred to as the catch-up dilemma [48]. Meticulous attention to providing optimal nutrition in the preterm period will reduce EUGR, catch-up growth and long-term metabolic derangements.

Acknowledgement The author would like to thank Ms Judith Kristensen, Pharmacy, King Edward Memorial Hospital for reviewing the manuscript.

References

1. Health Department of Western Australia (2010) The 13th Report of the Perinatal and Infant Mortality Committee of Western Australia for Deaths in the Triennium 2005–2007. Western Australia
2. Singhal A, Fewtrell M, Cole TJ, Lucas A (2003) Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 361:1089–1097

3. Hofman PL, Regan F, Jackson WE et al (2004) Premature birth and later insulin resistance. NEJM 351:2179–2186
4. Mathai S, Cutfield WS, Derraik JG et al (2012) Insulin sensitivity and B-cell function in adults born preterm and their children. Diabetes 61:2479–2483
5. Uthaya S, Thomas EL, Hamilton G, Doré CJ, Bell J, Modi N (2005) Altered adiposity after extremely preterm birth. Pediatr Res 57:211–215
6. Thomas EL Uthaya S, Vasu V et al (2008) Neonatal intrahepatocellular lipid. Arch Dis Child Fetal & Neonatal Ed 93:F382–F383
7. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK (2006) Growth in the NICU influences neurodevelopmental and growth outcomes of ELBW infants. Pediatrics 117:1253–1261
8. Regan FM, Cutfield WS, Jefferies C, Robinson E, Hofman PL (2006) The impact of early nutrition in premature infants on latter childhood insulin sensitivity and growth. Pediatrics 118:1943–1949
9. Ozanne SE, Hales CN (2004) Lifespan: catch-up growth and obesity in male mice. Nature 427:411–412
10. Clark RH, Thomas P, Peabody J (2003) Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics 111:986–990
11. Dusick AM, Poindexter BB, Ehrenkranz RA, Lemons JA (2003) Growth failure in the preterm infant: can we catch up. Semin Perinatol 27:302–310
12. Cooke RW, Foulder-Hughes L (2003) Growth impairment in very preterm and cognitive and motor performance at 7 years of age. Arch Dis Child 88:482–487
13. Embleton NE, Pang N, Cooke RJ (2001) Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 107:270–273
14. Senterre T and Rigo J (2011) Optimizing early nutritional support based on recent recommendations in VLBW infants and postnatal growth restriction. JPGN 53:536–542
15. Hay WW Jr, Lucas A, Heird WC et al (1999) Workshop summary: nutrition of the extremely low birthweight infant. Pediatrics 104:1360–1368
16. Saini J, MacMahon P, Morgan JB, Kovar IZ (1989) Early parenteral feeding of amino acids. Arch Dis Childhood 64:1362–1366
17. Denne SC, Poindexter BB (2007) Evidence supporting early nutritional support with parenteral amino acid infusion. Semin Perinatol 31:56–60
18. Radmacher PG, Lewis SL, Adamkin DH (2009) Early amino acids and the metabolic response of ELBW infants in three time periods. J Perinatol 29:433–437 (ePub 2009 Apr 2)
19. Poindexter BB, Langer JC, Dusick AM, Ehrenkranz RE (2006) Early provision of parenteral amino acids in extremely low birthweight infants: relation to growth and neurodevelopmental outcomes. J Pediatr 148:300–305
20. Stephens BE, Walden RV, Gargus RA et al (2009) First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123:1337–1343
21. Thureen PJ, Melara D, Fennessey PV, Hay WW (2003) Effect of low vs high intravenous amino acid intake on very low birthweight infants in the early neonatal period. Pediatr Res 53:24–32
22. Ibrahim HM, Jeroudi MA, Baier RJ, Dhanireddy R, Krouskop RW (2004) Aggressive early total parental nutrition in low-birth-weight infants. J Perinatol 24:482–486
23. te Braake FW, van den Akker CH, Wattimena DJ, Huijmans JG, van Goudoever JB (2005) Amino acid administration to premature infants directly after birth. J Pediatr 147:457–461
24. Clark RH, Chace DH, Spitzer AR (2007) Pediatrix Amino Acid Study Group. Effects of two different doses of amino acid supplementation on growth and blood amino acid levels in premature neonates admitted to the neonatal intensive care unit: a randomised controlled trial. Pediatrics 120:1286–1296
25. van den Akker CH, te Braake FW, Rövekamp-Abels WW, van Goudoever JB (2008) Quality of amino acid solutions for preterm infants. Pediatrics 121:865–866

26. Van den Akker CH, Schierbeek H, Rietveld T et al (2008) Human fetal albumin synthesis rates during different periods of gestation. Am J Clin Nutr 88:997–1003
27. Embleton ND (2007) Optimal protein and energy intakes in preterm infants. Early Hum Dev 83:831–837
28. ESPGHAN (2005) Guidelines on Paediatric Parenteral Nutrition. J Pediatr Gastroenterol Nutr 45: S5–S18
29. Parish A, Bhatia J (2008) Early aggressive nutrition for the preterm infant. Neonatology 94:211–214
30. Neu J (2009) Is it time to stop starving premature infants? (Editorial). J Perinatol 29:399–400
31. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart AL et al (2008) Early insulin therapy in very low birthweight infants. NEJM 359:1873–1884
32. Simmer K, Rao SC (2005) Early introduction of lipids to parenterally-fed preterm infants. Cochrane Database of Syst Rev 4:CD005256
33. Heird WC, Dell RB, Helms RA et al (1987) Amino acid mixtures designed to maintain normal plasma amino acid patterns in infants and children requiring parenteral nutrition. Pediatrics 80:401–408
34. Rigo J, Senterre J, Putet G, Salle B (1987) A new amino acid solution specially adapted to preterm infants. Clin Nutr 6:105–109
35. McIntosh N, Ventura V, Kempson C (1990) A new amino acid preparation for low birthweight infants. In: Intensive therapy and clinical monitoring
36. McIntosh N, Mitchell V (1990) A clinical trial of two parenteral nutrition solutions in neonates. Arch Dis Child 65:692–699
37. Deshpande G, Simmer K (2011) Lipids for parenteral nutrition in neonates. Curr Opin Nutr Metab Care 14:145–150
38. Gura KM, Lee S, Valim C et al (2008) Safety and efficacy of a fish-oil-based fat emulsion in the treatment of parenteral nutrition-associated liver disease. Pediatrics 21:e678–e686
39. Deshpande GC, Simmer K, Mori T, Croft K (2009) Parenteral lipid emulsions based on olive oil compared with soybean oil in preterm (< 28 weeks' gestation) neonates: a randomised controlled trial. J Pediatr Gastroenterol Nutr 49(5):619–625
40. van Kempen AA, van der Crabben SN, Ackermans MT, Endert E, Kok JH, Sauerwein HP (2006) Simulation of gluconeogenesis by intravenous lipids in preterm infants: response depends on fatty acid profile. Am J Physiol Endocrinol Metab 290:E723–E730
41. Makrides M, Gibson RA, McPhee AJ et al (2009) Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. JAMA 301:175–182
42. Sala-Vila A, Barbosa VM, Calder PC (2007) Olive oil in parenteral nutrition. Curr Opin Clin Nutr Metab Care 10:165–174
43. Lenclen R, Crauste-Manciet S, Narcy P et al (2006) Assessment of implementation of a standardized parenteral formulation for early nutritional support of very preterm infants. Eur J Pediatr 165:512–518
44. Iacobelli S, Bonsante F, Vintéjoux A, Gouyon JB (2010) Standardized parenteral nutrition in preterm infants: early impact on fluid and electrolyte balance. Neonatology 98:84–90
45. Peters O, Ryan S, Matthew L, Cheng K, Lunn J (1996) Randomised controlled trail of acetate in preterm neonates receiving parenteral nutrition. Arch Dis Child Fetal Neonatal Ed 77:F12–F15
46. Smolkin T, Diab G, Shohat I et al (2010) Standardized versus individualized parenteral nutrition in very low birth weight infants: a comparative study. Neonatology 98:170–178
47. Rigo J, Marlowe ML, Bonnot D et al (2012) Benefits of a new pediatric triple-chamber bag for parenteral nutrition in preterm infants. JPGN 54:210–217
48. Victora CG, Barros FC (2001) Commentary: the catch up dilemma: relevance of Leitch's 'low-high' pig to child growth in developing countries. Int J Epidemiol 30:217–220

Part IV
Catch up Growth/Developmental Origin of Adult Diseases

Chapter 14
Catch up Growth and the Developmental Origins of Health and Disease (DOHaD) in Preterm Infants

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Abstract Preterm infants are vulnerable to the effects of malnutrition in both the pre- and post-discharge period. On-going illness and immaturity result in a delay in the establishment of adequate nutrition. During this period, cumulative nutrient deficits are accrued and growth is poor. The majority of preterm infants are discharged with a weight lower than their birth centile, indicative of poor growth. Nutrition has the potential to promote catch-up growth, although growth acceleration in some situations is associated with increased risk of metabolic problems in the longer term. Controlled trial data show that early nutrient intakes may 'programme' a range of long term metabolic outcomes. The Developmental Origins of Health and Disease (DOHaD) theory amalgamates many areas of scientific study and encompasses a wide range of diverse disciplines from epidemiology to molecular biology. The mechanisms linking early growth to later outcomes include permanent structural changes, accelerated cellular ageing and epigenetic mechanisms. There are data to link faster early growth with decreased insulin sensitivity in children born preterm, but many other long-term effects do not demonstrate consistent associations with early growth. Despite such *potential* metabolic concerns, the current data suggest that promoting improved nutrient intake and catch up growth in the pre- and post-discharge period is likely to result in better neurocognitive outcomes.

Key Points

- Early nutrition affects cognition and metabolic function in later life through a range of mechanisms
- Preterm infants are at risk of poor nutritional status which may result in worse cognitive outcome
- Nutritional status (a composite of health status, requirements and demands) is not solely determined by nutrient intake

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_14,

- Growth acceleration or catch up growth may increase the risk of longer term metabolic harm in term infants, but the data in preterm infants are unclear
- Strong efforts must be taken to maximise the receipt of mother's own breast milk, both pre- and post-hospital discharge
- It is unclear whether post-discharge formula enrichment results in longer term benefit

1 Nutritional Vulnerability in Preterm Infants

Survival rates for infants born preterm have increased dramatically over the last three decades. This is likely to be attributable in part to better nutritional management. Closer attention is now being paid to longer-term outcomes, and in particular to their relationship with early growth and nutrient provision. In most neonatal intensive care units (NICUs) in developed countries, a survival rate in excess of 50 % is typically achieved for infants born at 24 weeks gestation, many of whom are born at a weight of around 500–750 g. Body composition studies provide data for the 'reference fetus' [1]. These show that a 24 week infant is composed of approximately 90 % water—at 500 g this means there is then just 50 g of 'dry' tissue, primarily protein and a tiny amount of mineral and lipid in neural structures and cell membranes. There are no fat stores or other tissues that could be considered dedicated energy stores. Basal metabolic rate calculated using calorimetry is around 50 kcal/kg/day in preterm infants, and energy requirements for the additional demands of common neonatal co-morbidities such as sepsis and respiratory distress syndrome may be substantial [2, 3]. Without sufficient exogenous energy the infants must use their own lean tissue to provide energy [4, 5]. Catabolism of lean tissue (mainly muscle and organs such as liver) will result in functional compromise—respiratory and diaphragm muscle function will be less effective, and synthesis of essential proteins may be impaired.

To support homeostasis and maximise function whilst enteral feeds are established, parenteral nutrition (PN) is now considered standard care for extremely low birth weight (ELBW) infants [6]. Although a degree of weight loss is inevitable (because of loss of extracellular water), nutritional management aims to minimise or avoid catabolism, and support and optimise growth from the first few postnatal days. In recent years though, concerns have been raised around whether growth promotion in these infants may be harmful, and whether 'aggressive' nutritional practices results in later metabolic harm in preterm infants [7, 8]. This is largely based on an extrapolation of data from studies in term babies (of whom some had in-utero growth restriction) within the Developmental Origins of Health and Disease (DOHaD) hypothesis. Extrapolation to the unique situation of prematurity is not straightforward. There is some limited data in preterm babies demonstrating associations between early nutrient provision and later metabolic outcomes [8–11], but this needs to be considered in the context of the clear neurocognitive advantages of optimising early nutrition.

2 Developmental Origins of Health and Disease (DOHaD)

DOHaD is a relatively new and immensely exciting discipline but has its roots in work performed more than half a century ago. Animal studies in the 1950s showed relationships between early growth and later outcomes. The classic cross-fostering rat studies of McCance, Widdowson and others [12] set the foundations for the study of early life nutrition. These studies showed that nutrient deprivation in very early postnatal life permanently affected growth potential, whereas later nutrient deprivation (after weaning) seemed not to result in permanent growth restriction. Over the next 2–3 decades various human studies suggested a link between growth in early life (both fetal and infant) and later metabolic outcomes. However, the discipline now amalgamated under the Developmental Origins of Health and Disease (DOHaD) umbrella did not receive widespread attention until the late 1980s and early 1990s when Barker et al. [13–20] showed consistent and large associations between birth weight and later risk of diabetes and cardiovascular health. This data was primarily epidemiological and focused on the relationship with birthweight, used as a proxy for fetal growth. The impact in scientific terms of these studies was immense such that the phenomenon was originally referred to as the 'Barker hypothesis', and subsequently the 'Fetal Origins of Adult Disease' [18]. It became apparent, however, that these mechanisms and effects were not restricted to fetal life and that nutrition and growth in infancy (and perhaps in later childhood) were also crucial, leading to the incorporation of elements of evolutionary biology and the adoption of the term DOHaD [21].

Barker's early work showed that term infants with lower birthweight had increased adult risk of type II diabetes and cardiovascular disease. There was also an apparent association with size at 1 year of age, again suggesting benefits of greater infant weight gain [13]. These findings have been replicated in studies conducted worldwide. Whilst the precise interpretation of these findings are still the subject of much debate, and the magnitude of the effect might still be less than lifestyle factors (e.g., smoking, diet etc.), it is important to appreciate that these epidemiological data primarily relate to term born infants. However, conflicting data do exist. More recent prospective, longitudinal cohort studies have failed to demonstrate a consistent role of the gestational environment or early postnatal growth in later insulin sensitivity in childhood [22]. Whether effects will appear later in life remains to be seen.

Birthweight in terms of the DOHaD hypothesis is important because it is a proxy for fetal growth. However, babies born with equivalent birth weights might have been exposed to very different in-utero nutritional environments. Fetuses with in-utero growth restriction (IUGR) are born at a lower weight than they would have been had pregnancy proceeded without problem, but there will never be any way of knowing what the 'appropriate' birthweight might have been for any individual. Given the relationship between the environment and genes, it is not even possible to imply that a 'genetically determined' birthweight exists for any single individual. IUGR implies fetal growth impairment, and although many infants born IUGR are also born small for gestational age (SGA) the terms are not synonymous. Birthweight

is an extremely accurate 'spot' measurement, but not necessarily a reliable indicator of fetal growth patterns.

Early life nutrition may affect later growth and disease through a variety of mechanisms including permanent structural change, clonal selection, accelerated cellular ageing, changes to homeostatic or endocrine systems (e.g., onset of puberty, or appetite control) and programming effects. There are a myriad of molecular processes involved that might explain the subsequent 'programming' effects of early growth, many of which have yet to be fully elucidated, but it seems likely that a substantial number of these will involve epigenetic processes [23]. It is not completely clear why early growth restriction results in adverse sequelae in later life, but, in its broadest sense, adaptations to a poor nutrient environment are probably made to attempt to maximise early and continuing survival to reproductive age [21, 24, 25]. Insulin resistance, higher blood pressure and decreased formation of comparatively 'energy-expensive' lean tissues are the 'trade-offs' made to help promote survival to reproductive age, even if this results in adverse, later-life outcomes. It is now clear that preterm birth is associated with increased risks of adverse metabolic outcomes. However, there are limited data to show that nutritional care during NICU stay for preterm infants increase those metabolic risks except perhaps during the first 2 weeks of life.

3 Early Nutrition and Growth

3.1 Parenteral Nutrition

The use of PN in infancy was first described in the late 1960s, and it has been widely used in preterm infants for the last 2–3 decades. Although there is little evidence of long-term neuro developmental benefit and the evidence base is still not substantial, studies in preterm infants have helped refine compositional requirements. PN is often introduced gradually because of concerns around metabolic tolerance despite few data to substantiate those concerns. Gradual introduction of PN (and enteral feeds) mean that ongoing requirements are not met, so each day a 'nutrient deficit' is accrued [26]. Over time the cumulative nutrient deficit might be substantial. It has been calculated that by the end of the 2nd postnatal week the protein deficit was such that only around 40 % of nutrient requirements was met during that period. Nutrient deficits are closely associated with growth failure, but probably only explain around half of the association between weight gain and intake [26].

A key study by Wilson et al. [27] showed that early 'aggressive' PN resulted in improved growth. Many would now consider the term 'aggressive' as a misnomer: even in this study intakes failed to match recommended intakes as suggested by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [28, 29]. Most authorities recommend immediate commencement of at least 2–3 g/kg/day (protein equivalent) of amino acids, and similar amounts of intravenous lipid [6, 28, 30]. Despite these recommendations most NICUs still fail to

Table 14.1 Potential adverse longer term effects of early parenteral nutrition

Concern	Potential adverse effect (example)
Sub-optimal amino acid composition in PN solution causing abnormal plasma amino acid (PAA) profile	PAA peaks may cause neuronal injury [34–36]
Aluminium contamination of PN solution in manufacturing process	Decreased bone mass in preterm born adolescents [37]
	Worse neurodevelopmental outcome [38]
Excess energy supplied from, and sub-optimal fatty acid blend of intravenous lipids	Abnormal aortic and myocardial function in young adults born preterm [39]

achieve those intakes and early growth is poor [31] although recent reports suggest some progress in limiting early growth failure [32]. The long-term impact of this early growth failure remains to be elucidated as few studies have been adequately powered to look at the long-term effects of early growth failure.

There are few studies to demonstrate the benefits of increased nutrient provision in the first few days but data do exist. In an observational study Stephens et al. [33] showed that first week intakes of protein and energy in preterm infants < 1000 g were closely related to developmental outcome at 18 months corrected age using the Bayley Scale of Infant Development (BSID). Each additional 10 kcal/kg intake was associated with a 4.6 point increase in Mental Development Index (MDI), and each 1 g/kg protein intake associated with an 8.2 point increase in MDI at 18 months. The apparent benefit of increased protein and calorie intakes was restricted to the first week but remained highly significant even after adjustment for likely confounders such as gestation, sex, parental education and illness severity. These are observational data but strongly argue *against* nutrient restriction in early postnatal life. However, some PN studies show inconsistent effects and concern remains around the potential for high peaks of specific amino acids to be associated with worse developmental outcome [34].

Most extremely low birth-weight (ELBW, < 1250 g) infants are likely to benefit from early PN, but it is unclear what weight cut-off should be used to guide the initiation of PN that most appropriately balances the nutritional benefits of PN against the known risks and complications. There are many short-term risks associated with PN use such as central venous line complications (e.g., sepsis, line mis-placement etc.), and PN associated cholestasis. Longer term DOHaD-type effects may also exist relating to the tissue-specific effects of the composition of early PN solutions (see Table 14.1).

Unfortunately it is difficult to avoid many of these risks. Although aluminium-free PN solutions could be made available and amino acid combinations could be improved, this requires consideration of cost/benefit ratio, and clinicians to work with industry to develop and design better solutions. Intravenous lipid is essential for both delivery of essential fatty acids and fat-soluble vitamins, and as a means of providing sufficient caloric intake without excess carbohydrate. Fatty acids are key components of cell membranes and neural structures and changes in supply may

affect a range of outcomes, especially in sick infants. The high content of omega-6 present in soybean predominant oil emulsions may be associated with increased production of vasoactive prostanoids and an increased inflammatory state. Newer lipid solutions based on combinations of fish and olive oils, and other sources, may result in better outcome. However, these have generally been developed as a response to the pro-inflammatory concerns in other population groups (e.g., adults) rather than being specifically designed, for example, to improve cognitive outcome in preterm infants. Although concerns exist, there may be no alternative to accepting some adverse vascular outcomes at present. Similar to many areas of nutrition for preterm infants, it should now be clear that a compromise needs to be attained between the positive nutritional benefits, and the risks of short term harm (e.g., from central venous catheters) and longer term adverse metabolic programming.

3.2 Feeding in the First Two Weeks and Later Outcomes

Despite potential neurodevelopmental advantages of early nutrient intakes from PN there are some data showing adverse metabolic effects of early growth promotion. Few studies have tracked preterm infants into adolescence or early adulthood, but such data are required if we are to interpret the longer term impacts of early nutrition. Whilst there is now a large body of literature demonstrating the increased incidence of insulin resistance, altered body composition, higher blood pressure, and earlier onset of puberty in children or adults born preterm, it is largely observational in nature and does not specifically examine these outcomes in relation to early growth. Observational data where the analyses have been adequately adjusted for the presence of confounding and interacting variables are important sources of data. However, all observational data suffer from the risks of reverse causation: it is possible that the apparent association of improved nutrient intakes with better neurodevelopmental outcome is due to something inherently different in the infants who benefit. Perhaps infants were more 'healthy' in some other way that allowed them to tolerate greater nutrient intakes and thus gain a neurodevelopmental advantage. In growth terms, it is possible that infants with specific genetic differences resulting in differing gene expression, transcription and protein production are those who grow fastest but also have the highest risk of insulin resistance. Disentangling nutrient effects from growth effects is not easy in retrospective studies.

Controlled, prospective studies are required to be certain of the direction of effect between early exposures such as weight gain or nutrient intakes, and later outcomes. In a series of seminal studies Lucas, Singhal and Fewtrell et al. [8, 10, 40, 47] have demonstrated longer term metabolic effects in a cohort of children born preterm and related this to specific epochs of growth. A detailed critique of these important studies is beyond the scope of this chapter, but the data are virtually unique in terms of their relevance to DOHaD in individuals born preterm. Two of the most important findings relate to insulin resistance and vascular health in 13–16 year old children who were born preterm (average birthweight $\sim$ 1.4 kg, gestation $\sim$ 31 weeks). The

cohort was divided into quartiles of weight gain (or loss) in the first 2 weeks. Fasting 32–33 split pro-insulin was used as a measure of insulin resistance in adolescence and showed 20 % higher levels in children who received a nutrient enriched diet versus those on a lower nutrient diet (7.2 vs. 5.9 pmol $p = 0.01$) [10]. Insulin levels also demonstrated a step-wise increase associated with more positive weight gain: children born preterm with higher rates of weight gain in just the first two postnatal weeks appeared to be more insulin resistant independent of birthweight, gestation and other relevant neonatal factors. Interestingly, a control group of children who were healthy and born at term had similar fasting 32–33 split pro-insulin concentrations (6.9 pmol) to the nutrient enriched group. Although a partly semantic argument, this could be interpreted as an advantage to early nutrient or growth restriction, rather than a disadvantage to nutrient enrichment.

In the same cohort and using the same quartiles of early weight gain, flow mediated brachial artery dilatation (FMD) assessed by high-resolution vascular ultrasound was used as a measure of vascular health. Higher FMD indicates more 'elastic' vessels associated with vascular health. Greater weight gain or linear growth in the first 2 weeks was associated with lower FMD independent of birthweight and other likely confounders. FMD was 4 % lower in adolescents with the highest rates of weight gain, a level of adverse effect similar to that seen for insulin dependent diabetes or smoking in adults. Similar to the data on insulin resistance, there were no differences in FMD between those in the highest weight gain group and term controls. Interestingly, although this study showed a similar step-wise association between early weight gain and later vascular health, there were no group differences between those receiving enriched versus standard diets. The interpretation of whether these are then 'catch up growth' effects or 'nutrient' effects remain uncertain. These data are important, but do not suggest that deliberate restriction of nutrient intake in preterm infants is the optimal strategy. Both 32–33 split pro-insulin and FMD are biomarkers rather than disease *per se*, so we cannot be certain of the true longer term morbidity associated with early patterns of growth. These data relate to a subset of the original studies, and it is also possible that these effects may change over time, becoming either more or less important during the life course.

Furthermore, it is important to re-emphasise that these data relate to weight gain in the first 2 postnatal weeks only. Analyses of other time epochs, for example change in weight between 2 weeks and discharge, did not show an association with later outcomes. Whether data will emerge in the future that demonstrate an association between later growth in the pre-discharge period and increased metabolic risks remains uncertain, but even then, consideration of the neurodevelopmental effects must remain. It seems inevitable that because the mechanisms that operate within a DOHaD context are designed to maximise reproductive fitness (in a Darwinian sense), compromises will always exist. There is unlikely, therefore, to be a strategy that enables both maximal brain development and optimal long-term metabolic health in preterm infants.

In healthy term born infants there appear to be no data suggesting better cognitive outcome from more rapid growth in early life, in which case avoiding early growth acceleration may be advantageous. In a comprehensive systematic review of the

literature combined with data from the large Avon Longitudinal Study on Parents and Children (ALSPAC) there was no association between rapid weight or length gain and intelligence quotient (IQ) measurements at 4 or 8 years after adjustment for potential confounders in term born appropriately grown infants [48]. Indeed, there is an increasing body of evidence linking early infant growth acceleration with later obesity [49, 50]. In a study ($n = 299$) of SGA (< 10th centile) term infants randomised to a standard or nutrient enriched diet, weight gain was greater in those on enriched formula [51]. BSID scores did not differ at 18 months of age, but were 4.6 points lower at 9 months in the enriched group with a greater disadvantage in girls than in boys [44]. A control group of breast fed infants showed the highest BSID scores.

4 Catch up Growth or Growth Acceleration?

Several authors have used the terms growth acceleration and catch up growth interchangeably and there is no consensus on definition. For the purpose of this chapter, **growth acceleration** is used to describe the phenomena where weight (or length) accelerates upwards from the birth centile. Typically, this is seen in term infants fed formula rather than breast milk over the first few days. Whether this is an effect of excess volume intake (breast fed term infants typically only consume 20–40 ml/kg/day over the first 1–2 days, compared to 60–80 ml/kg/day in formula fed infants) or excess protein intake is unclear. Higher levels of protein in infant formula were designed to compensate for differences in amino acid composition, but there is now evidence that higher protein intakes may 'drive' growth [52] perhaps via stimulation of insulin like growth factors (IGF).

Catch up growth is used to describe the increase in weight (or length) gain velocity demonstrated after a period of nutrient restriction. Preterm infants discharged on nutrient enriched formula demonstrate an upward crossing of centiles to regain birth centile. Whether growth acceleration and catch up are the same processes at a cellular level, and result in the same outcomes is unclear. However, it is clear that the catch up observed in preterm infants following discharge is occurring at a very different phase in the life course compared to term infants after birth, so it seems likely that the long term consequences will be different [53].

5 Nutrition Prior to Hospital Discharge and Long Term Outcomes

After the first 2 weeks of postnatal life there are no current data to show a metabolic advantage of slower growth pre-discharge. Although fetal growth curves and rates of weight gain have been proposed as the most appropriate growth reference for infants born preterm, a consensus does not exist. It is however, uncertain what data could

be used to determine the optimal growth pattern; so most authors and professional bodies continue to make comparisons to in-utero or fetal references. Because preterm delivery is frequently the end result of a compromised pregnancy (by for example, pregnancy induced hypertension, or placental insufficiency etc.) birth weights of infants born preterm are generally less than equivalent in-utero fetal weights. On average infants will need to gain weight at around 15–17 g/kg/day to remain on the same centile position on a weight chart. Weight gain though, is not the same as growth. Growth implies an increase in all relevant auxological parameters (weight, length and head circumference) whilst maintaining appropriate body composition (see later).

Despite the uncertainties and a focus on the use of PN in the scientific literature, preterm babies receive far more nutrition via the enteral route. Enteral nutrition is always preferable. The relationship between feeding and NEC is complex and although large trials will be needed, few exist [54]. A recent large study in preterm infants at high risk of NEC (< 35 weeks gestation, < 10th centile for weight and with antenatal ultrasound evidence of fetal growth restriction) randomised over 400 infants to either early or delayed feeds [55]. It showed no overall benefit to delaying feed introduction and no consistent effect on NEC, but significantly less cholestasis in the early feeding group. Although other similar studies show inconsistent effects, many NICUs now aim to initiate enteral feeds with breast milk from day 1 [56]. This practice is likely to both limit the duration and therefore the long term metabolic risks associated with PN, and accrue the maximum metabolic benefits of breast milk. Breast milk demonstrates a dose-exposure effect i.e., both increased intake volume and duration show associations with improved outcomes for metabolic health, so any strategy that increases receipt of breast milk is likely to be beneficial [57]. A recent study has shown that breast milk intake, despite a lower nutrient density than most formula, was the most important factor in determining bone mass in early adulthood [41]. Two key practical messages from this chapter then, are that 1) strong efforts must be taken to maximise the supply of mother's own breast milk, and 2) that nutritional status (a composite of health status, requirements and demands) is not solely determined by nutrient intake.

6 Nutrition and Longer Term Neurocognitive Outcome

Long-term follow up of controlled trials of nutrient interventions in early life provide the strongest data showing that improving intakes results in better cognition. Numerous observational studies show similar strong associations between nutrient intake and/or measures of growth e.g., weight gain, and later neurodevelopmental outcome, but even well designed studies risk bias from residual confounding. Recent data demonstrate the possibility of biological correlates that link early nutrition and later cognition, with studies suggesting that the caudate nucleus in particular might be vulnerable to under nutrition in preterm babies in early postnatal life [58–60]. Brain

growth is especially rapid during the third trimester when brain volume doubles, cortical gray matter increases four-fold and there are dramatic increases in sub-cortical gray matter and basal ganglia [58]. There are also dramatic increases in folding and gyrification, and a range of brain ontogenetic events are occurring at different times, so the long term brain effects of adverse exposures such as malnutrition will differ depending on timing and severity [61]. The data from animal studies, our knowledge of human brain biology, and long term follow up of controlled trials strongly argue against nutrient restriction in preterm babies in early life.

7 Nutrition and Body Composition—Longer Term Consequences

Fetal growth in humans is different from many other species with term infants having substantially more fat mass (around 15 %) compared to many other mammals. However, whilst there is limited fat accumulation in the fetus in the late 2nd and early 3rd trimester, most preterm infants ex-utero demonstrate much greater fat accumulation than in-utero peers. Fat provides mechanical support or protection, and provides thermal insulation in an ex-utero environment. It is difficult to determine the most appropriate pattern of fat deposition in preterm infants [62]. There is little data showing how catch up growth affects body composition, but some studies have provided important data. Higher protein:energy ratios are associated with greater lean mass deposition, and inappropriately high energy intakes are associated with markers of excess fat deposition [3]. It is difficult to be certain what standard or reference of lean and fat mass accretion to use in ex-utero preterm infants. However, current data suggests that higher protein:energy ratios (around 3.6 g per 100 kcal compared to 3 g per 100 kcal) [5, 63, 64] of feeds (either preterm formula, or fortified breast milk) are needed to meet protein requirements without providing excess energy (that might simply be deposited as fat) in ELBW preterm infants.

Accurate and precise measurement of fat mass in preterm infants is technically complicated especially prior to hospital discharge. Whilst Dual X-ray Absorptiometry (DXA) scanning is widely used there are numerous assumptions and variables used in the algorithms that mean it might not always provide an accurate figure, although in research studies it appears to provide a robust measure [65]. DXA scanning cannot reliably distinguish between subcutaneous and intra-abdominal fat, and cannot determine fat deposition within organs. Magnetic resonance scanning might provide a more accurate measure than DXA and can also provide data on intrahepatic lipid [66]. The few published studies show that preterm babies exhibit aberrant adiposity at discharge [66] and in later life, [67] but there are limited data to enable determination of how nutrient intakes influence these longer term phenotypes or how these effects may be modulated by catch up growth. Indeed, nutritional factors failed to explain a significant proportion of the variation, and authors suggested that fat deposition might reflect an altered response to stress [66].

8 Nutrition and Catch up in the Post-Discharge Period: Practical Recommendations

Preterm infants are typically discharged at around 36–37 weeks corrected gestation. At this stage, many are below the 10th centile for weight and length, and the majority are below their birth centile [31, 68]. Discharge is largely determined by non-nutritional factors: most babies who are able to maintain their temperature and feed by bottle or breast can be safely discharged. However, an ability to 'demand-feed' does not mean that these babies no longer require nutritional management [69, 70]. There is no evidence to suggest any advantage to formula feeding post-discharge compared to supporting continued breast feeding. If babies are breast fed post-discharge, they are likely to require supplemental iron and vitamin D [71] and possibly vitamin K.

8.1 Supporting Breast Feeding After Discharge

Continuing to breast feed babies, even if they have reduced weight-gain trajectory compared with those given formula milk, is likely to be associated with long term metabolic and cognitive advantage. The minimum rate of weight gain that is 'acceptable' has never been subject to meaningful study. Whilst interpretation of growth requires other measures, linear growth is often not reliably measured in non-hospital settings so weight gain is most useful in a practical sense. Weight gain of at least 20 g/day in an otherwise healthy breast fed preterm infant post-discharge is probably acceptable. If weight gain is poor, or anticipated to be sub-optimal after discharge then addition of a breast milk fortifier is possible and might promote catch up growth. Despite this being suggested by ESPHGAN, [72] there is scant evidence to support such an approach, and in most countries there are no commercially available products designed for the post-discharge setting. Two recent controlled studies provide useful data but both used different strategies and provide conflicting data on benefit [73, 74]. One small study suggested the possibility of growth advantages at 12 months corrected age, higher bone mineral density, and better visual outcomes [75, 76]. Whilst the data are too limited to make firm recommendations, there might be a role for fortifiers post-discharge especially if, they provide greater maternal reassurance to continue breast feeding [77].

8.2 Post-Discharge Formula Enrichment

Enriched formulas following discharge may improve growth, but a recent Cochrane review suggested the data were equivocal [77, 78]. However, several studies showed enhanced growth in the intervention groups (compared to control infants receiving

a term formula) with the exception of one study which appears to show an opposite effect [79]. Close examination of some of the studies show that many infants demonstrate quite marked catch up growth [43, 63, 80–84]. This effect is most marked in the first few weeks, with catch up peaking around a corrected age of term, and declining towards 3 months corrected age which may coincide with the introduction of weaning foods [80]. Growth differences for those continued on a preterm formula were usually greater in boys than girls. None of the studies showed any clear neurodevelopmental advantage [43, 80].

Detailed examination in our studies showed that infants discharged on a term formula consumed greater intake volumes than those on preterm formula. This meant that both groups had virtually identical caloric intake [81]. This suggests a number of important interpretations. Firstly, any group differences in growth were not due to energy, and are most likely due to the higher levels of protein intake (because protein: energy ratio differed). The corollary of this is that simply increasing caloric density of feeds in a post-discharge preterm baby feeding on demand may not result in improved nutrient intake; indeed it might reduce nutrient intake if it results in lower volumes consumed. Secondly, it appears that such infants may primarily regulate intake based on calories, so the intake of every other nutrient depends on the ratio with energy intake, and not the density per unit volume. Assessment of body composition using Dual X-ray Absorptiometry (DXA) showed that there were no group differences in percent fat mass (%FM) [65] so the greater rates of weight gain in the group maintained on preterm formula were not accompanied by excess FM deposition.

The lack of a group difference in developmental outcome in this and other similar trials is perhaps not surprising as the Bayley Scale of Infant Development (BSID) provides a global assessment of neurodevelopment. It was not designed to precisely assess cognitive function in infancy and might not be the optimal tool to assess the neurocognitive effects of nutritional interventions [85]. Similarly, and perhaps more importantly, a difference in brain growth might only be expected where there is a major lack of key nutrients; a slowing in the rate of somatic growth is likely to occur before a decrease in brain growth. Energy is likely to be the primary limiting nutrient for brain growth in the post-discharge period—if babies are feeding ad-libitum and up-regulating their intake to account for differences in nutrient density i.e., they are consuming the calories their brain 'needs', then brain effects are unlikely. Current data strongly suggest that inadequate protein intake is a key limiting nutrient in the first few postnatal weeks in preterm infants, but it is likely that following discharge lower protein intakes and protein:energy ratios (than needed between 24 and 36 weeks corrected age) might still support optimal brain growth. Higher protein:energy ratios (such as those in preterm or post-discharge formula) might be beneficial for catch up somatic growth and improved bone mineral density (BMD), even if they have no apparent benefit on neurodevelopment.

A recent study from Chile reported a historical cohort comparison between preterm infants discharged on term compared to preterm formula [86]. There were no differences in BMD or lean mass (LM) at 1 and 2 years, although total FM was lower in the post-discharge formula (PDF) group at 2 years (19.3 vs. 21.7 %, $p < 0.01$). Fasting insulin was also lower in the PDF group at 2 years (13.6 vs. 26.4 mI U/L, $p < 0.001$)

suggestive, perhaps, of metabolic benefit. Whilst the authors ascribed these potential benefits to the higher levels of DHA in the PDF [86], they might be secondary to higher protein intakes, or more appropriate protein:energy ratios.

Whilst there are animal data showing association between catch up growth, or growth acceleration [21], and later metabolic harm, humans differ from other mammals in many respects, most importantly in respect to brain size, growth and differentiation [87–91]. Few studies have considered metabolic and cognitive outcomes together, especially in the post-discharge period. In a post-hoc analysis of a data set of 911 infants born < 37 weeks gestation and < 2.5 kg, weight gain between term, 4 and 12 months corrected age was compared to blood pressure (BP) measured at 6.5 years of age, and cognition using Wechsler Intelligence Scale for Children (WISC III) at 8 years [92]. After adjusting for child gender, age, and race and maternal education, income, age, IQ, and smoking, for each standard deviation score of additional weight gain from term to 12 months, systolic BP was 0.7 mmHg higher and WISC-III total score was 1.9 points higher. Interestingly, the marginal increase in BP was not noted in infants born < 32 weeks gestation. Overall, the authors conclude that the modest neurodevelopmental advantages of more rapid weight gain in infancy were only associated with small BP-related effects. It is unclear how such changes may 'track' into later life. Effect sizes and clinical relevance may change over time. As this study was in relatively mature preterm infants it is not clear whether the cognitive advantages would be similar, greater or lesser in a more high risk group, and whether a similar level of metabolic 'trade off' would be observed.

A multitude of other nutrient and non-nutrient related processes might be important in the relationship between feeding and DOHaD outcomes in preterm infants. Individual nutrients that are likely to be of specific importance in determining long term outcomes include long chain fatty acids (especially DHA) [93], specific amino acids such as taurine [94], and non-protein nitrogenous compounds such as nucleotides [95]. There is also increasing evidence to support a role of the gastrointestinal microbiota in determining long-term outcomes, and for a role in chronic disease in adulthood, especially obesity [96].

9 Insulin Sensitivity and Catch up Growth in Preterm Infants

The 'metabolic syndrome' involves many biological systems [67], but insulin sensitivity or resistance is perhaps the area subject to the most detailed study in later life. Adults who were born preterm appear to be at a higher risk for type 2 diabetes in later life [97]. Studies examining the influence of birth weight on later health consistently show that in low birthweight born adults, there is decreased insulin sensitivity [98, 99], often combined with other features which contribute to the metabolic syndrome such as reduced glucose tolerance [98], hypertension, hyperlipidaemia and disordered postprandial physiology. There have been direct measurements of insulin sensitivity and glucose homeostasis in subjects born preterm, although studies have used different measures and methods that vary partly because of the participants' age at study (Table 14.2).

Table 14.2 Abnormal metabolic biomarkers and outcomes in children or adults born preterm

Decreased insulin sensitivity and increased type 2 diabetes
Increased cardiovascular disease markers: hypertension, vascular 'stiffness' and hyperlipidaemia
Changes in body composition: reduced bone mineral density; abnormal adipose tissue partitioning
Changes to biological control systems: earlier onset of puberty; altered response to stress

Most studies show that body composition at the time of study has a strong effect on measured insulin sensitivity [100] or glucose tolerance [100–106]. Children and adults with a higher BMI, increased body fat and/or truncal fat had worse glucose processing than their peers. There is some evidence that preterm infants who demonstrate increased growth might have altered insulin sensitivity compared to their peers. Increased height and weight standard deviation score (SDS or Z score) in infancy and early childhood [107–109] are associated with decreased insulin sensitivity in some studies. In addition to the data of Singhal et al. [110] linking higher weight gain in the first two weeks of life to later insulin resistance there are other data showing similar associations, but most are observational and subject to the possibility of confounding and reverse causation. Associations between greater weight gain and insulin resistance or higher serum glucose level were seen in a variety of epochs: birth to term [98], weeks 4–6 of life [111] birth to 9–12 years [106], and 2–21 years of age [108]. By contrast, in one study, preterm infants who were small for gestational age (SGA) who displayed appropriate catch-up growth showed less insulin resistance than term born SGA children when measured between 2 and 8 years old [112]. This emphasises that the mechanisms operating in preterm infants ex-utero are likely to differ from those born at term, and that catch up growth may have metabolic benefits for some preterm infants.

Insulin may play a direct role both as a neonatal growth factor in exaggerated (disordered) catch-up growth, and through additional pathways leading to metabolic problems in later life. Some studies appear to show an apparent beneficial effect of some growth restraint in either the prenatal (i.e., being SGA) or postnatal period (with less catch-up growth) with improved insulin sensitivity compared to those born AGA as young adults. The majority of studies suggest that preterm infants are, as a group, less insulin sensitive than term controls although in some gestational age was only significant when considered along with birth weight [113]. Some studies did not find an association with insulin sensitivity and gestation [101, 114], and differences between preterm infants who were SGA and AGA are not consistent. Some found no difference in insulin sensitivity between AGA and SGA preterm infants in the first week of life [115], and others also failed to find differences in children [104, 107, 112] and adults [98] between SGA and AGA preterm born subjects.

Some of the inter-study variation may be due to heterogeneity of methods of testing and modelling, and may reflect population differences in early nutritional and growth exposures. Many follow up studies include children who are pubertal, a period where increased insulin resistance is frequently observed. This may complicate comparisons with term born peers because pubertal onset also seems earlier in preterm children

Table 14.3 Examples of mechanisms linking early growth and nutrition to later outcomes in preterm infants

Mechanism	Examples	Potential outcome
Permanent structural change	Decreased pancreatic beta cell mass	Type II diabetes
	Bronchopulmonary dysplasia	Chronic obstructive pulmonary disease
Changes to control systems	Earlier onset of puberty	Decreased final adult height
Accelerated cellular ageing	Oxidative stress effects leading to telomere shortening	Range of chronic diseases
Epigenetic mechanisms	DNA methylation	Increased obesity

[116], but there are no current data to show how this might be modulated by catch up growth. Failure to observe a significant association between early growth (including catch up) and insulin resistance at some later point in time, does not exclude the possibility of effects later in the life course. Until the last 2–3 decades infants born < 28 weeks gestation rarely survived, so we simply do not know the full picture. Insulin sensitivity should also be considered a biomarker, and not a disease *per se*. The specific level of insulin sensitivity in childhood that has the highest predictive value for later disease (type 2 diabetes) may differ between those born preterm and term. Because of the life course nature of many DOHaD effects most studies do in fact, concentrate on biomarkers (and especially in preterm infants), despite the many pitfalls in interpretation [117].

10 Mechanisms Linking Catch up Growth and Later Metabolic Health (Table 14.3)

Early growth and nutritional exposures may have long term consequences due to a number of mechanisms including permanent structural changes, alterations to cellular ageing and/or longer term 'programming' effects (see table, based on references [118, 119]). Many of the programming effects may be modulated by epigenetic mechanisms such as DNA methylation and histone acetylation [23, 120]. These processes do not alter the nucleotide sequence in DNA, but result in differences in gene expression and transcription, and may also involve post-transcriptional effects on other processes such as protein translation. Early life growth and nutritional exposures appear to affect the 'cellular memory' and result in differences in later life phenotypes. Most of this work is still in the early stages but exciting data are already available.

In one study, methylation status of five key genes in umbilical cord blood had a strong association with measures of adiposity in term born infants at age 9 years, suggesting that a substantial amount of the variation in metabolic outcomes might be determined prenatally [121]. In other studies, differences in the methylation status of a specific candidate gene, TACSTD2, in adolescent children born preterm were

correlated with measures of early catch up growth and later phenotype (obesity) [122]. Several other gene candidates show differences in expression [123] and might be worthy of exploration [124]. Although reverse causation or confounding poses a major challenge when interpreting epigenetic data, especially in life course studies examining the effects of early catch up growth, a variation of Mendelian randomisation applied to the data may elucidate the direction of effect [123, 125]. In the study previously mentioned, the lack of an association between fat mass and a methylation proxy single nucleotide polymorphism (SNP) suggested that the association between TACSTD2 methylation was non-causal. However, the identified methylation patterns might still be useful predictors of later obesity [122]. The next decade will likely see a dramatic increase in the number of epigenetic studies that attempt to link early life effects to later outcomes.

11 Conclusion

Preterm infants are nutritionally vulnerable, and despite concerns surrounding longer term metabolic outcomes, the focus must be on optimising early health and neurocognitive outcomes. Preterm infants are at increased risk of a number of later life chronic diseases but there are no data to suggest that deliberate nutrient restriction will improve overall health outcomes. Whilst there are only a limited number of controlled studies, the current data argue in favour of early commencement of PN including amino acids and intravenous lipids, with the aim of achieving recommended intakes within the first few days. Early commencement of enteral feeds and the promotion of breast milk is important, with the use of fortifiers or formulas designed to meet nutrient requirements, and the use of supplemental vitamins and micronutrients over the first few postnatal weeks. After hospital discharge, nutritional management will still affect long-term outcomes and there should be continued support for breast milk. In the absence of breast milk, the role of specialised formula adapted for post-discharge feeding remains controversial and long term follow up studies are needed.

References

1. Ziegler EE, O'Donnell AM, Nelson SE, Fomon SJ (1976) Body composition of the reference fetus. Growth 40(4):329–341
2. Leitch CA, Denne SC (2000) Energy expenditure in the extremely low-birth weight infant. Clinics in Perinatology 27(1):181–195, vii–viii
3. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T et al (2010) Enteral nutrient supply for preterm infants: commentary from the European society of paediatric gastroenterology, hepatology and nutrition committee on nutrition. J Pediatr Gastroenterol Nutr 50(1):85–91
4. Denne SC (2007) Regulation of proteolysis and optimal protein accretion in extremely premature newborns. Am J Clin Nutr 85(2):621S–624S
5. Embleton ND (2007) Optimal protein and energy intakes in preterm infants. Early Hum Dev 83(12):831–837. PubMed PMID: 17980784. English

6. Tsang R, Uauy R, Zlotkin S, Koletzko B (eds) (2005) Nutritional needs of the preterm infant: scientific basis and practical guidelines. Digital Educational Publishing
7. Singhal A, Fewtrell M, Cole TJ, Lucas A (2003) Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 361(9363):1089–1097. PubMed PMID: 12672313. English
8. Singhal A, Cole TJ, Fewtrell M, Deanfield J, Lucas A (2004) Is slower early growth beneficial for long-term cardiovascular health? Circulation 109(9):1108–1113
9. Singhal A, Farooqi S, Cole TJ, O'Rahilly S, Fewtrell M, Kattenhorn M et al (2002) Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? Circulation 106(15):1919–1924
10. Singhal A, Fewtrell M, Cole TJ, Lucas A (2003) Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 361(9363):1089–1097
11. Singhal A, Wells J, Cole TJ, Fewtrell M, Lucas A (2003) Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? Am J Clin Nutr 77(3):726–730
12. McCance RA, Widdowson EM (1966) Protein deficiencies and calorie deficiencies. Lancet 2(7455):158–159
13. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ (1989) Weight in infancy and death from ischaemic heart disease. Lancet 2(8663):577–580
14. Barker DJ (1990) The fetal and infant origins of adult disease. BMJ 301(6761):1111
15. Hales CN, Barker DJP, Clark PMS, Cox LJ, Fall C, Osmond C et al (1991) Fetal and infant growth and impaired glucose tolerance at age 64. Br Med J 303(6809):1019–1022
16. Barker DJ (1992) The effect of nutrition of the fetus and neonate on cardiovascular disease in adult life. Proc Nutr Soc 51(2):135–144
17. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS (1993) Fetal nutrition and cardiovascular disease in adult life. Lancet 341(8850):938–941
18. Barker DJ (1995) The fetal and infant origins of disease. Eur J Clin Invest 25(7):457–463
19. Fall CH, Vijayakumar M, Barker DJ, Osmond C, Duggleby S (1995) Weight in infancy and prevalence of coronary heart disease in adult life. BMJ 310(6971):17–19
20. Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ (1999) Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ 318(7181):427–431
21. Gluckman PD, Hanson MA, Beedle AS (2007) Early life events and their consequences for later disease: a life history and evolutionary perspective. Am J Hum Biol 19(1):1–19
22. Jeffery AN, Metcalf BS, Hosking J, Murphy MJ, Voss LD, Wilkin TJ (2006) Little evidence for early programming of weight and insulin resistance for contemporary children: early bird diabetes study report 19. Pediatrics 118(3):1118–1123
23. Groom A, Elliott HR, Embleton ND, Relton CL (2011) Epigenetics and child health: basic principles. Arch Dis Child 96(9):863–869. PubMed PMID: 20656732. English
24. Gluckman PD, Hanson MA (2004) Living with the past: evolution, development, and patterns of disease. Science 305(5691):1733–1736
25. Gluckman PD, Hanson MA, Morton SMB, Pinal CS (2005) Life-long echoes: a critical analysis of the developmental origins of adult disease model. Biol Neonate 87(2):127–139
26. Embleton ND, Pang N, Cooke RJ (2001) Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 107(2):270–273. PubMed PMID: 11158457. English
27. Wilson DC, Cairns P, Halliday HL, Reid M, McClure G, Dodge JA (1997) Randomised controlled trial of an aggressive nutritional regimen in sick very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 77(1):F4–11
28. Koletzko B, Goulet O, Hunt J, Krohn K, Shamir R, Parenteral Nutrition Guidelines Working G et al (2005) 1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). J Pediatr Gastroenterol Nutr 41(Suppl 2):S1–87. PubMed PMID: 16254497. English

29. Agostini Cea (2010) Enteral nutrient supply for preterm infants. A comment of the ESPGHAN committee on nutrition. J Pediatr Gastroenterol Nutr. doi: 10.1097/MPG.0b013e3181adaee0
30. te Braake FW, van den Akker CH, Wattimena DJ, Huijmans JG, van Goudoever JB (2005) Amino acid administration to premature infants directly after birth. J Pediatr 147(4):457–461
31. Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL et al (1999) Longitudinal growth of hospitalized very low birth weight infants. Pediatrics 104(2 Pt 1): 280–289
32. Senterre T, Rigo J (2012) Reduction in postnatal cumulative nutritional deficit and improvement of growth in extremely preterm infants. Acta Paediatr 101(2):e64–70. PubMed PMID: 21854447. English
33. Stephens BE, Walden RV, Gargus RA, Tucker R, McKinley L, Mance M et al (2009) First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. Pediatrics 123(5):1337–1343
34. Blanco CL, Gong AK, Schoolfield J, Green BK, Daniels W, Liechty EA et al (2012) Impact of early and high amino acid supplementation on ELBW infants at 2 years. J Pediatr Gastroenterol Nutr 54(5):601–607
35. Van Den Akker CH, Vlaardingerbroek H, Van Goudoever JB (2010) Nutritional support for extremely low-birth weight infants: abandoning catabolism in the neonatal intensive care unit. Curr Opin Clin Nutr Metab Care 13(3):327–335
36. Vlaardingerbroek H, Goudoever JB van, Akker CHP van den (2009) Safety and efficacy of early and high-dose parenteral amino acid administration to preterm infants. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4(21):1–8
37. Fewtrell MS, Bishop NJ, Edmonds CJ, Isaacs EB, Lucas A (2009) Aluminum exposure from parenteral nutrition in preterm infants: bone health at 15-year follow-up. Pediatrics 124(5):1372–1379
38. Bishop NJ, Morley R, Day JP, Lucas A (1997) Aluminum neurotoxicity in preterm infants receiving intravenous-feeding solutions. N Engl J Med 336(22):1557–1561
39. Lewandowski AJ, Lazdam M, Davis E, Kylintireas I, Diesch J, Francis J et al (2011) Short-term exposure to exogenous lipids in premature infants and long-term changes in aortic and cardiac function. Arterioscler Thromb Vasc Biol 31(9):2125–2135
40. Fewtrell MS, Bishop NJ, Edmonds CJ, Isaacs EB, Lucas A (2009) Aluminum exposure from parenteral nutrition in preterm infants: bone health at 15-year follow-up (Pediatrics (2009) 124, 5 (1372–1379)). Pediatrics 124(6):1709
41. Fewtrell MS, Williams JE, Singhal A, Murgatroyd PR, Fuller N, Lucas A (2009) Early diet and peak bone mass: 20 year follow-up of a randomized trial of early diet in infants born preterm. Bone 45(1):142–149
42. Lucas A (2005) Long-term programming effects of early nutrition—implications for the preterm infant. J Perinatol 25(2):S2–6
43. Lucas A, Fewtrell MS, Morley R, Singhal A, Abbott RA, Isaacs E et al (2001) Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics 108(3):703–711
44. Morley R, Fewtrell MS, Abbott RA, Stephenson T, MacFadyen U, Lucas A (2004) Neurodevelopment in children born small for gestational age: a randomized trial of nutrient-enriched versus standard formula and comparison with a reference breastfed group. Pediatrics 113 (3 Pt 1):515–521
45. Morley R, Lucas A (2000) Randomized diet in the neonatal period and growth performance until 7.5–8 y of age in preterm children. Am J Clin Nutr 71(3):822–828
46. Singhal A, Cole TJ, Lucas A. (2001) Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. Lancet 357(9254):413–419. PubMed PMID: 11273059. English
47. Singhal A, Lucas A (2004) Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 363(9421):1642–1645. PubMed PMID: 15145640. English
48. Beyerlein A, Ness AR, Streuling I, Hadders-Algra M, Von Kries R (2010) Early rapid growth: no association with later cognitive functions in children born not small for gestational age. Am J Clin Nutr 92(3):585–593

49. Ekelund U, Ong KK, Linné Y, Neovius M, Brage S, Dunger DB et al (2007) Association of weight gain in infancy and early childhood with metabolic risk in young adults. J Clin Endocrinol Metab 92(1):98–103
50. Stettler N (2007) Nature and strength of epidemiological evidence for origins of childhood and adulthood obesity in the first year of life. Int J Obes 31(7):1035–1043
51. Fewtrell MS, Morley R, Abbott RA, Singhal A, Stephenson T, MacFadyen UM et al (2001) Catch-up growth in small-for-gestational-age term infants: a randomized trial. Am J Clin Nutr 74(4):516–523
52. Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, Giovannini M et al (2009) Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. American Journal of Clinical Nutrition. 2009 Jun;89(6):1836–1845. PubMed PMID: 19386747. English
53. Relton CL, Groom A, St. Pourcain B, Sayers AE, Swan DC, Embleton ND et al (2012) DNA Methylation Patterns in Cord Blood DNA and Body Size in Childhood. PLoS ONE 7(3). doi:10.1371/journal.pone.0031821
54. Embleton ND, Yates R (2008) Probiotics and other preventative strategies for necrotising enterocolitis. Semin Fetal Neonatal Med 13(1):35–43. PubMed PMID: 17974513. English
55. Leaf A, Dorling J, Kempley S, McCormick K, Mannix P, Linsell L et al (2012) Early or delayed enteral feeding for preterm growth-restricted infants: a randomized trial. Pediatrics 129(5):e1260–1268
56. Embleton ND, Tinnion RJ (2011) Enteral feeds in preterm infants: starting and increasing. Paediatr Child Health 21(10):476–477
57. Meier PP, Engstrom JL, Patel AL, Jegier BJ, Bruns NE (2010) Improving the use of human milk during and after the NICU stay. Clin Perinatol 37(1):217–245
58. Huppi PS (2008) Nutrition for the brain: commentary on the article by Isaacs et al. on page 308. Pediatr Res 63(3):229–231
59. Isaacs EB, Gadian DG, Sabatini S, Chong WK, Quinn BT, Fischl BR et al (2008) The effect of early human diet on caudate volumes and IQ. Pediatr Res 63(3):308–314
60. Abernethy LJ, Cooke RWI, Foulder-Hughes L (2004) Caudate and Hippocampal Volumes, Intelligence, and Motor Impairment in 7-Year-Old Children Who Were Born Preterm. Pediatr Res 55(5):884–893
61. de Graaf-Peters VB, Hadders-Algra M (2006) Ontogeny of the human central nervous system: what is happening when? Early Hum Dev 82(4):257–266. PubMed PMID: 16360292. Epub 2005/12/20. English
62. Sauer PJ (2007) Can extrauterine growth approximate intrauterine growth? Should it? Am J Clinical Nutr 85(2):608S–613S
63. Embleton ND, Cooke RJ (2005) Protein requirements in preterm infants: effect of different levels of protein intake on growth and body composition. Pediatr Res 58(5):855–860
64. Cooke R, Embleton N, Rigo J, Carrie A, Haschke F, Ziegler E (2006) High protein preterm infant formula: effect on nutrient balance, metabolic status and growth. Pediatr Res 59(2):265–270. PubMed PMID: 16439590. English
65. Cooke RJ, McCormick K, Griffin IJ, Embleton ND, Faulkner K, Wells JC et al (1999) Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res 46:461–464
66. Uthaya S, Thomas EL, Hamilton G, Dore CJ, Bell J, Modi N (2005) Altered adiposity after extremely preterm birth. Pediatr Res 57(2):211–215
67. Thomas EL, Parkinson JR, Hyde MJ, Yap IKS, Holmes E, Dore CJ et al (2011) Aberrant adiposity and ectopic lipid deposition characterize the adult phenotype of the preterm infant. Pediatr Res 70(5):507–512. PubMed PMID: 21772225. English
68. Dusick AM, Poindexter BB (2003) Ehrenkranz RA, Lemons JA. Growth failure in the preterm infant: can we catch up? Semin Perinatol 27(4):302–310
69. Hay WW (ed) (1996) Posthospital nutrition of the preterm infant requires improved predischarge nutrition. 106th Ross Conference on Pediatric Research

70. Hay WW, Lucas A, Heird WC, Ziegler E, Levin E, Grave GD et al (1999) Workshop summary: nutrition of the extremely low birth weight infant. Pediatrics 104:1360–1368
71. Leaf AA (2007) Vitamins for babies and young children. Arch Dis Child 92(2):160–164
72. Aggett PJ, Agostoni C, Axelsson I, De Curtis M, Goulet O, Hernell O et al (2006) Feeding preterm infants after hospital discharge: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 42(5):596–603
73. O'Connor DL, Khan S, Weishuhn K, Vaughan J, Jefferies A, Campbell DM et al (2008) Growth and nutrient intakes of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. Pediatrics 121(4):766–776
74. Zachariassen G, Faerk J, Grytter C, Esberg BH, Hjelmborg J, Mortensen S et al (2011) Nutrient enrichment of mother's milk and growth of very preterm infants after hospital discharge0. Pediatrics 127(4):e995–1003
75. Aimone A, Rovet J, Ward W, Jefferies A, Campbell DM, Asztalos E et al (2009) Growth and body composition of human milk-fed premature infants provided with extra energy and nutrients early after hospital discharge: 1-Year follow-up. J Pediatr Gastroenterol Nutr 49(4):456–466
76. O'Connor DL, Weishuhn K, Rovet J, Mirabella G, Jefferies A, Campbell DM et al (2012) Visual development of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. J Parenter Enteral Nutr 36(3):349–353
77. Henderson G, Fahey T, McGuire W (2007) Multicomponent fortification of human breast milk for preterm infants following hospital discharge. Cochrane Database Syst Rev (4):CD004866. PubMed PMID: 17943830. English
78. Henderson G, Fahey T, McGuire W (2007) Nutrient-enriched formula versus standard term formula for preterm infants following hospital discharge. Cochrane Database Syst Rev (4):CD004696. PubMed PMID: 17943826. English
79. Koo WWK, Hockman EM (2006) Posthospital discharge feeding for preterm infants: effects of standard compared with enriched milk formula on growth, bone mass, and body composition. Am J Clin Nutr 84(6):1357–1364
80. Cooke RJ, Embleton ND, Griffin IJ, Wells JC, McCormick KP (2001) Feeding preterm infants after hospital discharge: growth and development at 18 months of age. Pediatr Res 49(5): 719–722
81. Cooke RJ, Griffin IJ, McCormick K, Wells JC, Smith JS, Robinson SJ et al (1998) Feeding preterm infants after hospital discharge: effect of dietary manipulation on nutrient intake and growth. Pediatr Res 43(3):355–360
82. Carver JD, Wu PYK, Hall RT, Zeigler EE, Sosa R, Jacobs J et al (2001) Growth of preterm infants fed nutrient-enriched or term formula after hospital discharge. Pediatrics 107:683–9
83. Lucas A, Bishop NJ, King FJ, Cole TJ (1992) Randomised trial of nutrition for preterm infants after discharge. Arch Dis Child 67(3):324–327
84. Lucas A, King F, Bishop NB (1992) Postdischarge formula consumption in infants born preterm. Arch Dis Child 67(6):691–692
85. Colombo J, Carlson SE (2012) Is the measure the message: the BSID and nutritional interventions. Pediatrics 129(6):1166–1167
86. Pittaluga E, Vernal P, Llanos A, Vega S, Henrriquez MT, Morgues M et al (2011) Benefits of supplemented preterm formulas on insulin sensitivity and body composition after discharge from the neonatal intensive care unit. J Pediatr 159(6):926–32.e2
87. Dobbing J (1970) Undernutrition and the developing brain. The relevance of animal models to the human problem. Am J Dis Child 120(5):411–415
88. Dobbing J (1990) Vulnerable periods in developing brain. In: Dobbing J (ed) Brain, behaviour and iron in the infant diet. Springer-Verlag London Ltd., London, pp 1–25
89. Dobbing J, Hopewell JW, Lynch A (1971) Vulnerability of developing brain. VII. Permanent deficit of neurons in cerebral and cerebellar cortex following early mild undernutrition. Exp Neurol 32(3):439–447
90. Dobbing J, Sands J (1971) Vulnerability of developing brain. IX. The effect of nutritional growth retardation on the timing of the brain growth-spurt. Biol Neonate 19(4):363–378

91. Dobbing J, Smart JL (1974) Vulnerability of developing brain and behaviour. Br Med Bull 30(2):164–168
92. Belfort MB, Martin CR, Smith VC, Gillman MW, McCormick MC (2010) Infant weight gain and school-age blood pressure and cognition in former preterm infants. Pediatrics 125(6):e1419–1426
93. Innis SM, Gilley J, Werker J (2001) Are human milk long-chain polyunsaturated fatty acids related to visual and neural development in breast-fed term infants? J Pediatr 139(4):532–538
94. Wharton BA, Morley R, Isaacs EB, Cole TJ, Lucas A (2004) Low plasma taurine and later neurodevelopment. Arch Dis Child Fetal Neonatal Ed 89(6):F497–498
95. Cosgrove M, Davies DP, Jenkins HR (1996) Nucleotide supplementation and the growth of term small for gestational age infants. Archives of Disease in Childhood Fetal & Neonatal Edition 74(2):F122–125
96. Ley RE (2010) Obesity and the human microbiome. Curr Opin Gastroenterol 26(1):5–11. PubMed PMID: 19901833. English
97. Kajantie E, Osmond C, Barker DJP, Eriksson JG (2010) Preterm birth—a risk factor for type 2 diabetes? The Helsinki Birth Cohort study. Diabetes Care 33(12):2623–2625
98. Hovi P, Andersson S, Eriksson JG, Järvenpää AL, Strang-Karlsson S, Mäkitie O et al (2007) Glucose regulation in young adults with very low birth weight. N Engl J Med 356(20): 2053–2063
99. Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA et al (1997) Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab 82(2):402–406
100. Rotteveel J, Van Weissenbruch MM, Twisk JWR, Delemarre-Van De Waal HA (2011) Insulin sensitivity in prematurely born adults: Relation to preterm growth restraint. Horm Res Paediatr 75(4):252–257
101. Willemsen RH, Leunissen RWJ, Stijnen T, Hokken-Koelega ACS (2009) Prematurity is not associated with reduced insulin sensitivity in adulthood. J Clin Endocrinol Metab 94(5): 1695–1700
102. Willemsen RH, Willemsen SP, Hokken-Koelega ACS (2008) Longitudinal changes in insulin sensitivity and body composition of small-for-gestational-age adolescents after cessation of growth hormone treatment. J Clin Endocrinol Metab 93(9):3449–3454
103. Finken MJJ, Keijzer-Veen MG, Dekker FW, Frölich M, Hille ETM, Romijn JA et al (2006) Preterm birth and later insulin resistance: Effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. Diabetologia 49(3):478–485
104. Hofman PL, Regan F, Jackson WE, Jefferies C, Knight DB, Robinson EM et al (2004) Premature birth and later insulin resistance. N Engl J Med 351(21):2179–2186
105. Singhal A, Fewtrell M, Cole TJ, Lucas A (2003) Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 361(9363):1089–1097
106. Fewtrell MS, Doherty C, Cole TJ, Stafford M, Hales CN, Lucas A (2000) Effects of size at birth, gestational age and early growth in preterm infants on glucose and insulin concentrations at 9–12 years. Diabetologia 43(6):714–717
107. Bo S, Bertino E, Bagna R, Trapani A, Gambino R, Martano C et al (2006) Insulin resistance in pre-school very-low-birth weight pre-term children. Diabetes Metab 32(2):151–158
108. Rotteveel J, van Weissenbruch MM, Twisk JW, Delemarre-Van de Waal HA (2008) Infant and childhood growth patterns, insulin sensitivity, and blood pressure in prematurely born young adults. Pediatrics 122(2):313–321. PubMed PMID: 18676549. English
109. Regan FM, Cutfield WS, Jefferies C, Robinson E, Hofman PL (2006) The impact of early nutrition in premature infants on later childhood insulin sensitivity and growth. Pediatrics 118(5):1943–1949
110. Singhal A, Fewtrell M, Cole TJ, Lucas A (2003) Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 361(9363):1089–1097
111. Bazaes RA, Alegría A Pittaluga E, Ávila A, Íñiguez G, Mericq V (2004) Determinants of insulin sensitivity and secretion in very-low-birth-weight children. J Clin Endocrinol Metab 89(3):1267–1272

112. Darendeliler F, Bas F, Bundak R, Coban A, Sancakli O, Eryilmaz SK et al (2008) Insulin resistance and body composition in preterm born children during prepubertal ages. Clin Endocrinol 68(5):773–779
113. Dalziel SR, Parag V, Rodgers A, Harding JE (2007) Cardiovascular risk factors at age 30 following pre-term birth. Int J Epidemiol 36(4):907–915
114. Gray IP, Cooper PA, Cory BJ, Toman M, Crowther NJ (2002) The intrauterine environment is a strong determinant of glucose tolerance during the neonatal period, even in prematurity. J Clin Endocrinol Metab 87(9):4252–4256
115. Leipala JA, Raivio KO, Sarnesto A, Panteleon A, Fellman V (2002) Intrauterine growth restriction and postnatal steroid treatment effects on insulin sensitivity in preterm neonates. J Pediatr 141(4):472–476. PubMed PMID: 12378184. English
116. Wehkalampi K, Hovi P, Dunkel L, Strang-Karlsson S, Jarvenpaa AL, Eriksson JG et al (2011) Advanced pubertal growth spurt in subjects born preterm: the Helsinki study of very low birth weight adults. J Clin Endocrinol Metab 96(2):525–533. PubMed PMID: 21147886. English
117. Yudkin JS, Lipska KJ, Montori VM (2011) The idolatry of the surrogate. BMJ 343:d7995. PubMed PMID: 22205706. English
118. Tarry-Adkins JL, Ozanne SE (2011) Mechanisms of early life programming: current knowledge and future directions. Am J Clin Nutr 94(6 Suppl):1765S–1771S. PubMed PMID: 21543536. English
119. Ozanne SE (2009) The long term effects of early postnatal diet on adult health. pp 135–144
120. Wiedmeier JE, Joss-Moore LA, Lane RH, Neu J (2011) Early postnatal nutrition and programming of the preterm neonate. Nutr Rev 69(2):76–82. PubMed PMID: 21294741. English
121. Godfrey KM, Sheppard A, Gluckman PD, Lillycrop KA, Burdge GC, McLean C et al (2011) Epigenetic gene promoter methylation at birth is associated with child's later adiposity. Diabetes 60(5):1528–1534. PubMed PMID: 21471513. English
122. Groom A, Potter C, Swan DC, Fatemifar G, Evans DM, Ring SM et al (2012) Postnatal growth and DNA methylation are associated with differential gene expression of the TACSTD2 gene and childhood fat mass. Diabetes 61(2):391–400. PubMed PMID: 22190649. English
123. Relton CL, Groom A, St. Pourcain B, Sayers AE, Swan DC, et al (2012) DNA methylation patterns in cord blood DNA and body size in childhood. PLoS ONE 7(3):e31821. doi:10.1371/journal.pone.0031821
124. Turcot V, Groom A, McConnell JC, Pearce MS, Potter C, Embleton ND et al (2012) Bioinformatic selection of putative epigenetically regulated loci associated with obesity using gene expression data. Gene 499(1):99–107
125. Relton CL, Davey Smith G (2012). Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. Int J Epidemiol 41(1):161–176. PubMed PMID: 22422451. English

Part V
Monitoring Growth and Development

Chapter 15
Growth Monitoring of Preterm Infants During Stay in the Neonatal Unit and into Early Childhood

Shripada Rao

Abstract Monitoring growth is important in preterm infants as they are at a high risk for postnatal growth restriction which can lead to impaired long term growth and neurodevelopment. In the absence of better charts, intrauterine growth charts are recommended by leading professional paediatric organisations for monitoring the growth of preterm infants. The aim when caring for preterm infants is to at least match the growth velocity from published best postnatal growth charts and strive towards reaching ideal growth velocities from intrauterine growth charts. The Fenton chart appears to be suitable for monitoring growth of preterm infants during their stay in the neonatal intensive care unit (NICU). Recently, Fenton charts have been updated using the WHO 2006 charts for the 40–50 weeks' post conception age group. Once a post-conception age of 40 weeks is reached, the WHO 2006 growth charts can be used for monitoring ongoing growth. The ongoing "Intergrowth-21st study" has the potential to overcome the deficiencies of all current growth charts. It will enable the establishment of prescriptive growth charts for monitoring the growth of preterm infants during and beyond their NICU stay into early childhood. Care should be taken to avoid excessive catch up growth which is associated with increased risk of diabetes, hypertension, and obesity in later life.

Key points

1. Growth charts are essential for defining health and nutritional status and early detection and management of growth disorders in infants and children.
2. Growth monitoring is especially important in preterm infants as they are at a high risk for postnatal growth restriction which can lead to impaired long term growth and neurodevelopment.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_15,

3. A ‘standard’ chart that represents the ideal healthy growth of a population is prescriptive whereas a ‘reference’ chart that describes the population without making claims about the health of its sample is descriptive in nature.
4. In the absence of ideal growth charts, intrauterine growth charts are considered suitable for monitoring the growth of preterm infants until they reach term.
5. In the absence of ideal charts, the WHO 2006 growth charts may be used for monitoring the growth of ex-preterm infants.
6. The International Fetal and Newborn Growth Consortium study is designed to produce a set of international standards (normative charts for fetal growth, birth weight for gestational age and postnatal growth of preterm infants) for clinical applications and monitoring trends in populations.

Disturbances in health and nutrition, regardless of their aetiology, almost always affect growth [1]. Hence, growth assessment using growth charts is a useful tool for defining health and nutritional status in children [2]. Growth monitoring helps to improve nutrition, educate the care givers, and enables early detection and referral for conditions manifested by growth disorders [3]. The most common measurements for evaluating growth are weight, length/height, head circumference and body mass index. Growth monitoring of preterm infants is even more important because, as described below, many studies have shown that (a) preterm infants suffer from postnatal growth restriction and (b) postnatal growth restriction is associated with long term adverse neurodevelopmental outcomes.

1 Preterm Infants Suffer from Postnatal Growth Restriction

1.1 In a retrospective longitudinal cohort study, Horemuzova et al. (Sweden) evaluated the physical growth of all infants born before 26^{+0} weeks of gestation and surviving to full-term age ($n = 162$), admitted to the NICU of Karolinska Hospital between January 1990 and December 2002 [4]. Body weight was recorded daily, head circumference (HC) weekly and length twice a month. The majority of the infants showed a pronounced postnatal growth restriction for all growth variables with increasing deviation from the reference with age. At discharge from NICU, 75 % of those initially appropriate for gestational age (AGA) infants were below − 2 standard deviation scores for at least one of the body size variables [4].

1.2 In a retrospective cohort study [5], 101 children with a BW ≤ 750 g, born between 1996 and 2005 in the University Hospital Utrecht, The Netherlands, were followed until 5.5 years. Height, weight, occipital-frontal circumference at birth, 15 months and 2 years corrected age and 3.5 and 5.5 years were measured. Between birth and 5.5 years catch-up growth in height, weight for height, weight and OFC was seen in 72.2, 55.2, 28.6 and 68.9 % respectively of the small for gestational age (SGA) infants. For AGA infants they found substantial catch-down growth in height (15.4 %) and weight (33.8 %).

2 Physical Growth and Neurodevelopmental Outcomes in Preterm Infants

2.1 Association Between Postnatal Growth During NICU Stay and Neurodevelopmental Outcomes

1. Ehrenkranz et al. [6] assessed the predictive value of in-hospital growth velocity on neurodevelopmental and growth outcomes at 18–22 months post-conceptional age among extremely low birth weight (ELBW) infants (501–1,000 g). Of the 600 discharged infants, 495 (83 %) were evaluated at a corrected age (CA) of 18–22 months. As the rate of weight gain increased from 12.0 to 21.2 g/kg per day, there was decrease in the incidence of cerebral palsy, Mental Developmental Index (MDI) < 70 and Psychomotor Developmental Index (PDI) < 70 on Bayley Scale of Infant Development (BSID), abnormal neurologic examination, neurodevelopmental impairment, and need for rehospitalisation. Similar findings were observed in relation to the rate of head circumference growth. They concluded that the growth velocity during an ELBW infant's NICU hospitalisation exerts a significant and possibly independent effect on neurodevelopmental and growth outcomes at 18–22 months of CA.

2. Franz et al. [7] evaluated the neurological outcomes of a total of 219 of 263 (83 %) long-term survivors at a median corrected age of 5.4 years. Increasing SD scores for weight and head circumference from birth to discharge were associated with a reduced risk for an abnormal neurologic examination.

3. Shah et al. [8] aimed to identify measure of postnatal growth failure associated with long-term outcome in preterm infants born at < 28 weeks' gestation. Four measures of defining postnatal growth failure at 36 weeks corrected gestational age: (1) weight $<$ 10th centile, (2) weight $<$ 3rd centile, (3) z score difference from birth > 1 and, (4) z score difference from birth > 2; were compared for their predictive values and strength of association with adverse neurodevelopmental outcomes at 18–24 months.

Postnatal growth failure defined as a decrease in z score of > 2 between birth and 36 weeks corrected gestational age had the best predictive values compared to other postnatal growth failure measures. However, it was significantly associated with PDI ($p = 0.006$) but not with MDI ($p = 0.379$). Postnatal growth failure defined by z score change influenced psychomotor but not mental tasks in this cohort.

2.2 Association Between Post-Discharge Growth and Neurodevelopmental Outcomes in Preterm Infants

1) Ramel et al. [9] reported that pre- and post-discharge linear growth suppression in very low birth weight (VLBW: Birth weight $< 1{,}500$ g) infants was negatively associated with developmental outcomes at 24 months CA. In their retrospective study,

weight, recumbent length and head circumference were recorded at birth, hospital discharge and at 4, 12 and 24 months CA in 62 VLBW infants. Standardized Z-scores for weight (WZ), length (LZ) and head circumference (HCZ) were calculated. Twenty-four-month neurodevelopmental function was analysed as a function of growth status. Controlling for WZ and HCZ at each age, lower LZ at 4 and 12 months CA was associated with lower cognitive function scores at 24 months CA ($p \leq 0.03$).

2) Ghods et al. [10] conducted a retrospective cohort study to determine whether head circumference (HC) catch-up is associated with improved neurocognitive development. 179 preterm very low birth weight (VLBW) (Birth weight $\leq 1{,}500$ g) infants were followed to the age of 5.5 years. The association between HC catch-up and neurodevelopmental outcome was assessed and perinatal risk factors, infant characteristics and nutritional practices associated with HC catch-up were determined. HC catch-up occurred in 59 (34 %) infants and was positively correlated with neurodevelopmental outcome. They concluded that among preterm VLBW infants, there is a close relation between HC growth and neurodevelopmental outcome.

3) Powers et al. [11] assessed the post-discharge growth and developmental progress of 135 VLBW preterm infants in a predominantly Hispanic population and reported that failure to thrive and microcephaly increased neurodevelopmental impairment risk at 3 years of age regardless of gestational age.

4) Kan et al. [12] aimed to determine the associations between weight and head circumference, at birth and postnatally, with cognitive, academic and motor outcomes at age 8 years for very preterm children free of neurosensory impairment. 179 very preterm infants (gestational age < 28 weeks) born in 1991 and 1992 who were free of neurosensory impairment were included in the study. At 8 years of age children had cognitive, academic and motor assessments. Weight and head circumference data were collected at birth, at the time of discharge (weight only), at 2 years of age and at 8 years of age, and growth restriction was calculated using Z-scores (standard deviation scores) relative to the expected mean for age using the British 1990 growth reference charts [13]. Weight at any age was mostly unrelated to any outcomes. While head circumference at birth was not related to school-aged outcomes, smaller head circumferences at ages 2 and 8 years were associated with poorer performance in most outcome measures. Catch-up growth in weight in early childhood was not associated with 8-year outcomes.

5) Latal-Hajnal [14] studied the significance of growth status at birth and postnatal growth on neurodevelopmental outcome in VLBW infants. Growth and neurodevelopment were examined in 219 VLBW ($< 1{,}250$ g) children, 94 small for gestational age (SGA) (< 10th percentile) and 125 appropriate for gestational age (AGA) (> 10th percentile). Outcome at age 2 was assessed with the Bayley Scales of Infant Development MDI, PDI and a standardized neurologic examination. After adjustment for co variables including cerebral palsy (CP), SGA children with weight < 10th percentile at age 2 had lower mean PDI than SGA children with catch-up growth to weight > 10th percentile (mean [SD], 89.9 [17.4] versus 101.8 [14.5]; $p < .001$). AGA children with catch-down growth (weight < 10th percentile at age 2) were, independent of CP, more likely to have lower mean MDI (94.9 vs. 101.7, $p = .05$) and PDI (81.9

vs. 95.1; $p < .001$) than AGA children remaining > 10th percentile at age 2. They also more frequently had severe CP (22.9 % vs. 1.2 %; $p = .008$). They concluded that in VLBW children, the course of postnatal growth rather than the appropriateness of weight for gestational age at birth determines later neurodevelopmental outcome.

6) Casey et al. [15] assessed the 8-year growth, cognitive, behavioural status, health status, and academic achievement in low birth weight preterm infants who had failure to thrive only, were SGA only, had failure to thrive plus were SGA, or had normal growth. A total of 985 infants received standardized evaluations to age 8; 180 infants met the criteria for failure to thrive between 4 and 36 months' gestational corrected age. The following outcome variables were collected at age 8: growth, cognitive, behavioural status, health status, and academic achievement. Multivariate analyses were performed among the 4 growth groups on all 8-year outcome variables. Children who both were SGA and had failure to thrive were the smallest in all growth variables at age 8, and they also demonstrated the lowest cognitive and academic achievement scores. The children with failure to thrive only were significantly smaller than the children with normal growth in all growth variables and had significantly lower IQ scores. Those who were SGA only did not differ from those with normal growth in any cognitive or academic achievement measures. There were no differences among the 4 groups in behavioural status or general health status. They concluded that low birth weight preterm infants who develop postnatal growth problems, particularly when associated with prenatal growth problems, demonstrate lower physical size, cognitive scores, and academic achievement at age 8 years.

3 Types of Growth Charts

A 'standard' chart represents the ideal healthy growth of a population and hence is of prescriptive nature. To derive such ideal healthy growth charts, the study population should be from a cohort of infants born to healthy mothers with uncomplicated pregnancy and delivery. In addition, the study infants should be raised under optimal environmental conditions including breastfeeding, immunisations and follow recommended dietary practices. The study infants should be free from any disease that could hinder growth. Longitudinal follow up and measurement of anthropometry of such infants will help derive the 'standard' growth charts which will be of prescriptive nature. The WHO 2006 growth charts (term infants) are standard growth charts.

In contrast, a 'reference' chart describes the population without making claims about the health of its sample and hence is descriptive in nature [16–18] (Table 15.1). The 'reference' charts are derived by measuring the anthropometry of a sample of infants and children at various ages and plotting them on graph. The sample is thus cross-sectional rather than longitudinal. In addition, health of the children in the study population is not taken into consideration. Majority of the currently available growth charts in full term infants and children are 'reference' charts.

Table 15.1 Differences between reference and standard charts

Reference charts	Standard charts
Simply describe the growth of a population without taking into consideration the health of the population	Provide guidance on how a child should grow; not just how a child is growing
Based on cross sectional data; relatively easy to acquire large sample size	Based on prospective and longitudinal monitoring of healthy growth; difficult to acquire large sample size
Increase in incidence of childhood obesity means future descriptive charts will enable more children to be classified as normal even though overweight/obese	Have the potential to identify overweight and obesity early, which can help bring in early interventions
Have the potential to over diagnose under nutrition, which in turn can lead to unnecessary overfeeding	Have the potential to avoid over diagnosis of under nutrition

4 Types of Growth Charts Currently Available for Preterm Infants During Stay in the Neonatal Unit

4.1 Standard Charts

At present, there are no prescriptive standard growth charts available for preterm infants. Theoretically speaking, infants born prematurely should continue to grow at intrauterine rates until they reach term. The American Academy of Pediatrics [17] and Canadian Pediatric society [18] recommend intra uterine growth rates as the ideal growth of preterm infants.

4.1.1 Considered Being, But Not Really "Intra Uterine Growth" Charts (Table 15.2)

There are more than 25 studies reporting on 'intrauterine growth charts'. These have been best summarized by Karna et al. [19].

Until recently, Lubchenko [20] and Babson und Benda [21] charts were commonly used in many neonatal units around the world. Fenton et al. [22] updated the Babson and Benda growth charts to develop contemporary 'intrauterine growth charts'. Using preset criteria, three recent large population based surveys of birth weight for gestational age were identified. The Canadian study by Kramer [23] which had a sample size of 676,605 infants delivered between 22–43 weeks was used for updating the intrauterine weight section. Two large studies from Sweden [24] and Australia [25] were used to update the intrauterine head circumference and length section. The data were averaged together using a weighted average based on total sample size to derive the 3rd, 10th, 50th, 95th and 97th percentiles and create one growth chart. CDC 2000 growth charts were used to generate the growth charts from corrected gestation of 40 weeks onwards. The Fenton chart appears to be useful in monitoring the growth of preterm infants during their NICU stay. It is used by many North

American, European and Australian centres. Recently Olsen et al. have published growth charts for New intrauterine growth charts based on United States data [26] and it will be useful if Fenton charts are updated incorporating this new information from USA. The latest updated Fenton charts have used WHO 2006 growth charts instead of CDC 2000 charts to generate growth charts from post-conceptional age of 40 weeks until 10 weeks post term (BMC Pediatrics, 2013, 13:59).

Inherent issues with intrauterine growth charts Even though they are called "intrauterine" charts, they are in fact cross sectional data derived from anthropometry measured at birth on preterm infants delivered at various gestations. It is well known that fetuses delivered prematurely may not have reached full growth potential due various maternal/fetal morbidities and hence do not reflect the "ideal" growth. Also, these charts do not take into consideration, the normal 5–8 % weight loss that occurs in healthy preterm infants in the first week of life.

4.1.2 'Fetal Growth Charts' (Table 15.2)

Strictly speaking, only charts derived from longitudinal studies should be called growth charts, growth being a process extended over time [27]. Hence it may appear logical that ideal 'intrauterine growth charts' should be derived from serial and longitudinal assessment of physical parameters of weight, length and head circumference using fetal ultrasound technique [28]. However, the drawback of this method is that fetal ultrasound is not very accurate in predicting the fetal weight. A systematic review which analysed data from 58 articles over 28 years found wide variability in diagnostic accuracy of ultrasound examination in predicting the fetal weight. Overall only 62 % (8,895/14,384) of the sonographic predictions were within 10 % of the actual weight. The accuracy was affected significantly by the time interval between examination and delivery, person doing the sonography (registered diagnostic medical sonographers had better accuracy than physicians or residents), and the gestation at assessment (assessment closer to term were more accurate compared to preterm patients) [29].

Another systematic review came to similar conclusions. The reviewers searched four important databases (MEDLINE, EMBASE, ZETOC, and The Cochrane Library). Studies including the estimation of fetal weight by 11 different research groups using different formulas were included in the review. No preferred method for the ultrasound estimation of fetal weight emerged from their review. They found that the size of the random errors was quite wide, with 95 % confidence intervals exceeding 14 % of birth weight in all studies. They concluded that the accuracy of EFW using fetal ultrasound is compromised by large intra- and inter-observer variability and efforts must be made to minimise this variability if EFW is to be clinically useful [30]. In addition, maternal morbidities can result in fetal growth restriction, which in turn can result in non- ideal growth charts.

In view of such limitations, fetal weight charts derived from the currently available ultrasound technology may not be appropriate for use as ideal postnatal growth

of preterm infants. However, recent advances in technology have resulted in more frequent use of 3-D ultrasound for fetal biometry measurements. Chan et al. [31] in a prospective study compared the inter- and intra-observer variation of fetal biometric measurements utilising two-dimensional (2D) and three-dimensional (3D) ultrasound imaging. Three pairs of doctors trained in sonography evaluated singleton pregnancies in the mid-trimester. Measurements of the biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) were taken in duplicate by each doctor using 2D imaging and then again using 3D volume data sets. Each set of paired doctors evaluated 12 patients. Inter- and intra-observer variations were calculated as the SD of the difference between paired measurements performed by the doctor pairs and by the individual doctors, respectively. Bland–Altman plots were used to visually compare measurement bias and agreement by 2D and 3D methods. The intra-observer variation of HC, AC, and FL was significantly lower for 3D compared with 2D ultrasound. Inter-observer variation was not significantly different when measured with 2D and 3D ultrasound, with the exception of FL, which was lower when measured with 3D ultrasound. They concluded that the use of 3D ultrasound significantly reduces intra-observer variation for HC, AC, and FL and reduces inter-observer variation for FL [31]. Schild et al. [32] in a prospective cohort study, obtained biometric data of 150 singleton fetuses weighing $\leq$ 1,600 g at birth by sonographic examination within 1 week before delivery. Exclusion criteria were multiple pregnancy, intrauterine death as well as major structural or chromosomal anomalies. Their new formula was compared with currently available equations for estimating weight in the preterm fetuses. They concluded that in fetuses weighing $\leq$ 1,600 g at birth, the new formula using 3D ultrasound is superior to weight estimation by traditional formulae using 2D measurements [32]. These data indicate that 3D ultrasonography may have the potential to be a more accurate measure of fetal anthropometry than the traditional 2D ultrasounds. If these preliminary promising findings are proven correct in multiple large studies, intrauterine growth curves derived from such method may have the potential to be used as ideal growth curves for monitoring preterm infants after birth.

4.2 Postnatal 'Reference' Growth Charts (Table 15.2)

Many reference charts that describe the actual longitudinal growth of preterm infants during the course of their stay in the NICU have been published [33, 34]. If these reference charts are used to monitor the ongoing growth of preterm infants, extrauterine growth retardation would be considered as normal. Hence they are not ideal for monitoring the growth of preterm infants. However, these charts give an idea of what can be achieved with the available resources and limits set by the morbidities of prematurity and can be used to compare the growth of preterm infants between different units.

Table 15.2 Growth charts for monitoring preterm infant growth until term

Intrauterine growth charts	Fetal growth charts	Postnatal growth charts
Not really intrauterine. Based on cross sectional data derived from anthropometry measured at birth on preterm infants delivered at various gestations.	Based on longitudinal assessment of healthy fetal growth; truly intrauterine	Describe the growth of preterm infants, without taking into consideration morbidities of prematurity; descriptive and not prescriptive
Recommended by American Academy of Pediatrics and Canadian Pediatric society; Commonly used charts	Ultrasound measurement of fetal anthropometry is subject to wide interpersonal variability; Fetal ultrasound is not very accurate in estimating fetal weight	Useful for comparing different units

5 A Note of Caution While Aiming to Achieve the Perfect Intrauterine Growth Rates

Even though the intra uterine growth charts may appear idealistic goals, one needs to decide if it is really feasible and safe to attain those parameters. Any attempts to promote physical growth by aggressive enteral and parenteral nutrition may potentially harm the sick preterm infant. Rapid increases in enteral feeding are known risk factor for necrotising enterocolitis (NEC) [35]. In ELBW infants, higher fluid intake and less weight loss during the first 10 days of life are associated with an increased risk of death and BPD [36, 37]. In addition excessive catch up growth in early neonatal period for may result in adverse cardiovascular outcomes later in life. Finken et al. [38] and Euser et al. [39] found that in subjects born very preterm, rapid infancy weight gain until 3 months was associated with trend towards higher insulin levels at 19 years. They also concluded that rapid weight gain in both infancy and early childhood is a risk factor for adult adiposity and obesity. Similar concerns have been raised by other investigators [40, 41].

6 Growth Charts to Monitor Preterm Infants from Post-Conception Age of 40 Weeks into Early Childhood

Until recently, many countries used the growth charts released by Centers for Disease Control and Prevention (CDC 2000) for monitoring the growth of term infants and children. The same charts are usually used for ongoing growth monitoring of preterm infants after reaching post conceptional age of 40 weeks. The inherent problem with the CDC 2000 and similar charts is that they are ‘reference’ charts, which simply describe the sample population without making any claims about the health

of the sample. Because of various environmental and lifestyle influences, the prevalence of overweight in children and adolescents has increased markedly over the past few decades. Hence, any new reference charts, which are derived from such population of overweight children, would accept these abnormally high weights-for-age as normal [42, 43]. Use of such charts would also result in more children being wrongly and frequently diagnosed as underweight resulting in unnecessary nutritional supplementation and may contribute to obesity and associated morbidities.

To some extent, the CDC 2000 growth charts addressed this by excluding the data derived from the National Health and Nutrition Examination Survey (NHANES) III for children 6 years of age for weight-for-age and body mass index (BMI)- for-age charts. This was carried out because they had identified that compared with the NHANES II (1976–1980), the NHANES III (1988–1994) children were of higher weight-for-age [44]. Despite this adjustment, the 97th and the 99.9th percentile charts (+ 2 and + 3 z-scores) are located very high on the CDC weight-for-age and BMI-for-age charts, meaning that fewer overweight and obese children and adolescents are identified as such because the norms have been raised. The lower centiles have also been shifted upwards, leading to overestimation of under nutrition, and thus advice leading to overfeeding [45]; also, precautions that were taken by the CDC cannot be confidently expected from innumerable number of 'reference' charts which are being published regularly from different countries all over the world.

To overcome the problems inherent with 'reference' charts, with a complete change in philosophy, the World Health Organization (WHO) conducted the Multicentre Growth Reference Study (MGRS) in order to establish the 'standard' growth charts for children between 0 and 6 years [46]. The MGRS was conducted between 1997 and 2003 in 6 countries from diverse geographical regions: Brazil, Ghana, India, Norway, Oman and the United States. The study combined a longitudinal follow-up of 882 infants from birth to 24 months with a cross-sectional component of 6,669 children aged 18–71 months. The study populations lived in socioeconomic conditions favourable to growth. The individual inclusion criteria for the longitudinal component were: no known health or environmental constraints to growth, mothers willing to follow MGRS feeding recommendations (i.e., exclusive or predominant breastfeeding for at least 4 months, introduction of complementary foods by 6 months of age and continued breastfeeding to at least 12 months of age), no maternal smoking before and after delivery, single-term birth and absence of significant morbidity. The eligibility criteria for the cross-sectional component were the same as those for the longitudinal component with the exception of infant feeding practices. A minimum of 3 months of any breastfeeding was required for participants in the study's cross-sectional component. Weight-for-age, length/height-for-age, weight-for-length/height and body mass index-for-age percentile and Z-score values were generated for boys and girls aged 0–60 months. The pooled sample from the 6 participating countries allowed the development of a truly international reference. The standards explicitly identify breastfeeding as the biological norm and establish the breastfed child as the normative model for growth and development. They also demonstrate that healthy children from around the world who are raised in healthy environments and follow recommended feeding practices have strikingly

Table 15.3 Rationale for advocating WHO 2006 (0–2 years) growth charts for post discharge monitoring of preterm infants

1	Based on exclusively or predominantly breastfed babies
2	Study population (both mother and baby) were in optimal health enabling optimal growth
3	Study population quite recent: 1996–2003
4	Study population was from multiple countries and multiple ethnicities
5	Sophisticated statistical analyses
6	Multiple and longitudinal measurements of the infants growth parameters
7	Conceptually, better than the other currently available charts

similar patterns of growth. In addition, to establish 'standard' prescriptive charts for older children and adolescents, the WHO reconstructed the 1977 National Center for Health Statistics (NCHS)/WHO growth reference using state-of-the-art statistical methods. The 1977 growth references were used because they were from a population prior to the occurrence of the current epidemic of childhood obesity. These new charts were released by the WHO in 2007 for general use [47]. These charts are recommendations for how children should grow. More than 125 countries including UK, USA, Canada and New Zealand have started using the WHO growth charts for full term infants [48] (Table 15.3).

The full set of tables and charts are available on the WHO website (www.who.int/childgrowth/en) together with tools such as software and training materials.

Since their publication, many studies have shown the usefulness of WHO growth charts in predicting obesity and other cardiovascular morbidities.

De Onis et al. [49] examined the association between cardiovascular risk and childhood overweight and obesity using the BMI cut-offs recommended by the WHO. Children were classified as normal weight, overweight and obese according to the WHO BMI-for-age reference. Blood pressure, lipids, glucose, insulin, homeostasis model assessment-insulin resistance (HOMA-IR) and uric acid levels were compared across BMI groups. The subjects were children (*n* 149) aged 8–18 years. About 37, 22 and 41 % of children were classified respectively as normal weight, overweight and obese. Obese children were 10·6 times more likely than normal-weight children to have hypertension; OR for other associations were 60·2 (high insulin), 39·5 (HOMA-IR), 27·9 (TAG), 16·0 (low HDL-cholesterol), 4·3 (LDL-cholesterol) and 3·6 (uric acid). Overweight children were more likely than normal-weight children to have hypertension (OR = 3·5), high insulin (OR = 28·2), high HOMA-IR (OR = 23·3) and high TAG (OR = 16·1). Nearly 92 and 57 % of the obese and overweight children, respectively, had one or more risk factor. They concluded that obesity and overweight defined using the WHO BMI-for-age cut-offs identified children with higher metabolic and vascular risk.

Shields et al. [50] compared prevalence estimates of excess weight among Canadian children and youth according to three sets of body mass index (BMI) reference

cut-points. The cut-points were based on growth curves generated by the WHO, the International Obesity Task Force (IOTF), and the CDC (USA). Prevalence estimates of overweight and obesity were produced for 2- to 17-year-olds using the three sets of BMI cut-points. Estimates were based on data from 8,661 respondents from the 2004 Canadian Community Health Survey and 1,840 respondents from the 1978/1979 Canada Health Survey. In both surveys, the height and weight of children were measured. They found that 2004 prevalence estimate for the combined overweight/obese category was higher (35 %) when based on the WHO cut-points compared with the IOTF (26 %) or CDC (28 %) cut-points. Estimates of the prevalence of obesity were similar based on WHO and CDC cut-points (13 %), but lower when based on IOTF cut-points (8 %).

In the absence of other ideal growth charts, it is appropriate to use the WHO growth charts to monitor the ongoing growth of preterm infants after reaching postconceptional age of 40 weeks.

6.1 Evidence Supporting the Use of WHO 2006 Growth Charts for Monitoring Preterm Infants After Discharge

Nash et al. [51] aimed to determine whether the pattern of growth of very low birth weight (VLBW) infants during the first 2 years, assessed using the WHO-GS or the traditional Centers for Disease Control and Prevention reference growth charts (CDC-RGC), is associated with neurodevelopment [51]. Pattern of weight, length, and head circumference gain of appropriate-for-gestation VLBW preterm infants ($n = 289$) from birth to 18–24 months corrected age was classified, using the WHO-GS and CDC-RGC, as sustained (change in Z-score ≤ 1 SD), decelerated (decline > 1 SD), or accelerated (incline > 1 SD). Development was assessed using the Bayley Scales of Infant and Toddler Development (BSID)-III at 18–24 months corrected age. Using the WHO-GS, children with a decelerated pattern of weight gain had lower cognitive (10 points), language (6 points), and motor (4 points) scores than infants with sustained weight gain ($p < 0.05$), even after adjustment for morbidities. No association was found using the CDC-RGC. They concluded that a decelerated pattern of weight gain, determined with the WHO-GS, but not the CDC-GRC, is associated with poorer neurodevelopment scores on the BSID-III than a pattern of sustained growth [51].

Belfort et al. [52] aimed to identify sensitive periods of postnatal growth for preterm infants relative to neurodevelopment at 18 months' corrected age. They studied 613 infants born at < 33 weeks' gestation who participated in the DHA for Improvement of Neurodevelopmental Outcome (DINO) trial. They calculated linear slopes of growth in weight, length, BMI, and head circumference from 1 week of age to term (40 weeks' postmenstrual age), term to 4 months, and 4–12 months using the WHO growth charts, and estimated their associations with Bayley Scales of Infant Development, 2nd Edition, MDI and PDI in linear regression. The median gestational age was 30 weeks. Mean $\pm$ SD MDI was 94 ± 16, and PDI was 93 ± 16. From 1 week

to term, greater weight gain (2.4 MDI points per z score [95 % confidence interval (CI): 0.8–3.9]; 2.7 PDI points [95 % CI: 1.2–0.2]), BMI gain (1.7 MDI points [95 % CI: 0.4–3.1]; 2.5 PDI points [95 % CI: 1.2–3.9]), and head growth (1.4 MDI points [95 % CI: − 0.0–2.8]; 2.5 PDI points [95 % CI: 1.2–3.9]) were associated with higher scores. From term to 4 months, greater weight gain (1.7 points [95 % CI: 0.2–3.1]) and linear growth (2.0 points [95 % CI: 0.7–3.2]) were associated with higher PDI. From 4 to 12 months, none of the growth measures was associated with MDI or PDI score. They concluded that in preterm infants, greater weight and BMI gain to term were associated with better neurodevelopmental outcomes. After term, greater weight gain was also associated with better outcomes, but increasing weight out of proportion to length did not confer additional benefit.

7 Future Research

As discussed above, neither "intrauterine growth charts" nor "fetal growth charts" nor "postnatal growth charts" are suitable for monitoring the growth of preterm infants till they become term. Similarly, CDC 2000 and WHO 2006 growth charts are also not ideal for monitoring the growth of ex-preterm infants.

In order to establish normative growth charts, the Inter Growth 21st study has been commenced by the International Fetal and Newborn Growth Consortium [53, 54]. The goal is to develop new "prescriptive" standards describing normal fetal and preterm neonatal growth over time and newborn nutritional status, and to relate these to neonatal health risk.

The primary objective is to produce a set of international Fetal and Newborn Growth Standards (fetal growth, birth weight for gestational age and postnatal growth of preterm infants) for practical applications in clinical use and for monitoring trends in populations.

The study aims to recruit 4,500 healthy women aged 18–35, who had regular menstrual cycles and conceived spontaneously and do not have major health issues and practice healthy lifestyles. Study participant women are being recruited from 9 countries across five continents. They undergo 6 scans in addition to the initial dating scans. They are scheduled at 5 weekly intervals: 14–18 weeks, 19–23 weeks, 24–28 weeks, 29–33 weeks, 34–38 weeks and 39–42 weeks. Apart from the additional scans, they receive the standardized antenatal care. Based on expected 9 % rate of prematurity, it is expected that around 360 infants would be born to these mothers (26–37 weeks gestation). Their longitudinal growth will be monitored for 8 months. This would include measuring weight, length and head circumference every 2 weeks for the first 8 weeks and then monthly until 8 months after birth. Those suffering from death or serious morbidities of prematurity such as NEC will be excluded. This study will enable the derivation of prescriptive intrauterine growth charts as well as postnatal growth charts from a diverse population across five continents.

References

1. de Onis M, Habicht JP (1996) Anthropometric reference data for international use: recommendations from a World Health Organization Expert Committee. Am J Clin Nutr 64(4):650–658
2. Secker D (2010) Promoting optimal monitoring of child growth in Canada: using the new WHO growth charts. Can J Diet Pract Res 71(1):e1–e3
3. Garner P, Panpanich R, Logan S (2000) Is routine growth monitoring effective? A systematic review of trials. Arch dis child 82(3):197–201
4. Horemuzova E, Soder O, Hagenas L (2012) Growth charts for monitoring postnatal growth at NICU of extreme preterm-born infants. Acta paediatrica 101(3):292–299 (Research Support, Non-U.S. Gov't)
5. Claas MJ, de Vries LS, Koopman C, Uniken Venema MM, Eijsermans MJ, Bruinse HW et al (2011) Postnatal growth of preterm born children ≤ 750 g at birth. Early Hum Dev 87(7):495–507
6. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK (2006) Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 117(4):1253–1261
7. Franz AR, Pohlandt F, Bode H, Mihatsch WA, Sander S, Kron M et al (2009) Intrauterine, early neonatal, and postdischarge growth and neurodevelopmental outcome at 5.4 years in extremely preterm infants after intensive neonatal nutritional support. Pediatrics 123(1):e101–e109
8. Shah PS, Wong KY, Merko S, Bishara R, Dunn M, Asztalos E et al (2006) Postnatal growth failure in preterm infants: ascertainment and relation to long-term outcome. J Perinat Med 2011 34(6):484–489
9. Ramel SE, Demerath EW, Gray HL, Younge N, Boys C, Georgieff MK (2012) The relationship of poor linear growth velocity with neonatal illness and two-year neurodevelopment in preterm infants. Neonatology 102(1):19–24
10. Ghods E, Kreissl A, Brandstetter S, Fuiko R, Widhalm K (2011) Head circumference catch-up growth among preterm very low birth weight infants: effect on neurodevelopmental outcome. J Perinat Med 39(5):579–586
11. Powers GC, Ramamurthy R, Schoolfield J, Matula K (2008) Postdischarge growth and development in a predominantly Hispanic, very low birth weight population. Pediatrics 122(6):1258–1265
12. Kan E, Roberts G, Anderson PJ, Doyle LW (2008) Victorian infant collaborative study G. The association of growth impairment with neurodevelopmental outcome at 8 years of age in very preterm children. Early Hum Dev 84(6):409–416 (Research Support, Non-U.S. Gov't)
13. Cole TJ, Freeman JV, Preece MA (1998) British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med 17(4):407–429
14. Latal-Hajnal B, von Siebenthal K, Kovari H, Bucher HU, Largo RH (2003) Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. J Pediatr 143(2):163–170
15. Casey PH, Whiteside-Mansell L, Barrett K, Bradley RH, Gargus R (2006) Impact of prenatal and/or postnatal growth problems in low birth weight preterm infants on school-age outcomes: an 8-year longitudinal evaluation. Pediatrics 118(3):1078–1086
16. Sices L, Wilson-Costello D, Minich N, Friedman H, Hack M (2007) Postdischarge growth failure among extremely low birth weight infants: correlates and consequences. Paediatr Child Health 12(1):22–28
17. American Academy of Pediatrics Committee on Nutrition (1985) Nutritional needs of low-birth-weight infants. Pediatrics 75(5):976–986
18. Nutrient needs and feeding of premature infants (1995) Nutrition Committee, Canadian Paediatric Society. CMAJ 152(11):1765–1785
19. Karna P, Brooks K, Muttineni J, Karmaus W (2005) Anthropometric measurements for neonates, 23–29 weeks gestation, in the 1990s. Paediatr Perinat Epidemiol 19(3):215–226

20. Lubchenco LO, Hansman C, Dressler M, Boyd E (1963) Intrauterine growth as estimated from liveborn birth-weight data at 24–42 weeks of gestation. Pediatrics 32:793–800
21. Babson SG, Benda GI (1976) Growth graphs for the clinical assessment of infants of varying gestational age. J Pediatr 89(5):814–820
22. Fenton TR (2003) A new growth chart for preterm babies: babson and benda's chart updated with recent data and a new format. BMC Pediatr 16(3):13
23. Kramer MS, Platt RW, Wen SW, Joseph KS, Allen A, Abrahamowicz M et al (2001) A new and improved population-based Canadian reference for birth weight for gestational age. Pediatrics 108(2):E35
24. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P (1991) An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977–1981). Acta Paediatr Scand 80(8–9):756–762
25. Beeby PJ, Bhutap T, Taylor LK (1996) New South Wales population-based birthweight percentile charts. J Paediatr Child Health 32(6):512–518
26. Olsen IE, Groveman SA, Lawson ML, Clark RH, Zemel BS (2010) New intrauterine growth curves based on United States data. Pediatrics 125(2):e214–e224
27. Bertino E, Milani S, Fabris C, De Curtis M (2007) Neonatal anthropometric charts: what they are, what they are not. Arch Dis Child Fetal Neonatal Ed 92(1):F7–F10
28. Johnsen SL, Rasmussen S, Wilsgaard T, Sollien R, Kiserud T (2006) Longitudinal reference ranges for estimated fetal weight. Acta Obstet Gynecol Scand 85(3):286–297
29. Chauhan SP, Hendrix NW, Magann EF, Morrison JC, Scardo JA, Berghella V (2005) A review of sonographic estimate of fetal weight: vagaries of accuracy. J Matern Fetal Neonatal Med 18(4):211–220
30. Dudley NJ (2005) A systematic review of the ultrasound estimation of fetal weight. Ultrasound Obstet Gynecol 25(1):80–89
31. Chan LW, Fung TY, Leung TY, Sahota DS, Lau TK (2009) Volumetric (3D) imaging reduces inter- and intraobserver variation of fetal biometry measurements. Ultrasound Obstet Gynecol 33(4):447–452
32. Schild RL, Maringa M, Siemer J, Meurer B, Hart N, Goecke TW et al (2008) Weight estimation by three-dimensional ultrasound imaging in the small fetus. Ultrasound Obstet Gynecol 32(2):168–175
33. Diekmann M, Genzel-Boroviczeny O, Zoppelli L, von Poblotzki M (2005) Postnatal growth curves for extremely low birth weight infants with early enteral nutrition. Eur J Pediatr 164(12):714–723
34. Bertino E, Coscia A, Mombro M, Boni L, Rossetti G, Fabris C et al (2006) Postnatal weight increase and growth velocity of very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 91(5):F349–F356
35. Berseth CL, Bisquera JA, Paje VU (2003) Prolonging small feeding volumes early in life decreases the incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics 111(3):529–534
36. Oh W, Poindexter BB, Perritt R, Lemons JA, Bauer CR, Ehrenkranz RA et al (2005) Association between fluid intake and weight loss during the first 10 days of life and risk of bronchopulmonary dysplasia in extremely low birth weight infants. J Pediatr 147(6):786–790
37. Wadhawan R, Oh W, Perritt R, Laptook AR, Poole K, Wright LL et al (2007) Association between early postnatal weight loss and death or BPD in small and appropriate for gestational age extremely low-birth-weight infants. J Perinatol 27(6):359–364
38. Finken MJ, Keijzer-Veen MG, Dekker FW, Frolich M, Hille ET, Romijn JA et al (2006) Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. Diabetologia 49(3):478–485
39. Euser AM, Finken MJ, Keijzer-Veen MG, Hille ET, Wit JM, Dekker FW (2005) Associations between prenatal and infancy weight gain and BMI, fat mass, and fat distribution in young adulthood: a prospective cohort study in males and females born very preterm. Am J Clin Nutr 81(2):480–487

40. Ekelund U, Ong K, Linne Y, Neovius M, Brage S, Dunger DB et al (2006) Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). Am J Clin Nutr 83(2):324–330
41. Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A et al (2007) Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 115(2):213–220
42. Khadilkar VV, Khadilkar AV, Cole TJ, Sayyad MG (2009) Crosssectional growth curves for height, weight and body mass index for affluent Indian children, 2007. Indian pediatrics 46(6):477–489 (Research Support, Non-U.S. Gov't)
43. de Onis M, Lobstein T (2010) Defining obesity risk status in the general childhood population: which cut-offs should we use? Int J Pediatr Obesity (Editorial) 5(6):458–460. (IJPO: an official journal of the International Association for the Study of Obesity)
44. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z et al (2002) 2000 CDC growth Charts for the United States. Methods Dev (Comparative Study) 246:1–190. (Vital and health statistics Series 11, Data from the national health survey)
45. Promoting optimal monitoring of child growth in Canada (2010) Using the new World Health Organization growth charts—Executive Summary. Paediatr Child Health 15(2):77–83
46. WHO (2006) WHO multicentre growth reference study. Acta paediatr 450:5–101
47. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J (2007) Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organiz 85(9):660–667
48. de Onis M, Onyango A, Borghi E, Siyam A, Blossner M, Lutter C (2012) Worldwide implementation of the WHO Child Growth Standards. Public Health Nutr 12:1–8
49. de Onis M, Martinez-Costa C, Nunez F, Nguefack-Tsague G, Montal A, Brines J (2012) Association between WHO cut-offs for childhood overweight and obesity and cardiometabolic risk. Public Health Nutr 31:1–6
50. Shields M, Tremblay MS (2010) Canadian childhood obesity estimates based on WHO, IOTF and CDC cut-points. Int J Pediatr Obesity 5(3):265–273. (IJPO: an official journal of the International Association for the Study of Obesity)
51. Nash A, Dunn M, Asztalos E, Corey M, Mulvihill-Jory B, O'Connor DL (2011) Pattern of growth of very low birth weight preterm infants, assessed using the WHO Growth Standards, is associated with neurodevelopment. Appl Physiol Nutr Metab 36(4):562–569
52. Belfort MB, Rifas-Shiman SL, Sullivan T, Collins CT, McPhee AJ, Ryan P et al (2011) Infant growth before and after term: effects on neurodevelopment in preterm infants. Pediatrics 128(4):e899–e906
53. The International Fetal and Newborn Growth Consortuim (2011) http://www.intergrowth21.org.uk/. Accessed 18 Sept 2011 (updated 18 Sept 2011; cited 2011 18 Sept)
54. Villar J, Knight HE, de Onis M, Bertino E, Gilli G, Papageorghiou AT et al (2010) Conceptual issues related to the construction of prescriptive standards for the evaluation of postnatal growth of preterm infants. Arch Dis Child 95(12):1034–1038 (Research Support, Non-U.S. Gov't Review)

Part VI
Breast Milk, Breast Feeding, and Donor Milk Banks

Chapter 16
Role of Breast Milk

Jacqueline C. Kent, Lukas Christen, Foteini Hassiotou and Peter E. Hartmann

Abstract Breast milk provides all the necessary macronutrients (fat, protein and carbohydrates) and micronutrients (vitamins, minerals, bioactive molecules) at concentrations that completely support the growth and development of the term infant for the first six months of life. This is underlined by the fact that growth charts produced by the World Health Organization are developed from data only from exclusively breastfed infants. In addition to its function as a source of nutrition, breast milk contains multiple components that provide the infant with protection from infection before its own defence mechanisms are fully developed. The rates of infection in exclusively breastfed infants are lower compared to infants fed artificial formula, not only in the developing world where standards of hygiene are less than ideal, but also in first world countries. Breast milk continues to provide both nutrients and protection from infection for at least the first year of life as complementary foods are introduced. Ongoing research continues to elucidate the beneficial effects of breast milk.

Although there has been no evolutionary pressure for the breast milk of mothers of preterm infants to adapt to the requirements of these vulnerable infants, preterm milk, at least initially, shows differences from term milk that are advantageous for the growth and development of the preterm infant. Additionally, the immune protective factors in breast milk have been shown to have a significant effect on decreasing the number of infections suffered by the preterm infant.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_16,

Key points

- contains macronutrients (fat, protein and carbohydrates) and micronutrients (vitamins, minerals, bioactive molecules) in ideal amounts for optimal growth and development of term infants
- contains immunomodulatory biomolecules (immunoglobulins, lactoferrin, lysozyme, cytokines) and maternal immune cells that protect the infant, whether term or preterm, from pathogens in its particular environment and supports the infant's immature immune system
- contains a hierarchy of cells, from stem cells to progenitor cells to more differentiated cells, that may be involved in optimal development of the infant
- requires targeted fortification for preterm infants

1 Introduction

The World Health Organization recommends that mothers worldwide exclusively breastfeed their infants for the first six months of life, with breastfeeding to be continued into and beyond the second year [1]. As the sole source of food for term infants, breast milk contains not only adequate nutritional components (fat, lactose, protein and micronutrients) at concentrations that support optimal growth of the human infant, but also biochemical and cellular components that allow optimal development of the infant and provide protection from infection. The components of breast milk are either synthesized by the lactocytes in the secretory tissue of the breast from substrates in the blood, or are selectively and actively transported from the blood through the lactocytes to the milk [2]. Compared with mothers of term infants, mothers of preterm infants produce breast milk with a slightly different composition, however, breast milk is also recommended for preterm infants [3, 4]. The different composition of preterm milk compared to term milk and the variations between milks of preterm mothers may be reflective of the developmental stage of the breast of the preterm mother.

2 Nutrition

Breast milk provides all the necessary macronutrients (fat, protein and carbohydrates) and micronutrients (vitamins, minerals, bioactive molecules) at concentrations that completely support the growth and development of the term infant for the first six months of life (Fig. 16.1). The fats are in the form of emulsified globules coated with a lipid bilayer membrane; some proteins and minerals are in colloidal dispersion as micelles; and the remainder of the proteins and minerals and all the carbohydrates in true solution.

Average quantity		Per 100 mL
Energy		320 kJ
Protein	- Total	1.0 g
	- Casein	0.4 g
	- Whey proteins	0.6 g
Fat	- Total	4.1 g
	- Saturated	1.6 g
	- Monounsaturated	1.4 g
	- Polyunsaturated	0.6 g
Carbohydrates	- Total	8.0 g
	- Lactose	6.0 g
	- Oligosaccharides	2.0 g
Minerals and trace elements		
	- Sodium	21 mg
	- Potassium	54 mg
	- Chloride	45 mg
	- Calcium	25 mg
	- Magnesium	3 mg
	- Phosphate	15 mg
	- Iron	51 µg
	- Zinc	200 µg
	- Copper	30 µg
	- Manganese	0.4 µg
	- Selenium	0.2 µg
	- Iodine	8.1 µg
	- Fluoride	1.0 µg
Vitamins A, B1, B2, B3, B5, B6, B7, B9, B12, C, D, E and K		
Live stem cells, lactocytes and leukocytes		$1x10^6 – 9x10^8$

Fig. 16.1 Contents of breast milk nutrition information (servings per package unlimited; serving size variable (up to 240 mL))

3 Fat

Breast milk fat provides 50–60 % of the caloric intake of the term infant [5]. The average fat content of milk is 41 g/L, but this ranges between 22 and 62 g/L both within and between mothers [6]. The total fat content of the milk is largely independent of the mother's diet [5], but varies in the short term with the degree of fullness of the breast when the milk sample is collected [7]. The emulsified fat globules are secreted by the lactocytes and consist of a milk fat globule membrane surrounding a core. The milk fat globule membrane is a lipid bilayer membrane similar to the apical membrane of the lactocyte and comprises phospholipids, cholesterol, glycolipids, proteins (including butyrophilin and xanthine oxidoreductase), and glycoproteins (including mucin and lactadherin). Over 98 % of the lipid in the core is in the form of triacylglycerols (TAG). The remainder are phospholipids, sterols and sterol esters, and non-esterified fatty acids (free fatty acids) [5]. The triacylglycerols are composed of saturated and unsaturated fatty acids esterified to a glycerol backbone [8, 9]. The fatty acids are short chain fatty acids (SCFA, $<$ 10-carbon chain), medium chain fatty acids (MCFA, 10- to 14-carbon chain) that are synthesized within the lactocyte, long chain fatty acids (LCFA, 16- to 24-carbon chain), and long chain polyunsaturated fatty acids (LCPUFA) including the omega-3 fatty acid docosahexaenoic acid (DHA) and the omega-6 fatty acid arachidonic acid (AA) that are derived from the blood. LCFA comprise 85 % of the fatty acids, MCFA comprise 13 % by weight of the fatty acids, while the remainder are SCFA. The mean concentration of DHA in breast milk is 0.32 % (range: 0.06–1.4 %) and that of AA is 0.47 % (range: 0.24–1.0 %) [10].

The roles of DHA and AA in infant nutrition are of particular importance because they accumulate specifically in the membrane lipids of the brain and retina, where they are important for visual and neural function [11]. Infants fed breast milk have been shown to have higher plasma concentrations of DHA and AA, higher DHA levels in the brain cortex, cerebral grey and white matter compared to infants fed artificial formula not containing LCPUFA [12]. The visual function of infants fed breast milk is enhanced compared with infants fed artificial formula provided the breast milk DHA concentration is not low ($\leq$ 0.15 %) [12]. In addition, infants fed breast milk have a higher IQ than infants fed artificial formula, and this effect persists up to 15 years of age, after adjustment for confounding factors [12]. This may be related to the unique fatty acid composition of human milk compared to cow's milk, which is the basis for infant formulas.

In contrast with the total fat content, the fatty acid composition of breast milk is affected by the mother's diet [13–15]. In particular, the milk of mothers consuming a low-fat high-carbohydrate diet has a higher concentration of MCFA, the milk of mothers who consume a low-omega-3 fatty acid diet contains a lower concentration of DHA, while the milk of mothers who consume a diet high in fish contains a high concentration of DHA [11].

3.1 Preterm

During the last trimester of gestation the foetus develops adipose tissue and there is extensive brain growth, associated with 80 % of the intrauterine accumulation of DHA and AA [12]. Thus, the provision of LCPUFA via the placenta is critical for the infant [16]. Preterm birth interrupts the supply of nutrients from the placenta so the nutrition of preterm infants by parenteral, enteral and/or oral feeding (depending on the degree of prematurity) is, therefore, of particular importance. The milk of mothers of preterm infants is 20–30 % higher in total energy and lipid content [17] and contains higher proportions of MCFA compared to that of mothers of term infants [18], with the proportion of MCFA being inversely related to the gestational age at delivery [19]. In addition, a greater proportion of LCFA, including DHA and AA, has been reported in preterm milk [20, 21], with values of DHA in preterm milk ranging from 0.21 to 0.36 %, and values of AA in preterm milk ranging from 0.36 to 0.91 % [20].

There have been several studies on the supplementation of artificial formula with LCPUFA for preterm infants [22]. Addition of 0.4 % DHA and 0.6 % AA to artificial formula has been shown to result in infant LCPUFA status comparable to that of infants fed breast milk. Preterm infants receiving this supplemented formula have shown improved visual attention and global cognitive development compared with preterm infants receiving unsupplemented formula [12].

Although the effect of supplementation of artificial formula with LCPUFA on the growth of preterm infants is equivocal, a recent study that fed very-low-birthweight infants with DHA and AA-supplemented formula up to 92 weeks post-gestational age showed better growth and development scores than those fed unsupplemented artificial formula [23].

3.2 Digestion

Hydrolysis of the TAG begins in the infant's stomach by lingual and gastric lipases. These lipases appear before 26 weeks' gestation and are therefore active in both term and preterm infants [24]. In the duodenum, gastric lipase is inactivated by the infant pancreatic proteases (trypsin and chymotrypsin). Further hydrolysis of the TAG occurs through the actions of pancreatic and breast milk bile salt-stimulated lipase (BSSL) [5, 25]. Pancreatic lipase hydrolyzes TAG at the first and third positions on the glycerol backbone, releasing those fatty acids as non-esterified fatty acids and the fatty acid at the second position as a 2-monoacylglycerol. BSSL hydrolyzes tri-, di-, and mono-acylglycerols, retinyl esters, cholesteryl esters, and diacylphosphatidylglycerols and releases non-esterified fatty acids and glycerol [25, 26]. Fatty acids commonly located at the second position such as palmitate are more easily absorbed by infants as 2-monoacylglycerols than as non-esterified fatty acids [27, 28]. This preferential absorption and the high proportion of palmitate esterified to the 2-position of the triacylglycerols in breast milk, compared to bovine milk and vegetable oils used to prepare artificial formula, may explain why formula-fed infants

experience more difficulty absorbing fat from their diet and have higher rates of constipation than breastfed infants [29].

In preterm infants, breast milk BSSL plays a particularly important role in hydrolysis of TAG [28] because these infants produce less than optimal levels of pancreatic lipase. The unsaturated fatty acids that are produced are better absorbed by preterm infants than saturated fatty acids [30]. The importance of breast milk BSSL is shown by the fact that preterm infants fed pasteurized donor breast milk have a lower fat absorption and growth rate compared to preterm infants fed mothers' own milk [31, 32], probably at least partly due to inactivation of BSSL through the thermal pasteurization process. In addition, MCFA are better absorbed than LCFA [33] and are an important source of nutrients and energy for the preterm infant [34]. Therefore, the higher concentration of MCFAs in the milk of preterm mothers may have a nutritional advantage for the preterm infant [5].

4 Carbohydrate

4.1 Lactose

The principal carbohydrate in breast milk is lactose. Its concentration in breast milk has a fairly consistent level of approximately 60 g/L [35] and provides approximately 30–40 % of the energy delivered to the infant [36, 37].

4.2 Preterm

No consistent difference has been reported in the levels of lactose between term and preterm milks [17, 38–40]. This inconsistency has been attributed to differing sample collection protocols and large inter-individual variability in milk composition between mothers [40].

4.3 Digestion

Lactose is digested by lactase in the small intestine of the infant to produce glucose and galactose, which are then transported to the liver via the hepatic portal vein. In newborn infants the majority of the glucose passes into the peripheral circulation and is a substrate for energy production. Of the galactose, 94 % is absorbed by the liver where it is phosphorylated by galactokinase to glucose-1-phosphate. Half is then converted to glucose and the other half is further metabolized to replenish liver glycogen stores [41].

Normally, lactase activity increases during the third trimester, and therefore, lactose malabsorption is a risk factor for very preterm infants. However, it has been shown that infants born at < 30 weeks gestation show increasing lactase activity between 34 weeks and 37 weeks post-gestational age [42].

4.4 Human Milk Oligosaccharides

Human milk oligosaccharides (HMO) are the third largest solid component in breast milk, after lactose and triacylglycerols, with a concentration of 20–25 g/L in colostrum, declining to 5–20 g/L in mature breast milk [43]. Oligosaccharides are complex carbohydrates that range in length from 3 to 10 monosaccharides and comprise combinations of glucose, galactose, N-acetylglucosamine, fucose, sialic acid and lactose, with lactose often found at the reducing end. There are over 200 different oligosaccharides and extreme diversity in the oligosaccharide content of breast milk, with milk from different mothers containing as few as 23 and as many as 130 different oligosaccharides [44]. HMO are predominantly neutral, with only 10 % being acidic [45]. The pattern of oligosaccharides in the milk depends on the mother's secretor (Se) and Lewis (Le) status [46]. The secretor gene codes for α1,2 fucosyl transferase and the Lewis gene codes for α1,3/4 fucosyl transferase [47]. Therefore the milk of Se + Le + mothers (approximately 69 % of Caucasians) contains HMO with α1,2, α1,3, and α1,4 linked fucosyl residues; while the milk of Se-Le + mothers (approximately 20 % of Caucasians) contains HMO with no α1,2 linkages; and the milk of most of the remaining mothers with an active secretor gene and an inactive Lewis gene (Se + Le-) contains HMO with no α1,4 linkages [46].

Preterm milk from Se + Le + mothers contains higher concentrations of HMO than that of both the other genetic groups and term mothers and remains at or above 20 g/L for at least the first month of lactation.

HMO are largely resistant to digestion in the stomach and small intestine and are excreted in the infant's faeces [43]. Supplementation of formula-fed preterm infants with neutral HMO, with or without the addition of acidic HMO, in concentrations similar to those of breast milk reduces stool viscosity [48]. A small amount (~ 1 %) of HMO is absorbed in the intestine and appears in the infant's urine [43] but the significance of this is as yet unknown. It is also possible that HMO, as a rich source of sialic acid, assists brain development of the neonate and contributes to the intellectual development of breastfed infants. Therefore, despite their high concentration HMO are not likely to be a major energy source for the infant. Rather, they have multiple roles in protection from infection, as described below [43].

5 Protein

Protein provides approximately 8 % of the energy delivered to the infant [49]. Although there is wide variation in the concentration of protein in milk expressed by individual mothers, the total protein concentration of breast milk is generally higher

in colostrum (30–70 g/L), before gradually declining to a stable level in mature milk (7–14 g/L) [39, 50, 51]. The proteins in breast milk can be divided into three distinct sub groups: caseins, which exist in micellar structures; whey proteins, which are water-soluble; and the proteins associated with the membrane of the milk fat globule.

In the milk of most mammals, caseins are the major proteins present and the characteristic white appearance of milk is due to the presence of casein micelles. The micelles are made up of several casein subunits, calcium phosphate and other ionic constituents. However, in breast milk casein comprises less than 10 % of the total protein content in colostrum, 40 % (3.5–5.5 g/L) in mature milk, and roughly 50 % in late lactation [52]. The low casein content of breast milk results in the formation of a soft curd in the stomach of the infant that is easily digested and therefore compatible with frequent feeding which often occurs when infants are fed on demand [53]. The concentration of casein in bovine milk is 27 g/L [54] (more than 10-fold that of breast milk) and forms a relatively hard curd that is difficult for infants to digest. Addition of demineralised whey protein to artificial formula can partially overcome this problem [55]. Moreover, the low casein content of breast milk is compatible with the low growth rate of the term infant compared to the young of other species, the milks of which generally have higher casein contents [56].

The whey proteins constitute the majority of the protein content in breast milk comprising more than 90 % of the total protein content in colostrum, 60 % in mature milk, and decreasing to roughly 50 % in late lactation [52]. The whey proteins comprise principally α-lactalbumin, lactoferrin (lactotransferrin), serum albumin, enzymes (BSSL, lysozyme), binding proteins (folate-binding protein, vitamin B_{12}-binding protein, vitamin D-binding protein, thyroxine-binding protein, corticosteroid-binding protein), tenascin, macrophage mannose receptor and immunoglobulins (secretory IgA, IgG, IgM) [50, 57, 58]. α-Lactalbumin is one of the major whey proteins of breast milk comprising 10–20 % of the total protein of breast milk (2–3 g/L) [50, 59]. It is not only a major nutritive protein, with a composition that is closely matched to the amino acid requirements of infants, but also essential for the synthesis of lactose.

Most of the milk proteins are synthesized by the rough endoplasmic reticulum from amino acids derived from the blood. They are then transferred to the Golgi apparatus where phosphorylation and casein micelle formation take place, after which they are packaged into secretory vesicles and secreted by exocytosis [60]. Other proteins (albumin, IgA, IgG) are taken up from the blood by endocytosis at the baso-lateral membrane and transported through the lactocyte. They may be released directly into the alveolar lumen or secreted with the milk proteins by exocytosis [60].

Non-protein nitrogen comprises 20–25 % of the total nitrogen of breast milk and includes free amino acids (mainly glutamate and glutamine), carnitine, taurine, amino sugars, nucleic acids, nucleotides, polyamines, urea and uric acid [61].

Amongst its bioactive factors, breast milk contains cytokines (from the Greek ‘cyto’ = cell and ‘kinos’ = movement), which may be produced by mammary epithelial cells, breast milk cells, and/or be transported into milk from the maternal blood circulation [62]. Cytokines are small cell-signalling molecules with a function in intercellular communication. They can be classified as peptides, proteins or glycoproteins, and include chemokines, interleukins and interferons. Cytokines

have been shown to have primarily immunomodulatory roles, but are also involved in development [63]. They exert their function by binding to their receptors on the cell surface, which stimulates a cascade of intracellular events that regulate gene expression and alter cell function. Thus, the presence of cytokines in breast milk suggests their involvement in early infant development and protection. It has been suggested that breast milk cytokines survive the gastrointestinal tract of the infant [64] which brings forward the hypothesis of passage to the systemic circulation and transfer to various organs, where they may exert their functions.

Thus, although it is established that breast milk contains a variety of cytokines, inter- and intra-individual variability in their abundance have been observed. Factors influencing this variability, such as gestational age at birth, the stage of lactation, the degree of fullness of the breast, maternal diet, and maternal and/or infant infection, still remain unclear [62]. Inconsistency of sampling and measurement protocols may in part explain the observed variation [62]. A recent preliminary study demonstrated an increase in breast milk proinflammatory cytokines with increasing physical exercise, suggesting a link with caloric expenditure, which merits further investigation [65]. Future studies should address intra- and inter-individual factors that may influence milk cytokine concentrations, which may provide further insight into the roles of these molecules both in the lactating breast and for the breastfed infant. This may allow improvement of the current recommendations for the nutrition of the preterm infant. Importantly, accurate and reliable techniques and protocols must be developed for measuring breast milk cytokines and for the sampling of milk for this purpose, which is not currently standardised.

5.1 Preterm

The most consistently reported difference between preterm milk and term milk is in the protein fraction. Many studies have shown that there are 15–20 % higher levels of total protein in preterm milk compared with term milk [17, 38, 39, 66, 67], although this finding was not universal [68]. Individual proteins do not show consistent differences between preterm milk and term milk. Epidermal growth factor and sIgA levels are higher in preterm milk [69, 70]. However, leptin (which regulates appetite) is present at lower concentrations in preterm milk compared to term milk [71], and other proteins appear to be unrelated to gestational age [72]. For the majority of studies reporting a significant effect of gestational age upon breast milk, the greatest compositional differences were found early in lactation. After a month of lactation, the differences between preterm and term milk appear to become much less pronounced.

5.2 Digestion

The digestive enzyme chymosin in the infant stomach cleaves a specific bond in κ-casein which destroys the micellar structure and causes casein to aggregate in the

stomach to form soft curds [73]. Pepsin in the stomach then hydrolyzes large protein molecules. In the small intestine, the action of enzymes such as trypsin and chymotrypsin produces small peptide fragments and amino acids that are absorbed by the intestinal mucosa. The casein is not only a nutritive protein per se, but the formation of phosphopeptides during digestion keeps the calcium soluble and facilitates its absorption. This allows significant amounts of calcium and phosphate to be transferred to the infant [74].

6 Micronutrients: Vitamins and Minerals

In addition to macronutrients, breast milk provides fat-soluble vitamins, water-soluble vitamins, minerals and trace minerals for the infant [61]. Unlike the macronutrients, the concentration in breast milk of many of these micronutrients is dependent on the mother's diet, and it is therefore critical that mothers consume a diet adequate in micronutrients, in particular thiamin, riboflavin, vitamins B-6 and B-12, vitamin A, iron and iodine [75, 76].

6.1 Calcium, Phosphate and Magnesium

The total calcium and phosphate concentrations in breast milk are 250 mg/L and 150 mg/L, respectively, and are independent of maternal dietary intake [76]. The calcium phosphate is maintained in a stable form in milk as an essential component of casein micelles. Calcium and phosphate are essential for bone mineralization, and the concentrations in breast milk are normally sufficient for bone mineralization of the term infant, providing vitamin D levels are adequate.

During pregnancy, the majority of bone mineralization occurs during the third trimester, when the accretion rates are 100–120 mg/kg/day for calcium and 50–65 mg/kg/day for phosphate [77]. Preterm infants have a significant risk of osteopenia of prematurity (decreased bone mineral content) that may lead to rickets (radiological evidence of bone demineralization). When preterm infants are breastfed 40 %, are affected by osteopenia of prematurity [78]. Preterm milk has a calcium concentration that is equal to or lower than term milk [66, 79, 80] and bone mineralization of preterm infants fed breast milk that is not supplemented with calcium and phosphorus is lower than the intrauterine rate. Therefore, breast milk for preterm infants requires fortification with calcium and phosphorus [81]. Intrauterine accretion rates for calcium and phosphorus can be achieved when preterm infants are fed breast milk supplemented with calcium gluconate-glycerophosphate [80]. Recommendations for calcium and phosphorus contents of preterm formulas range between 70 and 160 mg of calcium per 100 kcal, and between 50 and 108 mg of phosphorus per 100 kcal [82]. Although it is not clear whether supplementation of breast milk with magnesium is required, a low level of 6–12 mg per 100 kcal has been recommended [80, 82, 83].

6.2 Vitamin D

Adequate vitamin D is critical for the absorption of calcium from the diet. Newborn infants initially depend on vitamin D transferred across the placenta to meet requirements in early life. Breast milk normally has a very low concentration of vitamin D, but this can be increased by maternal vitamin D supplementation [84]. Thus, the vitamin D status of breastfed infants is determined by that of their mothers during pregnancy and lactation and by their own skin vitamin D synthesis [85]. Some infants are still presenting with rickets, particularly those with higher skin pigmentation and receiving insufficient exposure to sunlight. Vitamin D administration (2,000–10,000 IU daily) has been shown to be effective in healing of the rickets [86].

6.3 Trace Elements

Trace elements in breast milk include copper, zinc, barium, cadmium, cesium, cobalt, cerium, lanthanum, manganese, molybdenum, nickel, lead, rubidium, tin, strontium, and these show a high bioavailability [87]. During the first 8 weeks postpartum, preterm milk has a lower concentration of lead, but there are no consistent differences in the other elements [79]. The selenium, zinc and manganese concentrations of preterm milk are adequate, but the copper concentrations are lower than recommended [88].

7 Protection

The immune system of the infant, and in particular the preterm infant, is immature and receives significant protection from infection via breast milk. This has been demonstrated not only in countries where hygiene is less than optimal, but also in first-world countries with adequate hygiene standards [89].

8 Fat

The fat in breast milk has a major protective role, in addition to its nutritional value. Both the milk fat globule membrane [90] and the core components of the milk fat globule can provide protection against microorganisms.

Glycoproteins and glycolipids in the milk fat globule membrane in breast milk have been found to provide protection against several enteropathogens, predominantly through acting as soluble receptor homologues that inhibit the binding of pathogens to their host receptors. The glycoproteins, mucin and lactadherin, have been shown to protect against rotavirus and *E. coli* binding, respectively [91, 92]. They are resistant to conditions in the newborn's stomach and maintain their structure and function even at low pH and in the presence of pepsin [91, 93]. The membrane

protein butyrophilin has been found to play a number of immunomodulatory roles [94, 95]. Similarly xanthine oxidoreductase is able to synthesize the antimicrobial radical nitric oxide, which inhibits the growth of *E. coli* and *Salmonella enteritides* [96]. In addition, the membrane glycolipids have been shown to inhibit the toxins from both *Vibrio cholerae* and *E. coli* from binding to their epithelial receptors *in vitro* [97].

Some of the products of hydrolysis of the core triacylglycerols by digestive lipases are able to lyse enveloped viruses, bacteria, and protozoa. The most active products are the monoacylglycerols and the medium-chain lauric acid and the long chain linoleic acid and oleic acid [98–102].

9 Carbohydrate

9.1 Oligosaccharides

HMO acts as a prebiotic, promoting intestinal growth of certain beneficial bacteria, in particular *Bifidobacterium longum* subsp *infantis* and *B. bifidum* [103, 104]. In addition, HMO are further thought to block binding of enteric bacteria and viruses to the epithelial cells of the gut. For example, HMO with α1,2 linked fucosyl residues blocked *Campylobacter jejuni* binding to the intestinal mucosa of mouse pups and prevented infection [105]. Also, the milk of Se + Le + mothers blocks binding of norovirus to its receptor, providing a likely mechanism for the reduced incidence of norovirus diarrhea in the infants of mothers in this milk group [106, 107].

The incidence of necrotising enterocolitis is lower in breast-fed infants than formula-fed infants, and this has been attributed to HMO [108]. In a neonatal rat model, one HMO, a specific isomer of disialyllacto-N-tetraose, in concentrations within the physiological range reported for human milk, significantly reduced necrotising enterocolitis [108]. While the action of HMO reduces the incidence of enteric infection in both term and preterm infants, the effect is likely to have a stronger impact on preterm infants due to their greater vulnerability to necrotising enterocolitis [109].

10 Protein

10.1 Casein

Digestion of β-casein yields peptides that show cysteine protease inhibiting activity, suggesting a possible role in antiseptic and anti-infectious functions through protease inhibition of bacteria and viruses [110]. In addition, peptides generated from the digestion of κ-casein possess potent antibacterial activity against both gram-positive and gram-negative bacteria [111].

10.2 α-Lactalbumin

Digestion of α-lactalbumin releases polypeptides with bactericidal properties against a number of gram-positive bacteria [112]. Furthermore, multimeric α-lactalbumin has been shown to be lethal to tumour cells while not affecting mature epithelial cells [113].

10.3 Immunoglobulins

The major immunoglobulin in breast milk is sIgA, with a concentration of 0.5–1.0 g/L in term milk, while monomeric IgA, IgG and IgM are the minor immunoglobulins in breast milk [114]. Via the entero-mammary pathway, environmental antigens ingested by the mother will sensitize cells in the maternal intestine. These cells are transported via the lymphatic system and blood to the mammary gland and start the formation of antibodies (sIgA) specific to the pathogens to which the mother and infant have been exposed [114]. The specific sIgA is consumed by the infant. The sIgA is resistant to proteolysis in the infant's intestine and is therefore more able to persist inside the intestinal tract than other immunoglobulins, boosting the infant's immune defence system [115]. Interestingly, the concentration of sIgA is highest in colostrum, at the time when the infant is most vulnerable to infections because its own immune system is still immature [116].

10.4 Lactoferrin

Breast milk has a high lactoferrin concentration with about 9.7 g/L in colostrum and 2.9 g/L in mature milk [68]. *In vivo* studies have shown a protective effect of lactoferrin against many different types of infections [117–119]. Lactoferrin is an iron-binding protein that acts as a bacteriostatic by competing with iron-dependent pathogens for iron [120]. More recent research shows also that lactoferrin binds to the lipid-A portion of lipopolysaccharide on the cell surface, disrupting the bacterial cell membrane [121]. Furthermore, lactoferrin inhibits adherence and invasion of bacteria into mammalian cells. Although the effect of lactoferrin on enteric viruses such as rotavirus is not yet fully understood, it has been suggested that lactoferrin blocks viral attachment of feline calicivirus and prevents adenovirus replication [122].

Several activities of lactoferrin other than its primary defence against microbial and viral infections have also been described. Lactoferrin has several enzymatic activities such as phosphatase and malto-oligosaccharide hydrolysis. Additionally, there is evidence that it acts as the major deoxyribonuclease, ribonuclease and adenosine triphosphatase of breast milk [123]. Furthermore, lactoferrin inhibits iron-dependent lipid peroxidation, immunomodulation and cell growth regulation [124–126]. It also

binds to DNA and the binding to specific regions of the DNA drives gene transcription and could account for a second level of defense against invading organisms [127, 128]. Lactoferrin is an activator of natural killer cells and has an anti-tumour activity [129, 130].

Digestion of lactoferrin in the stomach releases lactoferricin, which not only has antimicrobial, antiviral, and antifungal activities but also is capable of stimulating the immune system and neutralizing endotoxin [131].

10.5 Lysozyme

The concentration of lysozyme in breast milk is 0.05–0.25 g/L, which is around 3,000 times higher than in bovine milk [132]. Lysozyme contributes to the bacteriostatic properties of milk, however, *in vivo* evidence for its immunological function in the infant is still not yet available. Lysozyme catalyzes the hydrolysis of specific bonds between N-acetylglucosamine and N-acetylmuramic acid in the cell walls of most gram-positive bacteria resulting in lysis [133]. Furthermore, an *in vitro* study showed that in the presence of lactoferrin, lysozyme is not only bacteriostatic, but also bactericidal and can kill some gram-negative bacteria. The mechanism of action is not fully understood but it has been suggested that following alteration of the gram-negative outer cell membrane by lactoferrin, lysozyme is able to break down the inner membrane, ultimately killing the bacteria [134]. Lysozyme also shows anti-HIV activity *in vitro* [135]. The mechanism is not fully understood but lysozyme may act on the free virus and not on the cell-associated virus [74]. Additionally, lysozyme, lactoferrin and sIgA, particularly in the absence of iron, will lyse amoebas [136]. Further research on this is warranted.

10.6 Cytokines

A recent preliminary study demonstrated a response of breast milk cytokines, such as IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ and TNF-α, to certain maternal and/or infant infections [137]. Immune cells in breast milk relate to maternal and infant infections, but further work is needed to establish the infection-specific response of breast milk cytokines and their roles in protecting the breastfed infant. These roles could range from anti-inflammatory actions, to regulating other cytokines and/or immunoglobulins, to controlling immune and epithelial cell maturation, to indirectly protecting cell surfaces and tissues against damage [62, 138, 139].

For example, IFN-γ is an immunomodulatory cytokine present in colostrum and mature breast milk, which regulates immune cell maturation, and has been suggested to be involved in the protective role of breast milk feeding against allergies [62, 140]. TGF-β is another cytokine with immunomodulatory functions present in varying levels in colostrum and mature breast milk [62], which is thought to act as an initiator of IgA production in neonates [138]. At the same time, TGF-β has been shown to

prevent atopic disease in breastfed infants [141]. TNF-α is a cytokine involved in systemic inflammation, which is believed to have immunologic functions both in the lactating breast and in the breastfed infant, with studies showing that it may be slightly higher in colostrum than in mature breast milk [62] and that it responds to infections of the breastfeeding dyad [137].

Cytokines of the interleukin family are also present in colostrum and breast milk at varying levels, and have either inflammatory or anti-inflammatory functions. IL-1β is an inflammatory cytokine that is also involved in cell proliferation, differentiation and apoptosis [142], and has been shown to be produced by breast milk cells [143]. IL-4 is antagonistic to IFN-γ and may decrease from colostrum to mature breast milk [62]. IL-6 is an anti-inflammatory breast milk cytokine that appears to be very responsive to maternal and/or infant infections [137] and is known to be involved in the activation of the acute phase of the immune response [144].

10.7 Preterm

Infants who are born preterm miss out on the transplacental transfer of maternal immunoglobulins that occurs after 34 weeks of gestation. The immune system of preterm infants is immature and unable to produce sufficient immunoglobulins [145]. The sIgA in breast milk is, therefore, particularly important to assist in protecting the preterm infant from infections. These infants are likely to benefit from the higher concentrations of sIgA that are found in preterm milk compared with to term milk [146].

The lactoferrin concentration of the colostrum of preterm mothers is 5.8 g/L, which is lower than that of the colostrum of term mothers (9.7 g/L). However, as the preterm milk matures, the concentration decreases only slightly to 4.6 g/L. A study of VLBW and ELBW neonates showed that the supplementation of breast milk with bovine lactoferrin (bLF) reduced the incidence of an episode of late-onset sepsis [146]. The effect was seen in gram-positive bacterial and in fungal late-onset sepsis but was not statistically significant for gram-negative bacterial sepsis. Overall late-onset sepsis occurred in the bLF group less frequently (5.9 %) compared to the control group (17.3 %). Gram-positive bacterial, gram-negative bacterial and fungal sepsis occurred less frequently in the bLF group (1.3 %, 4.6 % and 0 %, respectively) than in the control group (7.7 %, 10.1 % and 5.4 %, respectively) [147]. Additionally, the supplementation of breast milk with bLF commencing within the first 72 h of life reduced the incidence of invasive fungal infections in VLBW neonates [148]. Although fungal colonization in the bLF-supplemented group was 17.6 % compared to the placebo group with 18.5 %, an invasive fungal infection occurred in the bLF-supplemented group less frequently (0.7 %) compared to the placebo group (7.7 %). This demonstrates that bLF acts later in the process of the fungus-host interaction and prevents progressing from colonisation to infection. The study claims that the supplementation of bLF has the same impact on prevention of invasive fungal infections as the prophylactic application of fluconazole [148].

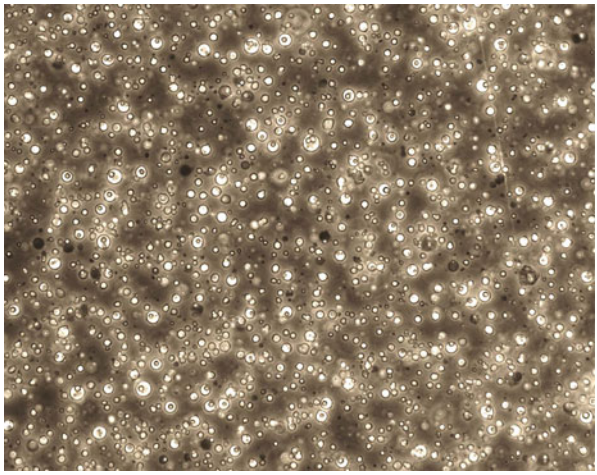

Fig. 16.2 Total cells isolated from freshly expressed breast milk. Trypan blue exclusion was used to discriminate between dead/dying cells (dark) and viable cells (the rest).

The concentration of lysozyme in preterm milk is similar to that in term milk [149]. Xanthine oxidoreductase and macrophage mannose receptor concentrations are lower in preterm compared with term milk, and the concentrations decrease further during lactation [58].

11 Cells in Breast Milk

In addition to biochemical components, breast milk contains maternal cells (Fig. 16.2). These include blood-derived leukocytes as well as cells of the mammary epithelium. Recent evidence supports a clear link between the leukocyte content of mature breast milk and maternal/infant infections. A longitudinal study analyzing colostrum and breast milk from breastfeeding dyads when they were healthy and when either the mother or the infant or both had an infection showed that although colostrum normally contains a significant proportion of leukocytes (13–70 % of total milk cells), mature breast milk from healthy dyads contains very few leukocytes (0–2 %) [150]. However, general or organ-specific infection of the mother and/or the infant was associated with a significant increase in the leukocyte content of mature breast milk, which returned to a low baseline level upon recovery from the infection. Interestingly, the response of milk leukocyte content to infection was observed also when only the infant had an infection and the mother was asymptomatic, and was rapid, and more consistent than either the immunoglobulin or lactoferrin milk response. This now suggests the use of breast milk leukocytes as a marker of the health status of both the lactating breast and the breastfeeding infant, and calls for investigation of the normal range of leukocytes in preterm milk, and the specific protective benefits they may provide to the preterm infant. In this connection, breast milk leukocytes may provide protection against NEC to the preterm infant, which needs further investigation.

In contrast to leukocytes, the total cell content of breast milk does not appear to significantly change with infections of the dyad with the exception of maternal breast infections [150]. The observed variability in the response of the total breast milk cell content to infection may be related to non-standardisation of sampling protocols, which have not usually considered the degree of breast fullness at each collection time point. Ongoing research is demonstrating that the cellular content of breast milk is dynamic, changing in response to infant feeding and breast drainage [151]. It has been previously known that pre-feed milk (from fuller breasts) has lower cell content than post-feed milk (from emptier breasts) [152]. Expanding on this, Hassiotou et al. [151] recently demonstrated that the maximum breast milk cell content can be seen within 30 min post-feeding. This has been so far shown for feeds where a milk volume of approximately 50 mL or more has been removed. Similar results were reported for breast milk fat content, suggesting a common or associated mechanism controlling both the cell and the fat content of breast milk. These findings can now be used to both standardize milk collection protocols in lactation studies and as a basis for improvement of the nutrition of preterm infants.

In addition to short-term changes associated with the degree of breast fullness and milk removal, the milk cellular content and composition also seems to be changing during the course of lactation, suggesting that it is reflective of modifications that occur in the mammary epithelium during lactation [153]. The latter is consistent with the notion that breastfeeding and lactation effect permanent changes in the breast, which have also been related to reduced breast cancer risk in the long term [154]. These changes are not yet known and merit further investigation.

Thus, it becomes evident that leukocytes are not the predominant cell population in mature breast milk from healthy dyads. The remaining milk cells are thought to be of mammary epithelial origin, although one cannot exclude the presence of other blood-derived cells in breast milk, such as hematopoietic stem cells or red blood cells [153]. Mammary epithelial cells end up in breast milk through the mechanical shear forces generated during breastfeeding/breast milk expression and/or via cell migration and turnover [155]. In either case, they originate from the mammary epithelium of the lactating breast, which contains an array of epithelial cell types, representing different developmental stages. These range from early-stage mammary stem cells, to progenitor cells, to the more differentiated myoepithelial cells and lactocytes, all of which can be seen in breast milk [153, 155–157]. Indeed, recent work has identified the presence of stem cells in breast milk and has also demonstrated a breast milk cellular hierarchy characteristic of the fully mature mammary gland [155–157]. A population of these stem cells has the potential to differentiate not only into the different mammary epithelial lineages under mammary differentiation conditions *in vitro*, but also into other cell types in corresponding microenvironments, such as bone cells, brain cells, liver cells, pancreatic beta cells, and heart cells [155]. This new discovery opens new avenues for exploration of the potential of breast milk-derived stem cells to be used in regenerative medicine, particularly given that they are not tumorigenic [155]. It also generates many other questions to be addressed, such as the potential function of breast milk stem cells for the breastfed infant, in which they may play important developmental roles.

Although the role of breast milk stem cells for either the term or the preterm infant has not yet been explored, it can be postulated that both these cells and regulatory components they produce, such as microRNAs, may pass unharmed through the gastrointestinal tract of the infant and enter the blood circulation, from which they can be transported to distant sites, assisting in tissue development [155, 158]. This has indeed been demonstrated for milk leukocytes in animals, including primate models [159, 160], and is thus conceivable that it also occurs in humans for other milk cells, such as the stem cells. This natural process of transfer of cells and genetic material from the mother to the infant via breastfeeding may be particularly important for the preterm infant, whose tissues are not as developed as those of the term newborn. In addition, breast milk stem cells could be used as a natural stem cell therapy for the treatment of fatal neonate diseases, such as chronic lung disease or hepatic steatosis. Therefore, the discovery of stem cells in breast milk now opens new avenues for improving the current understanding of developmental programming associated with breast milk feeding, and how the latter can be adjusted to provide maximal support for the survival and optimal growth of the preterm infant. In this connection, the effects of breast milk preservation and storage, such as pasteurisation and freezing, on cell activity need to be examined and taken into account when considering methods of breast milk preparation for the preterm infant.

12 Summary

Breast milk alone can meet the nutrient needs of term infants during the first 6 months, with the possible exception of vitamin D in certain populations and iron in infants of relatively low birth weight [161]. For preterm infants, the nutritional benefits of feeding breast milk, in terms of protein digestion, amino and fatty acid patterns, fat absorption, and lactose digestion and gastrointestinal function are recognized. Although the optimum nutrition of preterm infants is unknown, it has been demonstrated that breast milk for preterm infants needs to be supplemented with calcium and phosphorus, while protein and sodium supplements may also be needed [80]. Research is needed to determine the precise quantity of nutrients to be added as supplements [162].

Compared with preterm formula, the feeding of fortified breast milk may provide significant protection to the preterm infant from infection and NEC. The potential stimulation of an enteromammary pathway through skin-to-skin contact provides species-specific antimicrobial protection for preterm infants, including nosocomial pathogens [162]. Thus, for preterm infants, neonatal centers should encourage the feeding of fortified breast milk, together with skin-to-skin contact, as reasonable methods to enhance milk production while potentially facilitating the development of an enteromammary response [162]. Facilitation of feeding of a mother's own milk should be adopted by neonatal units [163].

Acknowledgments PEH, FH and JCK are recipients of an unrestricted research grant from Medela AG (Switzerland). LC receives scholarship support from Carag AG (Switzerland). FH also received scholarship support from the Women and Infants Research Foundation, Western Australia.

References

1. Gartner LM, Morton J, Lawrence RA et al (2005) Breastfeeding and the use of human milk. Pediatrics 115:496–506
2. McManaman JL, Neville MC (2003) Mammary physiology and milk secretion. Adv Drug Deliv Rev 55:629–641
3. Lawrence PB (1994) Breast milk. Best source of nutrition for term and preterm infants Pediatr Clin North Am 41:925–941
4. Maas YG, Gerritsen J, Hart AA et al (1998) Development of macronutrient composition of very preterm human milk. Br J Nutr 80:35–40
5. Jensen RG (1999) Lipids in human milk. Lipids 34:1243–1271
6. Kent JC, Mitoulas LR, Cregan MD, Ramsay DT, Doherty DA, Hartmann PE (2006) Volume and frequency of breastfeeds and fat content of breast milk throughout the day. Pediatrics 117:e387–e395
7. Daly SE, Di Rosso A, Owens RA, Hartmann PE (1993) Degree of breast emptying explains changes in the fat content, but not fatty acid composition, of human milk. Exp Physiol 78:741–755
8. Innis SM (2011) Dietary triacylglycerol structure and its role in infant nutrition. Adv Nutr 2:275–283
9. Martin JC, Bougnoux P, Antoine JM, Lanson M, Couet C (1993) Triacylglycerol structure of human colostrum and mature milk. Lipids 28:637–643
10. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM (2007) Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr 85:1457–1464
11. Innis SM (2007) Human milk: maternal dietary lipids and infant development. Proc Nutr Soc 66:397–404
12. Fleith M, Clandinin MT (2005) Dietary PUFA for preterm and term infants: review of clinical studies. Crit Rev Food Sci Nutr 45:205–229
13. Innis SM (2004) Polyunsaturated fatty acids in human milk: an essential role in infant development. Adv Exp Med Biol 554:27–43
14. Nasser R, Stephen AM, Goh YK, Clandinin MT (2010) The effect of a controlled manipulation of maternal dietary fat intake on medium and long chain fatty acids in human breast milk in Saskatoon, Canada. Int Breastfeed J 5:3
15. Ribeiro P, Carvalho FD, Abreu Ade A, Sant'anna Mde T, Lima RJ, Carvalho Pde O (2012) Effect of fish oil supplementation in pregnancy on the fatty acid composition of erythrocyte phospholipids and breast milk lipids. Int J Food Sci Nutr 63:36–40
16. Larque E, Demmelmair H, Gil-Sanchez A et al (2011) Placental transfer of fatty acids and fetal implications. Am J Clin Nutr 94:1908S–1913S
17. Anderson GH, Atkinson SA, Bryan MH (1981) Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. Am J Clin Nutr 34:258–265
18. Genzel-Boroviczeny O, Wahle J, Koletzko B (1997) Fatty acid composition of human milk during the 1st month after term and preterm delivery. Eur J Pediatr 156:142–147
19. Bitman J, Wood L, Hamosh M, Hamosh P, Mehta NR (1983) Comparison of the lipid composition of breast milk from mothers of term and preterm infants. Am J Clin Nutr 38:300–312

20. Bokor S, Koletzko B, Decsi T (2007) Systematic review of fatty acid composition of human milk from mothers of preterm compared to full-term infants. Ann Nutr Metab 51:550–556
21. Kovacs A, Funke S, Marosvolgyi T, Burus I, Decsi T (2005) Fatty acids in early human milk after preterm and full-term delivery. J Pediatr Gastroenterol Nutr 41:454–459
22. Fewtrell MS, Abbott RA, Kennedy K et al (2004) Randomized, double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in preterm infants. J Pediatr 144:471–479
23. Clandinin MT, Van Aerde JE, Merkel KL et al (2005) Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. J Pediatr 146:461–468
24. Hamosh M, Bitman J, Fink CS et al (1985) Lipid composition of preterm human milk and its digestion by the infant. Elsevier Science Publishers BV (Biomedical Division)
25. Hernell O, Blackberg L (1984) Human milk bile salt-stimulated lipase: functional and molecular aspects. J Pediatr 125:S56–S61
26. Fredrikzon B, Hernell O, Blackberg L, Olivecrona T (1978) Bile salt-stimulated lipase in human milk: evidence of activity in vivo and of a role in the digestion of milk retinol esters. Pediatr Res 12:1048–1052
27. Filer LJ, Jr., Mattson FH, Fomon SJ (1969) Triglyceride configuration and fat absorption by the human infant. J Nutr 99:293–298
28. Innis SM, Dyer R, Nelson CM (1994) Evidence that palmitic acid is absorbed as sn-2 monoacylglycerol from human milk by breast-fed infants. Lipids 29:541–545
29. Straarup EM, Lauritzen L, Faerk J, Hoy Deceased CE, Michaelsen KF (2006) The stereospecific triacylglycerol structures and fatty acid profiles of human milk and infant formulas. J Pediatr Gastroenterol Nutr 42:293–299
30. Jensen C, Buist NR, Wilson T (1986) Absorption of individual fatty acids from long chain or medium chain triglycerides in very small infants. Am J Clin Nutr 43:745–751
31. Andersson Y, Savman K, Blackberg L, Hernell O (2007) Pasteurization of mother's own milk reduces fat absorption and growth in preterm infants. Acta Paediatr 96:1445–1449
32. Williamson S, Finucane E, Ellis H, Gamsu HR (1978) Effect of heat treatment of human milk on absorption of nitrogen, fat, sodium, calcium, and phosphorus by preterm infants. Arch Dis Child 53:555–563
33. Hamosh M, Bitman J, Liao TH et al (1989) Gastric lipolysis and fat absorption in preterm infants: effect of medium-chain triglyceride or long-chain triglyceride-containing formulas. Pediatrics 83:86–92
34. Odle J (1997) New insights into the utilization of medium-chain triglycerides by the neonate: observations from a piglet model. J Nutr 127:1061–1067
35. Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL, Hartmann PE (2002) Variation in fat, lactose and protein in human milk over 24 h and throughout the first year of lactation. Br J Nutr 88:29–37
36. Hambraeus L (1984) Human milk composition. Nutr Abstr Rev 54:219–236
37. Neville MC (1999) Physiology of lactation. Clin Perinatol 26:251–279, v
38. Gross SJ, Geller J, Tomarelli RM (1981) Composition of breast milk from mothers of preterm infants. Pediatrics 68:490–493
39. Saarela T, Kokkonen J, Koivisto M (2005) Macronutrient and energy contents of human milk fractions during the first six months of lactation. Acta Paediatr 94:1176–1181
40. Atkinson SA (1995) Effects of gestational stage at delivery on human milk components. In: Jensen RG (ed) Handbook of milk composition. Academic Press, San Diego, pp 222–237
41. Czank C, Mitoulas LR, Hartmann PE (2007) Human milk composition—carbohydrates. In: Hale TW, Hartmann PE (eds) Textbook of human lactation. Hale Publishing, Amarillo, pp 69–73
42. Shulman RJ, Wong WW, Smith EO (2005) Influence of changes in lactase activity and small-intestinal mucosal growth on lactose digestion and absorption in preterm infants. Am J Clin Nutr 81:472–479

43. Bode L (2012) Human milk oligosaccharides: every baby needs a sugar mama. GlycoBiology 22:1147–1162
44. German JB, Freeman SL, Lebrilla CB, Mills DA (2008) Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. Nestle Nutr Workshop Ser Pediatr Program 62:205–218; discussion 218–222
45. Stahl B, Thurl S, Zeng J et al (1994) Oligosaccharides from human milk as revealed by matrix-assisted laser desorption/ionization mass spectrometry. Anal Biochem 223:218–226
46. Thurl S, Henker J, Siegel M, Tovar K, Sawatzki G (1997) Detection of four human milk groups with respect to Lewis blood group dependent oligosaccharides. Glycoconj J 14:795–799
47. Oriol R, Le Pendu J, Mollicone R (1986) Genetics of ABO, H, Lewis, X and related antigens. Vox Sang 51:161–171
48. Westerbeek EA, Hensgens RL, Mihatsch WA, Boehm G, Lafeber HN, van Elburg RM (2011) The effect of neutral and acidic oligosaccharides on stool viscosity, stool frequency and stool pH in preterm infants. Acta Paediatr 100:1426–1431
49. Butte NF, Lopez-Alarcon MG, Garza C (2002) Nutrient adequacy of exclusive breastfeeding for the term infant during the first 6 months of life. World Health Organization, Geneva
50. Lönnerdal B, Atkinson SA, Robert GJ (1995) Human milk proteins. In: Jensen RG (ed) Handbook of milk composition. Academic Press, San Diego, pp 351–368
51. Khan S, Hepworth AR, Prime DK, Lai CT, Trengove NJ, Hartmann PE (2012) Variation in fat, lactose, and protein composition in breast milk over 24 h: associations with infant feeding patterns. J Hum Lact 29:81–89
52. Kunz C, Lönnerdal B (1992) Re-evaluation of the whey protein/casein ratio of human milk. Acta Pædiatrica 81:107–112
53. Hartmann PE (1991) The breast and breast feeding. In: Philipp E, Setchell M, Ginsburg JG (eds) Scientific foundations of obstetrics and gynaecology, 4th edn. Heinemann, London, pp 378–390
54. Davies DT, Holt C, Christie WW (1983) The composition of milk. In: Mepham TB (ed) Biochemistry of lactation. Elsevier, Amsterdam, pp 71–117
55. Newton ER (2004) Breastmilk: the gold standard. Clin Obstet Gynecol 47:632–642
56. Bounous G, Kongshavn PA, Taveroff A, Gold P (1988) Evolutionary traits in human milk proteins. Med Hypotheses 27:133–140
57. Froehlich JW, Dodds ED, Barboza M et al (2010) Glycoprotein expression in human milk during lactation. J Agric Food Chem 58:6440–6448
58. Molinari CE, Casadio YS, Hartmann BT et al (2012) Proteome mapping of human skim milk proteins in term and preterm milk. J Proteome Res 11:1696–1714
59. Lien EL, Davis AM, Euler AR (2004) Growth and safety in term infants fed reduced-protein formula with added bovine alpha-lactalbumin. J Pediatr Gastroenterol Nutr 38:170–176
60. Neville MC, Allen JC, Watters C (1983) The mechanisms of milk secretion. Plenum Press, New York
61. Picciano MF (2001) Nutrient composition of human milk. Pediatr Clin North Am 48:53–67
62. Agarwal S, Karmaus W, Davis S, Gangur V (2011) Immune markers in breast milk and fetal and maternal body fluids: a systematic review of perinatal concentrations. J Hum Lact 27:171–186
63. Saito S (2001) Cytokine cross-talk between mother and the embryo/placenta. J Reprod Immunol 52:15–33
64. Calhoun DA, Lunoe M, Du Y, Staba SL, Christensen RD (1999) Concentrations of granulocyte colony-stimulating factor in human milk after in vitro simulations of digestion. Pediatr Res 46:767–771
65. Groër MW, Shelton MM (2009) Exercise is associated with elevated proinflammatory cytokines in human milk. J Obstet Gynecol Neonatal Nurs 38:35–41
66. Butte NF, Garza C, Johnson CA, Smith EO, Nichols BL (1984) Longitudinal changes in milk composition of mothers delivering preterm and term infants. Early Hum Dev 9:153–162
67. Lemons JA, Moye L, Hall D, Simmons M (1982) Differences in the composition of preterm and term human milk during early lactation. Pediatr Res 16:113–117

68. Ronayne de Ferrer PA, Baroni A, Sambucetti ME, Lopez NE, Ceriani C JM (2000) Lactoferrin levels in term and preterm milk. J Am Coll Nutr 19:370–373
69. Dvorak B, Fituch CC, Williams CS, Hurst NM, Schanler RJ (2003) Increased epidermal growth factor levels in human milk of mothers with extremely premature infants. Pediatr Res 54:15–19
70. Montagne P, Cuilliere ML, Mole C, Bene MC, Faure G (1999) Immunological and nutritional composition of human milk in relation to prematurity and mother's parity during the first 2 weeks of lactation. J Pediatr Gastroenterol Nutr 29:75–80
71. Bielicki J, Huch R, von Mandach U (2004) Time-course of leptin levels in term and preterm human milk. Eur J Endocrinol 151:271–276
72. Velona T, Abbiati L, Beretta B et al (1999) Protein profiles in breast milk from mothers delivering term and preterm babies. Pediatr Res 45:658–663
73. Rutherfurd KJ, Gill HS (2000) Peptides affecting coagulation. Br J Nutr 84(Suppl 1): S99–S102
74. Lönnerdal B (2003) Nutritional and physiologic significance of human milk proteins. Am J Clin Nutr 77:1537S–1543S
75. Allen LH (2005) Multiple micronutrients in pregnancy and lactation: an overview. Am J Clin Nutr 81:1206S–1212S
76. Kent JC, Arthur PG, Mitoulas LR, Hartmann PE (2009) Why calcium in breastmilk is independent of maternal dietary calcium and vitamin D. Breastfeed Rev 17:5–11
77. Sparks JW (1984) Human intrauterine growth and nutrient accretion. Semin Perinatol 8:74–93
78. Takada M, Shimada M, Hosono S et al (1992) Trace elements and mineral requirements for very low birth weight infants in rickets of prematurity. Early Hum Dev 29:333–338
79. Friel JK, Andrews WL, Jackson SE et al (1999) Elemental composition of human milk from mothers of premature and full-term infants during the first 3 months of lactation. BiolResearch 67:225–247
80. Schanler RJ, Abrams SA (1995) Postnatal attainment of intrauterine macromineral accretion rates in low birth weight infants fed fortified human milk. J Pediatr 126:441–447
81. Chan GM, Mileur L, Hansen JW (1988) Calcium and phosphorus requirements in bone mineralization of preterm infants. J Pediatr 113:225–229
82. Demarini S (2005) Calcium and phosphorus nutrition in preterm infants. Acta Paediatr Suppl 94:87–92
83. Loui A, Raab A, Obladen M, Bratter P (2002) Calcium, phosphorus and magnesium balance: FM 85 fortification of human milk does not meet mineral needs of extremely low birthweight infants. Eur J Clin Nutr 56:228–235
84. Wagner CL, Hulsey TC, Fanning D, Ebeling M, Hollis BW (2006) High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study. Breastfeed Med 1:59–70
85. Fraser DR (1995) Vitamin D. Lancet 345:104–107
86. Pugliese MT, Blumberg DL, Hludzinski J, Kay S (1998) Nutritional rickets in suburbia. J Am Coll Nutr 17:637–641
87. Fransson G-B, Lönnerdal B (1982) Zinc, copper, calcium, and magnesium in human milk. J Pediatr 101:504–508
88. Kim SY, Park JH, Kim EA, Lee-Kim YC (2012) Longitudinal study on trace mineral compositions (selenium, zinc, copper, manganese) in Korean human preterm milk. J Korean Med Sci 27:532–536
89. Howie PW, Forsyth JS, Ogston SA, Clark A, Florey CdV (1990) Protective effect of breast feeding against infection. Br Med J 300:11–16
90. Schroten H (1998) The benefits of human milk fat globule against infection. Nutrition 14: 52–53
91. Schroten H, Hanisch FG, Plogmann R et al (1992) Inhibition of adhesion of S-fimbriated Escherichia coli to buccal epithelial cells by human milk fat globule membrane components: a novel aspect of the protective function of mucins in the nonimmunoglobulin fraction. Infect Immun 60:2893–2899

92. Yolken RH, Peterson JA, Vonderfecht SL, Fouts ET, Midthun K, Newburg DS (1992) Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis. J Clin Invest 90:1984–1991
93. Peterson JA, Patton S, Hamosh M (1998) Glycoproteins of the human milk fat globule in the protection of the breast-fed infant against infections. Biol Neonate 74:143–162
94. Stefferl A, Schubart A, Storch M et al (2000) Butyrophilin, a milk protein, modulates the encephalitogenic T cell response to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis. J Immunol 165:2859–2865
95. Yamashiro H, Yoshizaki S, Tadaki T, Egawa K, Seo N (2010) Stimulation of human butyrophilin 3 molecules results in negative regulation of cellular immunity. J Leukoc Biol 88:757–767
96. Stevens CR, Millar TM, Clinch JG, Kanczler JM, Bodamyali T, Blake DR (2000) Antibacterial properties of xanthine oxidase in human milk. Lancet 356:829–830
97. Otnæss AB, Laegreid A, Ertresvag K (1983) Inhibition of enterotoxin from Escherichia coli and Vibrio cholerae by gangliosides from human milk. Infect Immun 40:563–569
98. Batovska DI, Todorova IT, Tsvetkova IV, Najdenski HM (2009) Antibacterial study of the medium chain fatty acids and their 1-monoglycerides: individual effects and synergistic relationships. Pol J Microbiol 58:43–47
99. Hamosh M, Peterson JA, Henderson TR et al (1999) Protective function of human milk: the milk fat globule. Semin Perinatol 23:242–249
100. Irons LI, Perkins DJ (1962) Studies on the interaction of magnesium, calcium and strontium ions with native and chemically modified human serum albumin. Biochem J 84:152
101. Isaacs CE (2005) Human milk inactivates pathogens individually, additively, and synergistically. J Nutr 135:1286–1288
102. Newburg DS, Ruiz-Palacios GM, Morrow AL (2005) Human milk glycans protect infants against enteric pathogens. Annu Rev Nutr 25:37–58
103. Bode L (2012) Human milk oligosaccharides: every baby needs a Sugar Mama. Glycobiology 22:1147–1162
104. Zivkovic AM, German JB, Lebrilla CB, Mills DA (2011) Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci USA 108(Suppl 1):4653–4658
105. Ruiz-Palacios GM, Cervantes LE, Ramos P, Chavez-Munguia B, Newburg DS (2003) Campylobacter jejuni binds intestinal H(O) antigen (Fuc alpha 1, 2Gal beta 1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. J Biol Chem 278:14112–14120
106. Jiang X, Huang P, Zhong W et al (2004) Human milk contains elements that block binding of noroviruses to human histo-blood group antigens in saliva. J Infect Dis 190:1850–1859
107. Morrow AL, Ruiz-Palacios GM, Altaye M et al (2004) Human milk oligosaccharides are associated with protection against diarrhea in breast-fed infants. J Pediatr 145:297–303
108. Jantscher-Krenn E, Zherebtsov M, Nissan C et al (2012) The human milk oligosaccharide disialyllacto-N-tetraose prevents necrotising enterocolitis in neonatal rats. Gut 61:1417–1425
109. Neu J (2005) Neonatal necrotizing enterocolitis: an update. Acta Paediatr Suppl 94:100–105
110. Ohashi A, Murata E, Yamamoto K et al (2003) New functions of lactoferrin and beta-casein in mammalian milk as cysteine protease inhibitors. Biochem Biophys Res Commun 306:98–103
111. Liepke C, Zucht HD, Forssmann WG, Standker L (2001) Purification of novel peptide antibiotics from human milk. J Chromatogr B Biomed Sci Appl 752:369–377
112. Pellegrini A, Thomas U, Bramaz N, Hunziker P, von Fellenberg R (1999) Isolation and identification of three bactericidal domains in the bovine alpha-lactalbumin molecule. Biochim Biophys Acta 1426:439–448
113. Hakansson A, Zhivotovsky B, Orrenius S, Sabharwal H, Svanborg C (1995) Apoptosis induced by a human milk protein. Proc Natl Acad Sci USA 92:8064–8068
114. Lönnerdal B (1985) Biochemistry and physiological function of human milk proteins. Am J Clin Nutr 42:1299–1317

115. Goldman AS, Goldblum RM (1995) Defense agents in milk A. Defense agents in human milk. In: Jensen RG (ed) Handbook of milk composition. Academic Press, San Diego, pp 727–745
116. Goldman AS, Garza C, Nichols BL, Goldblum RM (1982) Immunologic factors in human milk during the first year of lactation. J Pediatr 100:563–567
117. Drago-Serrano ME, Rivera-Aguilar V, Resendiz-Albor AA, Campos-Rodriguez R (2010) Lactoferrin increases both resistance to Salmonella typhimurium infection and the production of antibodies in mice. Immunol Lett 134:35–46
118. Mosquito S, Ochoa TJ, Cok J, Cleary TG (2010) Effect of bovine lactoferrin in Salmonella ser. Biometals 23:515–521
119. Yen CC, Shen CJ, Hsu WH et al (2011) Lactoferrin: an iron-binding antimicrobial protein against Escherichia coli infection. Biometals 24:585–594
120. Fransson G-B, Lönnerdal B (1980) Iron in human milk. J Pediatr 96:380–384
121. Appelmelk BJ, An YQ, Geerts M et al (1994) Lactoferrin is a lipid A-binding protein. Infect Immun 62:2628–2632
122. Ochoa TJ, Cleary TG (2009) Effect of lactoferrin on enteric pathogens. Biochimie 91:30–34
123. Kanyshkova TG, Babina SE, Semenov DV et al (2003) Multiple enzymic activities of human milk lactoferrin. Eur J Biochem 270:3353–3361
124. Bennett RM, Merritt MM, Gabor G (1986) Lactoferrin binds to neutrophilic membrane DNA. Br J Haematol 63:105–117
125. Gutteridge JM, Paterson SK, Segal AW, Halliwell B (1981) Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. Biochem J 199:259–261
126. Zimecki M, Mazurier J, Spik G, Kapp JA (1995) Human lactoferrin induces phenotypic and functional changes in murine splenic B cells. Immunology 86:122–127
127. Fleet JC (1995) A new role for lactoferrin: DNA binding and transcription activation. Nutr Rev 53:226–227
128. He J, Furmanski P (1995) Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA. Nature 373:721–724
129. Bezault J, Bhimani R, Wiprovnick J, Furmanski P (1994) Human lactoferrin inhibits growth of solid tumors and development of experimental metastases in mice. Cancer Res 54:2310–2312
130. Shau H, Kim A, Golub SH (1992) Modulation of natural killer and lymphokine-activated killer cell cytotoxicity by lactoferrin. J Leukoc Biol 51:343–349
131. Hunter HN, Demcoe AR, Jenssen H, Gutteberg TJ, Vogel HJ (2005) Human lactoferricin is partially folded in aqueous solution and is better stabilized in a membrane mimetic solvent. Antimicrob Agents Chemother 49:3387–3395
132. Chandan RC, Parry RM, Shahani KM (1968) Lysozyme, lipase, and ribonuclease in milk of various species. J Dairy Sci 51:606–607
133. Lönnerdal B, Atkinson SA (1995) Nitrogenous components of milk. A. Human milk proteins. In: Jensen RG (ed) Handbook of milk composition. Academic Press, San Diego, pp 351–368
134. Ellison RT 3rd, Giehl TJ (1991) Killing of gram-negative bacteria by lactoferrin and lysozyme. J Clin Invest 88:1080–1091
135. Lee-Huang S, Huang PL, Sun Y, Kung HF, Blithe DL, Chen HC (1999) Lysozyme and RNases as anti-HIV components in beta-core preparations of human chorionic gonadotropin. Proc Natl Acad Sci USA 96:2678–2681
136. Léon-Sicairos N, Lopez-Soto F, Reyes-Lopez M, Godinez-Vargas D, Ordaz-Pichardo C, de la Garza M (2006) Amoebicidal activity of milk, apo-lactoferrin, sIgA and lysozyme. Clin Med Res 4:106–113
137. Hassiotou F, Metzger P, Trengove NJ, Lai CT, Hartmann PE, Filgueira L (2011) Immune cells in breastmillk relatte to maternal and infant infections. Combined Biological Sciences Meeting, Perth, Australia
138. Ogawa J, Sasahara A, Yoshida T et al (2004) Role of transforming growth factor-beta in breast milk for initiation of IgA production in newborn infants. Early Hum Dev 77:67–75
139. Bettelli E, Carrier Y, Gao W et al (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 441:235–238

140. Nakae S, Iwakura Y, Suto H, Galli SJ (2007) Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. J Leukoc Biol 81:1258–1268
141. Kalliomaki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E (1999) Transforming growth factor-beta in breast milk: a potential regulator of atopic disease at an early age. J Allergy Clinical Immunol 104:1251–1257
142. Dinarello CA (1996) Biologic basis for interleukin-1 in disease. Blood 87:2095–2147
143. Hawkes JS, Bryan DL, James MJ, Gibson RA (1999) Cytokines (IL-1beta, IL-6, TNF-alpha, TGF-beta1, and TGF-beta2) and prostaglandin E2 in human milk during the first three months postpartum. Pediatr Res 46:194–199
144. Xing Z, Gauldie J, Cox G et al (1998) IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest 101:311–320
145. Brandtzaeg P (2003) Mucosal immunity: integration between mother and the breast-fed infant. Vaccine 21:3382–3388
146. Lönnerdal B, Lien EL (2003) Nutritional and physiologic significance of alpha-lactalbumin in infants. Nutr Rev 61:295–305
147. Manzoni P, Rinaldi M, Cattani S et al (2009) Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. JAMA 302:1421–1428
148. Manzoni P, Stolfi I, Messner H et al (2012) Bovine lactoferrin prevents invasive fungal infections in very low birth weight infants: a randomized controlled trial. Pediatrics 129:116–123
149. Koenig A, de A Diniz EM, Barbosa SF, Vaz FA (2005) Immunologic factors in human milk: the effects of gestational age and pasteurization. J Hum Lact 21:439–443
150. Hassiotou F, Hepworth AR, Metzger P, Lai CT, Trengove N, Hartmann PE, Filgueira L (2013) Maternal and infant infections stimulate a rapid leukocyte response in breastmilk. Clin Transnatl Immunol 2:e3
151. Hassiotou F, Filgueira L, Hepworth AR, Trengove NJ, Lai CT, Hartmann PE (2012) Coordinated response of the fat and cellular content of breastmilk to the degree of fullness of the breast. Experimental biology. San Diego pp 108
152. de Mello TRB (2000) Characterization of mammary epithelial cells in human milk. In: Biochemistry. The University of Western Australia, Australia
153. Hassiotou F, Geddes D (2012) Anatomy of the human mammary gland: current status of knowledge. Clin, Anat 26:29–48
154. Kotsopoulos J, Lubinski J, Salmena L et al (2012) Breastfeeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res 14:R42
155. Hassiotou F, Beltran A, Chetwynd E et al (2012) Breastmilk is a novel source of stem cells with multilineage differentiation potential. Stem Cells 30:2164–2174
156. Cregan MD, Fan Y, Appelbee A et al (2007) Identification of nestin-positive putative mammary stem cells in human breastmilk. Cell Tissue Res 329:129–136
157. Thomas E, Zeps N, Cregan M, Hartmann P, Martin T (2011) 14-3-3sigma (sigma) regulates proliferation and differentiation of multipotent p63-positive cells isolated from human breastmilk. Cell Cycle 10:278–284
158. Kosaka N, Izumi H, Sekine K, Ochiya T (2010) MicroRNA as a new immune-regulatory agent in breast milk. Silence 1:7
159. Jain L, Vidyasagar D, Xanthou M, Ghai V, Shimada S, Blend M (1989) In vivo distribution of human milk leucocytes after ingestion by newborn baboons. Arch Dis Child 64:930–933
160. Schnorr KL, Pearson LD (1984) Intestinal absorption of maternal leucocytes by newborn lambs. J Reprod Immunol 6:329–337
161. Dewey KG (2001) Nutrition, growth, and complementary feeding of the breastfed infant. Pediatr Clin North Am 48:87–104
162. Schanler RJ (2001) The use of human milk for premature infants. Pediatr Clin North Am 48:207–219
163. Schanler RJ (1995) Suitability of human milk for the low-birthweight infant. Clin Perinatol 22:207–222

Chapter 17
Breastfeeding the Preterm Infant

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Abstract Based on the evidence for its short and long term benefits, full breastfeeding is the optimal aim for the preterm infant-mother dyad. The preterm infant's transition to full oral feeding is frequently complicated by neurological and developmental immaturity as well as accompanying co-morbidities. Currently there is limited evidence available to guide feeding strategies for these infants, and few validated diagnostic tools available to assist health professionals in assessing feeding progress for these infants. The current knowledge of breastfeeding the preterm infant is summarised in this chapter. We look forward to more research elucidating the most effective means of achieving and sustaining full breastfeeding in this population.

Keywords Breast/breastfeeding · Breast milk · Lactation · Preterm · Premature · Infant · Feeding · Suck/ing · Swallow/ing · Breathing · Respiration · Breast expression

Key points

- Early and frequent expression of milk improves milk production.
- Preterm infants are at high risk of feeding difficulties.
- Frequent feeding at the breast improves breastfeeding outcomes.
- Infant feeding efficiency and effectiveness improves with increased ability to create a vacuum.
- Breastfeeding support is often required post discharge to achieve full breastfeeding.

1 Introduction

Breastfeeding is a multi-dimensional relationship between the mother and infant with a wide range of factors contributing to its establishment and continuation [1]. These include nutritional, biological, psychological, demographic, cultural and social components (Table 17.1). For breastfeeding to be biologically successful it is essential

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_17,

Table 17.1 Factors implicated in breastfeeding duration. (Adapted from Thulier and Mercer [172])

Variable	Association with breastfeeding
Demographic	
Race	Asian, White, Hispanic and Black women ranked in order of success (US)
Age	Increased age is positively associated with breastfeeding duration
Martial status	Married women have higher rates and duration of breastfeeding
Level of education	Higher levels of education associated with higher rates and duration of breastfeeding
Socioeconomic status	Women of lower socioeconomic status are less likely to feed and for shorter periods
Biological	
Insufficient milk supply	Real and perceived insufficient milk supply is strongly associated with early weaning
Infant health	Mothers of sick or preterm infants are at high risk of early weaning
Maternal obesity	Obese women are more likely to terminate breastfeeding earlier than women within the normal weight range
Physical challenges	Pain and disruption of feeding due to sore nipples, engorgement, blocked ducts are common reason for weaning within the first 6 weeks postpartum
Maternal smoking	Maternal smoking is negatively associated with duration of breastfeeding
Parity	Generally multiparity and previous breastfeeding experience (more than 4 months) is associated with longer breastfeeding durations
Mode of delivery	Some studies show a negative association between caesarean section delivery and breastfeeding duration and others show no effect
Social	
Maternal work	Generally paid work has been associated with lower breastfeeding duration however the hours of work may have an impact
Support from significant others	Studies are limited to the father's support and lactation education, which has been shown to have a positive effect on duration of breastfeeding There are indications that high stress marital relationships and responsibility for most home/family tasks have a negative impact on duration of breastfeeding Peer support tends to be more effective than professional support. Inconsistent advice/support is implicated in early weaning
Professional support	Prenatal educational support may or may not improve breastfeeding rates Appropriate professional support postpartum has a positive influence on breastfeeding duration
Psychological	
Prenatal maternal intention	Intention to breastfeed is related to longer durations of breastfeeding
Maternal value of breastfeeding and confidence	Both maternal interest and confidence in feeding are associated with longer breastfeeding duration

that the infant removes adequate amounts of breast milk to ensure optimal infant growth and continuing stimulation of milk synthesis [2, 3]. Furthermore, safe and efficient feeding requires adequate coordination of sucking, swallowing and breathing [4–6]. Many preterm infants encounter feeding difficulties, due to neurological and developmental immaturity which may be further compounded by co-morbidities. Generally, 'late preterm' infants born at 35–37 weeks' gestation experience a relatively short period of feeding difficulties that may be impacted by conditions such as respiratory distress syndrome (RDS) before transitioning to full breastfeeding prior to discharge. The feeding trajectory of very preterm infants (born < 33 weeks) is more likely to be impacted by immaturity and co-morbidities such as chronic lung disease, intracranial haemorrhage and anaemia. For all preterm infants, feeding support should be focused toward breastfeeding as the best possible outcome for the mother and infant.

2 Milk Production

2.1 Initiation

The initiation of lactation (secretory activation) is triggered by the withdrawal of progesterone after the delivery of the placenta and therefore observed 30–40 h after birth [7]. Other hormones necessary for secretory activation include prolactin, insulin and cortisol [8] and are permissive rather than a trigger for initiation. Secretory activation is tightly coupled to the birth to ensure adequate provision of nutrition and perhaps more importantly for infant protection. Colostrum is produced prior to the closure of paracellular pathways allowing the transfer of high molecular weight molecules. This results in a fluid rich in anti-inflammatory and anti-infective components similar to those in amniotic fluid and appears to impact the digestive system enormously stimulating rapid growth of the intestinal mucosa and inducing the secretion of digestive enzymes [9]. Colostrum contains high concentrations of lactoferrin, sIgA, oligosaccharides, antioxidants and growth factors. Women who deliver preterm produce colostrum with the highest concentration of immunoprotective components for longer periods than mothers of term infants [9].

Mothers of preterm infants that exclusively express their milk may experience a delay in initiation and tend to produce less milk at secretory activation than breastfeeding mothers of term infants [10, 11]. The reason for delay and low milk production in preterm mothers is unclear however factors such as maternal Type 1 diabetes and obesity, maternal medications, mode of delivery, anaesthetic agents, maternal-infant separation, maternal stress and frequency of pumping have been implicated [12].

Mean milk output (37–169 mL/day) for a pump dependent preterm mother in the first 2 days is similar to volumes taken by the term infant [13]. However at day 5 milk productions of women that delivered at 31–35 weeks' gestation ranged from 20–550 mL/day [14] compared to 240–560 mL/day produced by the full term

breastfeeding mother at the same time point [15]. By the 2nd week of lactation a preterm mother pumps 350–500 mL/day [16–19] whereas term infants receive 500–1,000 mL/day [20]. Pumping studies show that milk production in the preterm mother tends to be maintained around 340–640 mL/day [16, 21] rather than increase over time.

2.2 Control of Milk Production

For the breastfeeding term infant breast milk production generally matches infant demand during established lactation [22]. The amount of milk stored within the breast determines the rate of milk synthesis i.e., a full breast has a lower rate of synthesis compared to a breast drained of milk [23]. It is assumed that the rate of milk synthesis is controlled by the accumulation of a protein called the feedback inhibitor of lactation (FIL) [24]. The rate of synthesis fluctuates in term breastfed infants due to variable feed volumes. Term mothers have been shown to increase milk production by expressing the remaining milk after breastfeeds [25]. Thus complete drainage of the breast is essential for the pump dependent preterm mother in order to increase milk synthesis and production. Other factors that influence milk production and recommended management are shown in Table 17.2.

3 Milk Ejection

Breast milk is synthesized and stored mainly within the alveoli of the breast. The milk ejection reflex makes milk available to be removed by either the infant or expressing. This reflex is critical as little milk is removed from the breast in its absence [26]. Stimulation of the nipple conveys nervous impulses to the hypothalamus which stimulate the posterior pituitary gland to release oxytocin into the bloodstream. Oxytocin binds to receptors on the myoepithelial cells surrounding the alveoli causing them to contract thereby forcing milk into the milk ducts. The milk ducts expand and intra-ductal pressure increases further facilitating removal of milk by the infant or pump [26, 27]. The milk ejection reflex can be conditioned and often mothers eject in response to the sight or cries of their infant [28]. Stress has a detrimental effect by limiting the amount of oxytocin released and thereby reducing the amount of milk delivered to the infant [29] or removed by the pump [30]. The preterm mother is subject to considerable amounts of stress and therefore efforts should be made to reduce its levels. Pumping at the infant's bedside has been shown to be beneficial for milk production although some mothers prefer privacy. Whichever is more comfortable for the mother is the most conducive to milk ejection. Relaxation techniques such as massage and listening to music have also been shown to increase milk output [30, 31].

Table 17.2 Factors known to affect milk production and the recommended management

Factors affecting milk production	Recommended management
Retained placental fragments	Surgical removal of fragments to decrease maternal blood progesterone levels
Delayed initiation of lactation	Early removal of colostrum and subsequent frequent pumping Use of a variable pumping pattern Massage prior to pumping
Low prolactin levels	Prescribed galactogogues such as domperidone
Interruption of mammary development	Increase frequency of pumping with/without galactogogue
Ineffective breast emptying: Engorgement Blocked ducts Mastitis	Instruction in effective breast pumping—duration of pumping, appropriate vacuum levels, simultaneous pumping and breast massage both prior to during pumping
Infrequent pumping (< 5 expressions/day)	Increase number of expressions per day, simultaneous pumping
Maternal infant separation	Increase frequency and duration of skin-to-skin contact
Poor positioning and attachment	Ensure breast is relatively full to improve milk transfer Experimentation with techniques to provide support for the infant's weak neck and facial muscles Deformation of the breast to assist the baby to latch Breast massage to encourage milk flow whilst feeding
Dysfunctional suck (incl. inability to create adequate vacuum and poor coordination)	Use of nipple shield Feeding from a less full breast
Maternal breast anomalies: Primary hypoplasia Post radiation Scarring from breast surgery, trauma, nipple piercing Post severe abscess	More frequent and effective pumping Consideration of galactogogues
Stress and fatigue	Pumping at the bedside or in a relaxed place
Drugs: Alcohol, opiates Pseudoephedrine	Avoid known medications that affect milk production Timing and dosage of drugs in relation to breastfeeding/expression may limit effects on the infant

4 Expression of Breast Milk

The relationship between the health and cost benefits of human milk is dose dependent in that the greatest benefits are associated with higher doses [9, 32]. Many preterm infants cannot breastfeed in the weeks following birth and their mothers are dependent on a breast pump to initiate and establish lactation. Unfortunately many women struggle to produce a full milk supply [9, 11, 21]. Early and frequent breast milk

expression is critical to the initiation of lactation after preterm birth with frequency of expressions positively correlated with milk volume [33]. Mothers expressing fewer than 6 times/day produce lower daily milk volumes than women expressing more frequently [18]. This effect is consistent with the short-term control of milk synthesis in that extended intervals between expressions slow the rate of milk synthesis. Despite current recommendations to pump 8–10 times/day most women pump 6 times/day on average [34].

Simultaneous (double) pumping has been shown to extract greater volumes of milk compared to sequential (single) pumping in mothers of preterm [35, 36] and term infants [37] with the advantage of halving pumping time. Pumping at the infant's bedside or in a relaxed environment may also be conducive to better pumping performance [38] by decreasing maternal stress thereby facilitating milk ejection.

Other factors have been shown to promote milk production in mothers of preterm infants. Skin-to-skin contact not only stimulates milk production but also extends the breastfeeding duration [34, 39–41]. Non-nutritive sucking at the breast is thought to stimulate the maternal release of prolactin and oxytocin and have a positive effect on milk production [42]. More recently two additional interventions have been reported to have a positive effect on milk expression. One study compared the effects of a standard 2-phase pumping pattern on a hospital grade electric breast pump with a pumping pattern similar to that of the breastfeeding infant in the first 3 postnatal days on milk production in mothers of preterm infants. Mothers who used the 'newborn' pumping pattern produced significantly more milk from day 6–14 than mothers using the standard pumping pattern [17]. The inclusion of breast massage/expression during pumping is also effective in increasing milk volume [43, 44] and caloric content [45].

5 Physiology of Oral Feeding

Due to neurological and motor immaturity preterm infants are at risk for feeding difficulties particularly those with underlying complications such as gastro-oesophageal reflux, chronic respiratory disease [46] and neurological impairment [47]. Attainment of full oral feeding is often the sole unmet criteria that delays discharge [48]. A fundamental knowledge of the physiology of feeding is therefore essential to develop interventions that support the preterm infant as well as minimise long term behavioural eating disorders [49, 50].

6 Intragastric Tube Feeding

Preterm infants are often fed via a nasogastric or orogastric feeding tube as they transition to full oral feeds. Supplementation of breastfeeds via a tube is associated with higher rates of breastfeeding [51] compared to bottle-feeding at discharge. However the presence of the feeding tube also impacts bottle-feeds resulting in lower minute

ventilation, tidal volume, heart rate and oxygen saturation accompanied by more prolonged desaturations [52, 53] compared to feeding without the tube in situ [53]. Pacifiers are often used during tube feeding although evidence of positive effects is conflicting. Positive influences such as increased intestinal transit time, rapid weight gain [54] and calmer behavioural state [55] have been demonstrated. A Cochrane review did not find a consistent benefit of non-nutritive sucking although a reduction in transition time from tube to bottle-feeds and improved bottle-feeding performance was noted. Rapid progression to full feeds is highly desirable as prolonged tube feeding is associated with poor sucking ability and increased risk of feeding problems [56–58]. Oro-gastric tubes have also been shown to cause deep palatal grooves, high arched palates, lip or gum clefts, laryngeal trauma and subglottic stenosis that can impede successful oral feeding and increase later dental complications [58]. This transition process may be further complicated and protracted due to serious illness or the necessity to deliver frequent feeds [59].

7 Readiness for Oral Feeding

Many NICUs use corrected gestational age [46] or infant weight as criteria for initiating oral feeding with infant behaviour as an additional indicator of readiness [60]. Others have employed a developmental assessment approach where recognition of the behavioural organization of the infant is considered to be reflective of central nervous system maturation [61]. This approach that utilises the assessment of infant alertness and the ability to maintain this state as a cue to begin oral feeding, has been shown to decrease the duration of parenteral nutrition and transition to oral feeding [62]. Feeding readiness behaviours such as mouthing, hand sucking, tongue sucking, hand to mouth and hand swipes are also predictive of feeding efficiency [63]. More recently others have taken similar approaches to encourage earlier oral feeding [64]. Thoyre et al. [65] investigated the use of a co-regulated approach, involving acoustic enhancement of infant breathing and swallowing during feeding allowing identification of signature feeding patterns thereby promoting individualised feeding support. They found that the person feeding the infant was more responsive to the infant leading to improved infant behavioural state and physiological responses (e.g., less time in a desaturated state). Nyqvist however recommends that breastfeeding can be initiated by facilitating competence of the infant rather than assessing readiness for oral feeding [59].

8 Initiation and Establishment of Breastfeeding

Once the infant has demonstrated the ability to successfully attach to the breast, the mother is encouraged to offer breastfeeds daily, increasing in frequency in response to the infant's growing alertness, stamina and ability to feed. Until the infant is able

to suck two consecutive oral feeds, alternating breastfeeds with interim measures such as intra-gastric tube feeding provides the opportunity for infant rest between breastfeeds. However the challenges of travel and family commitments make this difficult for some mothers. Hospital facilities allowing the parents to stay with their infant are conducive to achieving breastfeeding rapidly. Parent involvement in infant care is acknowledged to be integral to both the smooth transition of feeding and settling into family life [66].

Full oral feeding is a criterion for discharge for most neonatal nurseries therefore many preterm infants receive a combination of breast and bottle feeds, with full breastfeeding achieved in the weeks following discharge home. Complementary feeds are necessary as most very preterm infants are discharged before reaching full term and breastfeeding at discharge is often inefficient in that adequate feed volumes are not consistently taken [67]. Breastfeeding support is essential during the nursery stay and beyond discharge to guide families in the transition to full breastfeeding [68].

9 Breastfeeding

Defining the normal breastfeeding relationship provides a framework by which success can be measured. For the term breastfeeding relationship the 4 key factors that are considered critical to success are:

1. Breastfeeding provides a comfortable (absence of pain/discomfort) and satisfying nurturing experience for both the mother and her infant.
2. Breastfeeding provides optimal nutrition (adequate milk supply) for normal infant growth.
3. Breastfeeding provides innate immune protection and facilitates optimum infant development.
4. Breastfeeding facilitates a mutually beneficial physical, cognitive and emotional interaction for mother and infant.

Considering the preterm infant's immaturity, breastfeeding is often difficult and presents a challenge to their caretakers. Nevertheless the aim is to improve the infant's functional sucking skills by enhancing neurological and motor development to attain full breastfeeding.

Breastfeeding requires a complex interaction and coordination of the jaw, hyoid bone, tongue, palate, pharynx and, larynx to coordinate rhythmic patterns of infant sucking, swallowing and breathing [69, 70]. Humans exhibit two types of sucking patterns, nutritive sucking (NS), where milk is consumed in long bursts with short rest periods [4]; and non-nutritive sucking (NNS) which is characterised by a series of regulated burst and rest periods with little removal of milk [4].

Tongue function is of major importance during infant sucking as it is integral to both the removal of milk from the breast and its safe clearance from the oral cavity [71], yet exactly how the tongue functions during sucking is often the subject

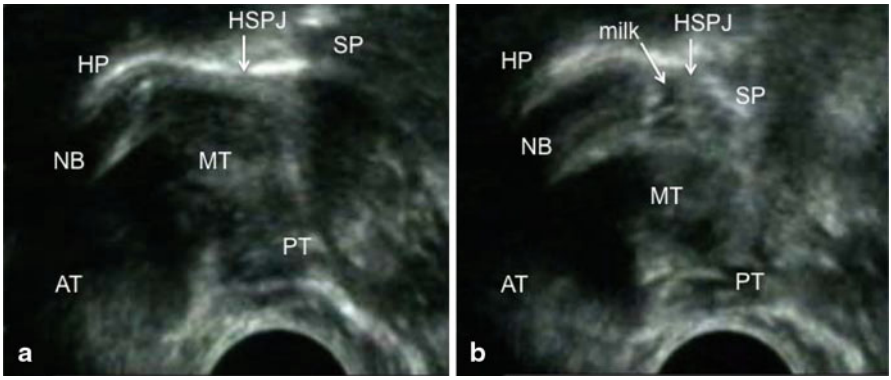

Fig. 17.1 Sagittal ultrasound images of a breastfeeding term infant. *AT*-anterior tongue, *MT*—mid tongue, *PT*—posterior tongue, *HP*—hard palate, *SP*-soft palate, *HSPJ*—hard soft palate junction, *NB*-nipple base. (**a**) tongue up—note the tongue is in apposition with the hard and soft palate. (**b**) tongue down—as the tongue lowers vacuum increases, the nipple expands evenly and moves towards the HSPJ and milk flows into the oral cavity

of considerable debate. Two theories describing the sucking mechanism exist: the Stripping Action theory [72, 73] and the Vacuum theory [74].

The Stripping Action theory suggests that compression of the breast by the superior ridge of the infant's mandible followed by a peristaltic tongue movement squeezes milk from the nipple [73]. This hypothesis was based on the presence of lactiferous sinuses that were believed to hold significant milk volumes. Recent studies show that the main milk ducts are small, not holding large amounts of milk [75, 76]. Negative intra-oral pressure created by depression of the posterior tongue is secondary and believed to allow refilling of the sinuses with milk.

The Vacuum theory of milk removal suggests that the creation of an intra-oral vacuum is the primary mechanism of milk removal [74]. Intra-oral vacuum is generated by the movement of the tongue downwards away from the palate to create a negative pressure resulting in milk flow. More evidence is rapidly accumulating emphasising the importance of adequate vacuum for effective feeding in preterm infants, infants with bronchopulmonary disease and cleft lip and palate infants. Recent ultrasound studies have not shown marked peristaltic tongue actions during the milk removal phase of the suck cycle during breastfeeding (Fig. 17.1) [77–79].

10 Nipple Shields

Full breastfeeding in the preterm infant is often hindered by immature feeding skills and reduced energy levels manifested by difficulty in achieving and sustaining attachment to the breast, very short suck bursts and falling asleep after a short time. Nipple shields were originally designed to assist breastfeeding in women with inverted nipples or nipple pain but are often employed to enable the preterm infant

Fig. 17.2 Preterm infant feeding with a nipple shield. Copyright Medela AG reprinted with permission

to attach to the breast and improve milk transfer. Meier demonstrated a significant increase in mean milk intake with the nipple shield compared to without (3.9 vs. 18.4 mL). Although the long-term effect of nipple shields is not established, Meier's data suggest that they do not decrease duration of breastfeeding in preterm infants [80].

The shield should be fitted so that the nipple fits the shield easily and positioned so that the cut out is placed where the infant's nose will be when feeding. A little sterile water or expressed breast milk expressed onto the shield facilitates attachment to the skin of the breast. As the infant opens his/her mouth the shield is gently guided over the tongue without forcing it into the mouth. The hand supporting the infant's head can be used to guide the infant onto the shield so that his/her nose is almost touching the breast. The aim is to have the infant's mouth opened wide so that the lips are close to the base of the shield (Fig. 17.2). Compression of the breast during feeding will help to improve milk removal.

11 Bottle-Feeding

Bottle-feeding is not physiologically equivalent to breastfeeding and therefore not generally encouraged as a replacement of the breast. Bottle-feeding provides fixed feed volumes at regular intervals, which is not conducive to establishing or maintaining breastfeeding during which the mother responds to the infant's feeding cues and differing milk volumes are taken at each feeding depending on infant satiety and availability of milk. There are concerns that 'nipple confusion' may occur when bottle-feeding is introduced despite little evidence of its existence.

It is important to note that the delivery of milk via a bottle is markedly different to that from the breast. Milk is not available from the breast unless milk ejection occurs. Milk ejection is a transient process ($\sim$ 2 min in duration) and milk flow increases sharply followed by a gradual decrease until the next milk ejection. Multiple milk ejections are common during breastfeeding and pumping and represent the amount

of time milk available for removal [26, 27]. In contrast bottles deliver milk freely under the influence of gravity and milk flow rate is governed by the size of the hole at the end of the teat. Different tongue movements have been demonstrated on teats of different flow rates [81]. Further facial muscles are engaged differently during bottle-feeding compared to breastfeeding [82]. Vacuums applied by term infants during breastfeeding are approximately twice that applied to bottles. This may be because the nipple must be elongated and positioned optimally to enable successful removal and control of the oral bolus or it may be a reflection of the variable milk flow rates during breastfeeding.

Bottle-feeding is still implemented as the preferred alternative to intra-gastric tube feeding when the preterm infant is able to suck feeds, in the absence of the mother and to supplement breastfeeding as it is being established. Bottle feeding is more efficient and delivers larger quantities of milk per feed compared to the breast [83], however breastfeeding success has been achieved by the routine measurement of milk intake by test weighing infants with an accurate scale before full breastfeeding has been established.

When choosing to bottle-feed the preterm infant there is some debate on the most suitable type of teat. No universal type of teat has been identified yet and this may be due to the infant's ability to adapt and modify their sucking to maintain a desirable rate of transfer that is conducive to good suck-swallow-breathe coordination [84]. Fucile et al. however demonstrated that for VLBW infants a controlled-flow vacuum-free bottle system improved feed volumes and rate of milk transfer, reduced feed duration and infants tolerated faster milk flow rates compared to a standard bottle [85].

12 Alternative Methods of Supplementation

Whilst bottle-feeding is perhaps the most common form of supplementation to breastfeeding, several alternatives exist. Cup feeding is used in some nurseries to avoid nipple confusion. Different types of vessels are used and can be effective, depending on the care giver's experience. In some settings a significant spillage of milk has been reported, with infants receiving reduced volumes [86] whereas, others have shown more positive effects such as less desaturation episodes and a greater incidence of breastfeeding at 3 months compared to bottle-feeding [87]. Supplemental feeding systems can be employed and consist of two feeding tubes connected to a milk reservoir. The feeding tubes are taped alongside the nipple allowing supplementation while the infant is breastfeeding. Finger feeding is also an option particularly in cases where the infant is not able to suck at the breast. A feeding tube is taped to the finger and connected to a milk-filled syringe or reservoir and the infant siphons the milk while sucking on the mother's finger. It is believed that this method reduces nipple confusion however little data is available other than one study that showed an increase in breastfeeding rates at discharge [88]. The advantages and disadvantages of these techniques are summarised in Table 17.3.

Table 17.3 Advantages and disadvantages of alternative feeding methods to breastfeeding for preterm infants

Feeding method	Advantages	Disadvantages
Supplemental feeding systems	Oral-tactile experiences are at the breast	Cumbersome equipment may reduce maternal compliance
	Less opportunity to develop nipple confusion	Flow rate may be difficult to adjust
	Increased opportunity to develop breastfeeding skills	Infant sensitivity to the feeding tube
	Stimulation of mother's nipples to promote milk production	Difficulty achieving optimal breastfeeding position and attachment to the breast whilst positioning the tube for good milk flow
Finger feeding	Believed to reduce nipple confusion as the infant sucks milk	Reduced gape of the mouth—conditioning may be problematic for breastfeeding where the mouth should be opened wide
	Potential to encourage improved tongue movements	The lack of elasticity of the finger compared to the nipple and the lack of necessity to hold the finger in position in the mouth may not be conducive to breastfeeding
Cup feeding	Lapping of milk improves co-ordination of swallowing and breathing	Potential risk of aspiration with poor technique
	Potential to avoid nipple confusion	Longer feed times
	Increased oxygenation and reduced energy expenditure due to the slower pace of feeding	Milk wastage due to milk spilt during feeding and difficulty assessing volume consumed by the infant
		Possible breast refusal related long term cup feeding
Bottle-feeding	Ease of feeding compared to the breast (vacuum applied is lower than that applied during breastfeeding)	Ease of this method may diminish motivation to breastfeed particularly due to the confidence that the prescribed amount of milk is actually delivered to the infant
	Large variation in teat shapes, sizes and flow rates which gives flexibility in choice to match the infant's feeding ability	

13 Suck-Swallow-Breathe Coordination

Suck-swallow-breathe (SSB) co-ordination is one of the most complex tasks required of the infant and is therefore often underdeveloped and a major impediment to oral feeding in the preterm infant. No studies have precisely described the temporal relationship of SSB co-ordination for either breast or bottle-feeding. Often oral feeding is not initiated before 32 weeks and sometimes as late as 34–36 weeks based on the premise that SSB co-ordination is poor before 34 weeks. Few studies exist documenting the maturation of the SSB reflex. Mizuno and Ueda showed significant maturation between 33 and 36 weeks gestation in a cohort of infants 32–36 weeks' gestation [89]. Lau tracked maturation in infants from 34 to 42 weeks' post menstrual age and their results suggest that good SSB coordination is reliant on two factors; a consistent suck-swallow ratio of 1:1 or 2:1 and timing of the swallow during a safe point in the respiratory cycle which is at the beginning of either the inspiration or expiration [90]. It should be noted that these studies have not been performed on breastfeeding preterm infants where milk is ejected from the breast in a sporadic fashion with variable rates of milk flow. Research in this area is urgently required to provide a basis for supporting breastfeeding.

14 Interventions to Enhance Feeding

In recent years a number of studies have attempted to accelerate maturation of oral feeding by providing different types of stimulation. Fucile et al. [91] applied oral or non-oral stimulation, or a combination of both, to enhance feeding performance. The oral intervention consisted of NNS for 3 min with a pacifier and two sessions of stroking of the cheeks, lips, gums and tongue for 12 min [92]. Non-oral stimulation comprised of two sessions of stroking the head, neck, back, arms and legs (10 min) and passive movement of the limbs (5 min) [93]. All three interventions improved the effectiveness and efficiency of feeding as measured by volume taken in 5 min, total volume taken, rate of transfer of milk (mL/min), and time taken to transition to oral feeding. It is important to note that sensorimotor stimulation remote from the oral area has beneficial effects on feeding reflecting the multifaceted dimensions of feeding involving the neurological, cardiorespiratory and gastrointestinal systems. Further in an extension of this study protocol to examine the effects of oral and non-oral interventions on SSB coordination it was found that oral stimulation alone improved sucking and expression amplitudes and swallow-respiration coordination was improved for all three groups (oral, non-oral and combined) [94].

In the majority of the studies designed to promote oral feeding the researchers or hospital staff have carried out the intervention. Furthermore assessments of feeding performance have been made exclusively on bottle-feeds. In the context of supporting breastfeeding it is logical to involve the mother in any intervention that might promote oral feeding. A small pilot study endeavoured to do so and the intervention group achieved full oral feeds 3 days earlier than the control group, were discharged 5

days earlier and had significant improvement in oral motor function as assessed by Neonatal Oral Motor Assessment Score (NOMAS). No details were provided on the effect on breastfeeding for these infants. However these results are encouraging in that developmental interventions can be implemented effectively by a parent or carer in a family centred approach [95].

Effective SSB coordination is essential to avoid aspiration. While NNS sucking is considered reflective of sucking skills it is not necessarily indicative of a well coordinated swallow [90]. Patterned orocutaneous simulation or entrainment has been shown to be beneficial in enhancing oral feeding skills beyond that expected by maturation alone particularly in infants with sucking difficulties and those with RDS [96, 97]. This approach is centered on the effect of sensory neural activity during the critical periods of late gestation and early infancy in the development of ororhythmic and deglutition networks [98, 99]. A pacifier is actively controlled to produce a burst-pause pattern similar to NNS bursts measured in infants and the stimulation of the oral mechanoreceptors imputing into the trigeminal system primes swallow circuits and improves development of orofacial motor control [100].

15 Opportunity to Feed

Several studies have confirmed that increasing the opportunity to feed results in significant improvements in sucking parameters such as increased suck bursts and sucking rates [101, 102] and a shorter time to full oral feeds [103–105]. Whilst bottle-feeding at the same frequency of breastfeeding is more efficient and effective it is possible that the infant is more able to coordinate sucking, swallowing and breathing at the breast [83] which is reflected in better oxygen saturation levels [106]. Sucking at an empty breast at less than 32 weeks [38] has been promoted based on the benefits of NNS at a pacifier [107]. Semi demand feeding however has been shown to promote the age at which full breastfeeding is attained in VLBW infants [108]. Furthermore there is evidence that the introduction of oral feeds should be based on cardiorespiratory stability rather than weight, age or maturational development [109]. The addition of frequent skin-to-skin contact increases the exposure of the infant to the breast and encourages more frequent attempts at breastfeeding leading to greater rates of exclusive breastfeeding [110].

16 Assessments of Feeding

The majority of specific feeding assessments have been developed for the bottle-fed preterm infant. Only a few are specifically designed for breastfeeding. These assessments aim to provide a valid means of monitoring feeding development facilitating both the design and the monitoring of interventions. All published feeding assessments for preterm infants lack adequate psychometric testing due to the difficulty

in standardising clinical assessments. More objective measures are likely to be less affected by observer error. Research in this area is only beginning to emerge.

The Neonatal Oral Motor Assessment Score (NOMAS) was designed to categorize oral-motor patterns conducive to poor feeding. Results pertaining to the validity of the scale are mixed in that while da Costa showed satisfactory intra-rater reliability, inter-rater reliability was fair. A recent study reported that NOMAS was a poor predictor of the timing of full oral feeding compared to baseline measures such as gestation, birth weight and efficiency of first feeding [111]. Da Costa et al used NOMAS to assess sucking longitudinally in term breastfed and bottle-fed infants. Most of the disorganised episodes of sucking were observed in bottle-fed infants [112]. They also employed NOMAS to show that small for gestational age preterm infants tend to reach mature sucking patterns at term corrected age [113] and that infants with bronchopulmonary dysplasia often have difficulty coordinating swallowing and breathing even at term corrected age [114].

The Infant Breastfeeding Assessment Tool (IBFAT) [115, 116] subjectively assesses infant behaviour, attachment, effective feeding and mother's experience of feeding. When evaluated for use in VLBW preterm infants, IBFAT had variable reliability and lacked predictive validity, and scores were weakly associated with milk intake [117]. The inability to differentiate between adequate and inadequate milk intake at the breast somewhat limits the usefulness of IBFAT.

Preterm Infant Breastfeeding Behavior Scale (PIBBS) [64] assesses infant behaviour, attachment, sucking bursts and swallowing. Inter-rater reliability between health professionals is adequate but poor between mothers and health professionals. Drawbacks are lack of differentiation between low and adequate milk transfer.

The Early Feeding Skills assessment (EFS) focuses on assessment skills specific to feeding including the ability to remain engaged, organisation of oral motor movements, SSB coordination and the maintenance of physiological stability [118]. EFS consists of a 36 item checklist divided into 3 parts. The first measures oral feeding readiness, the second oral feeding skill, and the third oral feeding recovery. This assessment has not been validated.

Test weighing before and after breastfeeding to determine infant milk intake, has been validated in preterm infants [119] and is more accurate and reliable than clinical indices [120]. It has proven successful in several settings and increases the mother's confidence as infant milk intakes progressively increase [59]. One study found that infants who were test weighed reached exclusive breastfeeding faster than those assessed 'clinically' suggesting that this technique has value in grading the reduction of supplementation as breastfeeding is established [121]. Mothers that test weighed their preterm infants at home experienced less stress and anxiety about their breastfeeding despite similar infant weight gains to those infants that were not test weighed [122].

17 Maternal Breastfeeding Difficulties

17.1 Pain/Discomfort

Maternal pain or discomfort is one of the most common reasons cited for early weaning [123, 124] and many term mothers experience nipple tenderness in the first few days of breastfeeding [33]. Most preterm mothers are pump-dependent at least initially and pain and/or trauma to the nipples may also occur during pumping. Pain is known to interfere with the milk ejection reflex and potentially decrease the volumes of milk expressed which may inhibit milk synthesis to the point where the mother is not reaching her full milk production potential. Methods of expression may influence the level of pain experienced by mothers. A Japanese study has shown that more preterm mothers experience pain during manual expression compared to pumping (Symphony, Medela AG) in the first 48 h postpartum [125], in contrast to a study of term mothers where manual expression tended to be more comfortable (Ameda, Illinois, USA) [126]. Differing milk expression practices and pumping equipment may account for the differences observed. Differences in the vacuum patterns applied by different electric pumps also influence comfort as well as effectiveness and efficiency of pumping [127].

More commonly, pumping practice is thought to be the main cause of sore nipples. Breast shields that are too small, particularly for those women with large or wide nipples are considered problematic due to excessive friction of the nipple against the shield tunnel [128]. Sore nipples have also been attributed to too high a pumping vacuum. Recommendations include increasing the vacuum level until it becomes uncomfortable and then reducing it until comfort is achieved and further reducing the vacuum during the pumping session if it becomes uncomfortable again [128, 129]. The pump should be switched off prior to removing the shield. If trauma occurs, there is a risk of bacterial infection and subsequent mastitis. Manual or hand expression is therefore often used for some time to facilitate healing [128].

17.2 Engorgement

Breast fullness is common in the post-partum period during secretory activation in mothers of term infants, but may be variable following preterm birth as initiation is often delayed [8, 11]. Engorgement can be considered from pathological and physiological perspectives. Pathological engorgement is characterised by complications such as bilateral, uniformly distended, firm, painful [130], shiny, warm breasts with or without a low-grade fever caused by vascular engorgement and early onset of milk production. Oedema occurs secondary to swelling and obstruction of the lymphatic drainage [130]. General consensus is that engorgement can be prevented by frequent, effective breast drainage. Cold packs and simple analgesics such as paracetamol or ibuprofen may assist in pain management.

17.3 Blocked Duct/s

Blocked milk ducts manifest as a tender breast lump that varies from the size of a pea to a large wedge-shaped area [131] without accompanying systemic illness or inflammation. Although causes are not well established they have been associated with delayed breast emptying, mechanical restriction (e.g., bras), duct disruption (e.g., previous surgery, tumour) and low-grade infection [132]. Elimination of any mechanical obstructions, frequent breast emptying [133], gentle massage of the lump towards the nipple whilst baby feeds or when pumping with pain relief such as cold packs and simple analgesics suitable for lactation (e.g., paracetamol, ibuprofen) often remedy the problem. In cases of chronic obstruction therapeutic ultrasound may help. However no systematic studies have assessed its effectiveness compared to conservative treatment apart from a small pilot study that has shown no difference between the two treatments [134].

17.4 Mastitis

Mastitis is characterised by localised breast inflammation that is either non-infective or infective. Symptoms of non-infective mastitis include pain, swelling, heat and redness at the site, without fever and are often attributed to milk stasis [135]. Features of infective mastitis include rapid onset, fever > 38°C, chills and flu-like myalgia [136]. A temporary reduction in milk production may be experienced [137]. Risk factors for mastitis include damaged nipples, milk stasis (e.g., blocked duct), maternal stress or fatigue and maternal or infant illness [138]. Early treatment is important to prevent recurrence and/or breast abscess [135].

Management of non-infective mastitis is identical to that for a blocked duct. If symptoms have not resolved after 12 h or fever and/or acute illness has ensued, treatment for infective mastitis should commence [135]. Identification of the causative pathogen by nipple swabs and breast milk culture is recommended as well as immediate commencement of a 10–14 days course of appropriate antibiotics for *Staphylococcus aureus* (most common cause) [138–140]. Whilst there is a plethora of research into mastitis in species such as the cow, investigation of the incidence and impact of mastitis is largely restricted to mothers of term infants despite the importance of breast milk for the preterm infant [141].

17.5 Breast Abscess

Breast abscess presents as a mobile, tender, palpable mass usually accompanied by fever and malaise [140]. Most are caused by *S. aureus* although the incidence of methicillin-resistant *S. aureus* is increasing [142]. Ultrasound is utilized to confirm diagnosis and plan treatment [131]. Initial management involves drainage by needle

aspiration and appropriate antibiotic therapy. However large (> 3 cm) recurrent or multiple abscesses may require surgical incision and drainage [140].

18 Infant Problems Impacting on Breastfeeding

18.1 Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD) is a chronic pulmonary disease that develops as a result of initial injury to the lung and ensuing treatment of the injury. BPD is typically preceded by positive pressure ventilation in the early postnatal period and is characterised by supplemental oxygen and abnormal respiratory function beyond 28 days of age, accompanied by diffuse lung changes on chest x-ray [143]. The incidence of BPD is higher in VLBW infants with approximately 50 % of infants weight under 1,000 g at birth developing BPD [144]. Feeding difficulties are very common with increasing severity of BPD and include lower intra-oral vacuums, sucking frequency and short suck burst durations resulting in low feeding efficiency during bottle-feeding [145]. Decreased feeding endurance and efficiency is attributed to the increased energy expenditure for respiration and poor coordination [146]. Low oxygenation saturation levels and growth delay tend to persist into infancy (up to 6 months corrected age) for those with severe BPD [147]. Bronchodilators administered prior to feeding may increase pulmonary compliance and lower airway resistance making it easier for the infant to breathe during feeding, and supplemental oxygen during feeding is also beneficial [148]. Infants with BPD that are discharged home with supplemental oxygen have demonstrated improved weight gains [149].

18.2 Ankyloglossia (Tongue-Tie)

Ankyloglossia is typically characterised by a sublingual frenulum that changes the appearance and/or function of the infant's tongue. The frenulum is short, lacks elasticity or the attachment to the inferior surface of the tongue is too distal (Fig. 17.3). Severity varies between infants and ankyloglossia has been associated with infant feeding difficulties, speech problems, poor dental hygiene, orthodontic and mandibular abnormalities and psychological problems [150–152]. Complications related to feeding such as nipple pain and/or trauma, prolonged feeding times, problems maintaining attachment to the breast, and poor milk transfer with subsequent slow infant weight gain have been documented [153, 154]. Frenotomy, the surgical release of the frenulum, restores movement of the tongue (Fig. 17.4) with a marked improvement in breastfeeding performance [151,155–157]. There are few risks to the procedure that can be performed without a general anaesthetic on infants under the age of 6 months. Excessive bleeding is the main risk if the large blood vessel is cut.

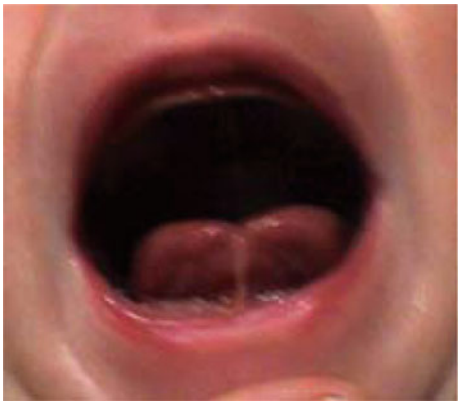

Fig. 17.3 Classical presentation of an infant with ankyloglossia. Note the short frenulum and heart shaped tongue

Despite lack of evidence that frenotomy is detrimental controversy still exists over treatment of tongue-tie and is most likely due to the lack of a universally agreed definition and a diagnostic tool to assess the degree to which ankyloglossia impairs breastfeeding [158]. The impact of ankyloglossia on feeding in the preterm infant has not been investigated yet. Given that many preterm infants are weak, have

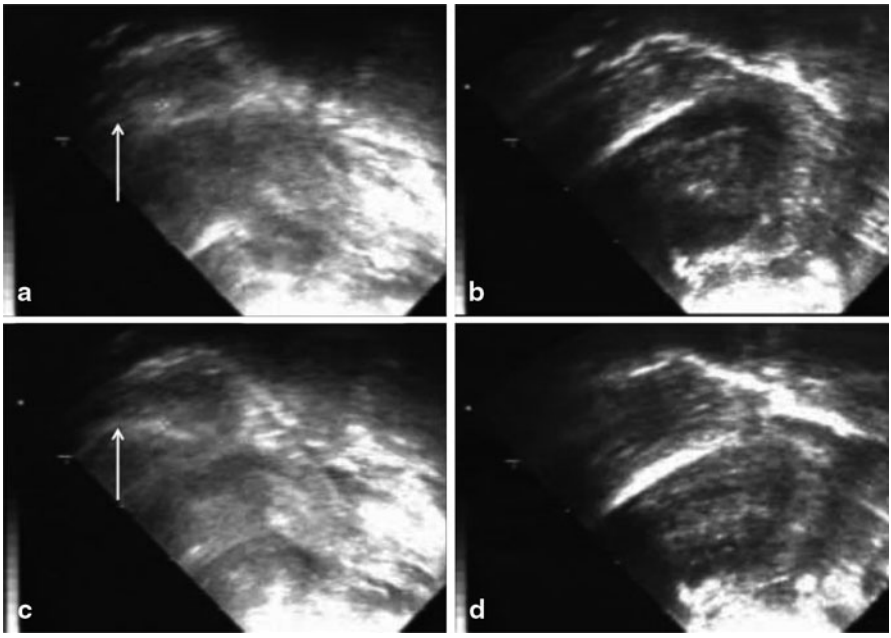

Fig. 17.4 Sagittal ultrasound images pre and post frenotomy of an infant with ankyloglossia. (**a**) Tongue up pre frenotomy—note the compression of the nipple base (**b**) Tongue up post frenotomy—the nipple has a even shape (**c**) Tongue down pre frenotomy—the compression becomes excessive when the tongue is lowered (**d**) Tongue down post frenotomy—no compression of the base of the nipple and even expansion of the nipple allowing better milk flow

respiratory problems, are developmentally immature and face more feeding difficulties than the term infant, ankyloglossia may restrict tongue function enough to compromise feeding even further. It seems ankyloglossia did not present as readily as an impediment to feeding when breastfeeding rates were at their lowest possibly due to the difference in the delivery of milk between the breast and bottle. The preterm infant is discharged when full suck feeds are achieved often feeding from both the breast and bottle. Mixed feeding methods increases the possibility that breastfeeding difficulties associated with ankyloglossia may not be detected until breastfeeding frequency is increased, often after discharge home.

18.3 Gastro-Oesophageal Reflux

Gastro-oesophageal reflux (GOR) is a normal physiologic event that occurs frequently in healthy term infants and therapy is not necessary if there are no complications [159]. Gastro-oesophageal reflux disease (GORD) is diagnosed when symptoms cause difficulty or complications. Clinical signs of GORD in preterm infants include frequent vomiting, effortless regurgitation, and occasional pulmonary aspiration. Signs may be subtle with apnoeas and bradycardias being attributed to GORD although there is a lack of firm supporting evidence [160, 161]. Despite this some studies have shown the cessation of apnoeas following treatment of GORD [162, 163]. In the preterm infant GORD presents even more diagnostic and therapeutic challenges due to the lack of accurate diagnostic tests. The non-availability of such tests has further compromised progress of research in this population.

Oesophageal pH monitoring is generally considered the gold standard diagnostic test for GORD in preterm infants but only measures acid reflux. Multiple intraluminal electrical impedance monitoring detects both acid and non-acid reflux and the multiple detection sites confirm the direction of the bolus [164]. Contrast imaging is not considered accurate for detection of all GORD and oesophageal ultrasound has not been validated in this population. Isotope scintigraphy is rarely employed clinically [161].

Management of GORD often includes a combination of strategies such as prone or left lateral positioning of the infant [165–167], elevation of the head of the cot, increasing feed frequency and reduced feed volumes. Whilst thickeners are perceived to have a beneficial effect there is little evidence to support this thus a trial of thickened feeds is considered reasonable provided there are no symptoms of feed intolerance [161]. Recent evidence suggests that the effectiveness of proton pump inhibitors to suppress acid production is questionable. These therapies may predispose to enteric and respiratory infections by interfering with defence against pathogens provided by the acidic stomach environment particularly in the preterm infant and therefore should be used only for infants with acid-induced disease [168, 169].

Prokinetic agents (metoclopramide, domperidone, cisapride) have been used in the past however potential side effects such as cardiac arrhythmias outweigh potential benefits. Gastric-acid buffering agents, and alginate or sucralfate are not

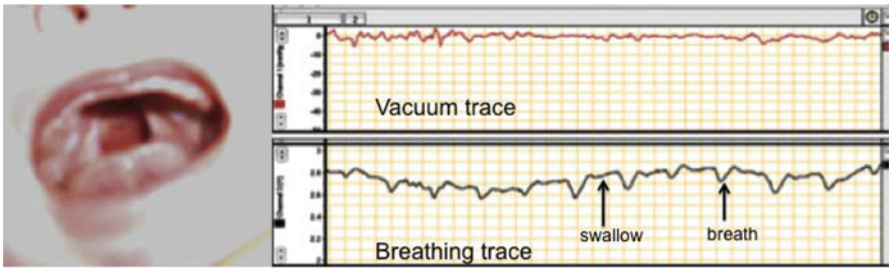

Fig. 17.5 Infant with a cleft of the soft palate. Vacuum and respiratory traces of the infant feeding from a bottle. The top trace shows the inability to produce a vacuum during bottle-feeding. The lower trace shows good rhythmical respiration and swallowing

recommended for long-term use. Surgery is considered only in exceptional circumstances where medical treatments fail, neurological compromise suggests that reflux will not resolve spontaneously and there is significant recurrent pulmonary aspiration [159].

18.4 Cleft Lip/Palate

Cleft lip occurs due to incomplete fusion of the upper lip (5th week gestation) whereas failure of growth and fusion of the midline palate (7–8 weeks gestation) results in a cleft palate. Both cleft lip and cleft palate occur in isolation or together, can be unilateral or bilateral and vary enormously in their severity. Submucosal clefts of the palate are the least severe where the mucosal surface is intact but the muscles have not closed across the velum. Typical cleft palates are completely open and are often U or V shaped. The incidence of cleft lip and/or palate is approximately 1 in 600 live births and may be associated with other congenital anomalies in up to 40 % of infants. Feeding for cleft infants can be a major hurdle, or less so depending on the severity of the cleft. Feeding problems include poor sucking, low milk intake, long feed durations, nasal regurgitation, choking/gagging and increased air intake.

Cleft lip: The ability to feed effectively depends mainly on the ability to maintain a seal with the breast and generate sufficient vacuum. This will vary between infants however small cleft lips can often be assisted by repositioning the breast to fill the cleft, enabling maintenance of the attachment.

Cleft palate: Frequently the infant with cleft palate is unable to generate any/adequate vacuum to effectively remove milk from the breast unless the cleft is small or posterior. Further the absence of an opposing surface makes compression movements ineffective (Fig. 17.5). However the ability to produce compression with the jaws and tongue may facilitate bottle-feeding in these infants. If the cleft is very anterior, the breast or a thin silicone nipple shield may seal it. If the cleft is too large or extends too far into the palate an obturator is made to cover the cleft and assist during feeding [170]. There is some evidence that a few infants are able to transfer

milk from the breast but the mechanism is unknown. Holding the infant firmly between the shoulders and then guiding and holding the breast in the infant's mouth assists breastfeeding. The mother can then manually compress the breast expressing milk directly into the infant's mouth [170].

18.5 Micrognathia and Pierre-Robin Malformation Sequence

Micrognathia is defined as a small or posteriorly placed mandible giving the appearance of a receding chin. The tongue is often posteriorly positioned increasing the possibility of obstructing the pharyngeal airway. The tongue can be restricted with limited elevation of the mid to posterior portion [170]. The combination of micrognathia, cleft palate and upper airway obstruction is referred to as Pierre-Robin Malformation Sequence. The degree of structural and functional impairment will influence the feeding method most appropriate for the infant. Nipple shields may assist the infant in maintaining attachment to the breast. Breastfeeding positions that encourage head and neck extension such as an asymmetrical latch, side lying and prone positioning (to manage milk flow) may be useful [170]. Infants with a short tongue may apply excessive compression to the nipple causing vasospasm characterised by specific tri or biphasic nipple colour changes (white, purple, red) along with intense pain [171]. If compression cannot be minimised pharmacologic treatment with calcium channel blockers may reduce pain associated with vasospasm [170].

A myriad of factors have the potential to affect the breastfeeding success of the preterm infant that have not been discussed here such as cardiac abnormalities. Often the assessment and support of breastfeeding in difficult cases requires a multidisciplinary team (e.g., speech pathology, lactation consultant, physiotherapy) working with the neonatologist to achieve the best possible outcome. More research is critical in this area to assist clinicians in providing the best possible care at a critical period that has both short and long term effects on the mother's and infant's health.

Acknowledgments M Boss and D Geddes receive salaries from an unrestricted research grant provided by Medela AG. S Perrella also receives a partial PhD scholarship from Medela AG.

References

1. Webb K, Marks G, Lund-Adams M, Rutishauser I, Abraham B (2001) Towards a national system for monitoring breastfeeding in Australia: recommendations for population indicators, definitions and next steps. Australian Food and Nutrition Monitoring Unit. Commonwealth Dept of Health and Aged Care, Canberra
2. Winberg J (2005) Mother and newborn baby: mutual regulation of physiology and behavior—a selective review. Dev Psychiatry 47:217–219
3. Kent JC (2007) How breastfeeding works. J Midwifery Wom Heal 52:564–570
4. Wolff PH (1968) The serial organization of sucking in the young infant. Pediatrics 42:943

5. Weber J, Woolridge M, Baum J (1986) An ultrasonographic organisation of sucking and swallowing by newborn infants. Dev Med Child Neurol 28:19–24
6. Mathew OP, Bhatia JJ (1989) Sucking and breathing patterns during breast and bottle-feeding in term neonates. Effects of nutrient delivery and composition. Am J Dis Child 143:588–592
7. Neifert MR (1999) Clinical aspects of lactation. Promoting breastfeeding success. Clin Perinatol 26:281–306
8. Pang WW, Hartmann PE (2007) Initiation of human lactation: secretory differentiation and secretory activation. J Mam Gl Biol Neopl 12:211–221
9. Meier PP, Engstrom JL, Patel AL, Jegier BJ, Bruns NE (2010) Improving the use of human milk during and after the NICU stay. Clin Perinatol 37:217–245
10. Saint L, Smith M, Hartmann PE (1984) The yield and nutrient content of colostrum and milk of women from giving birth to 1 month post-partum. Brit J Nutr 52:87–95
11. Cregan MD, De Mello TR, Kershaw D, McDougall K, Hartmann PE (2002) Initiation of lactation in women after preterm delivery. Acta Obstet Gyn Scan 81:870–877
12. Hartmann PE, Ramsay DT (2005) Mammary anatomy and physiology. In: King C, Jones E (eds) Feeding and nutrition in the preterm infant. Elsevier Churchill Livingstone, Edinburgh, pp 53–68
13. Kent JC (2007) How breastfeeding works. J Midwifery Womens Health 52:564–570
14. Paul VK, Singh M, Deorari AK, Pacheco J, Taneja U (1996) Manual and pump methods of expression of breast milk. Indian J Pediatr 63:87–92
15. Arthur PG, Smith M, Hartmann PE (1989) Milk lactose, citrate, and glucose as markers of lactogenesis in normal and diabetic women. J Pediatr Gastr Nutr 9:488–496
16. Hill PD, Aldag JC, Chatterton RT (1999) Effects of pumping style on milk production in mothers of non-nursing preterm infants. J Hum Lact 15:209–216
17. Meier PP, Engstrom JL, Janes JE, Jegier BJ, Loera F (2012) Breast pump suction patterns that mimic the human infant during breastfeeding: greater milk output in less time spent pumping for breast pump-dependent mothers with premature infants. J Perinatol 32:103–110
18. Hill PD, Aldag JC, Chatterton RT (2001) Initiation and frequency of pumping and milk production in mothers of non-nursing preterm infants. J Hum Lact 17:9–13
19. Engstrom JL, Meier PP, Jegier B, Motykowski JE, Zuleger JL (2007) Comparison of milk output from the right and left breasts during simultaneous pumping in mothers of very low birthweight infants. Breastfeed Med 2:83–91
20. Sakalidis VS, Williams TM, Hepworth AR et al. (2013) A comparison of early sucking dynamics during breastfeeding after cesarean section and vaginal birth Breastfeed Med 8(1):79–85
21. Hill PD, Aldag JC, Chatterton RT, Zinaman M (2005) Comparison of milk output between mothers of preterm and term infants: the first 6 weeks after birth. J Hum Lact 21:22–30
22. Daly SE, Hartmann PE (1995) Infant demand and milk supply. Part 1: Infant demand and milk production in lactating women. J Hum Lact 11:21–26
23. Daly SE, Owens RA, Hartmann PE (1993) The short-term synthesis and infant-regulated removal of milk in lactating women. Exp Physiol 78:209–220
24. Peaker M, Wilde CJ (1996) Feedback control of milk secretion from milk. J Mammary Gland Biol 1:307–315
25. Dewey KG, Lonnerdal B (1986) Infant self-regulation of breast milk intake. Acta Paediatr Scand 75:893–898
26. Ramsay DT, Kent JC, Owens RA, Hartmann PE (2004) Ultrasound imaging of milk ejection in the breast of lactating women. Pediatrics 113:361–367
27. Cobo E, De Bernal M, Gaitan E, Quintero C (1967) Neurohypophyseal hormone release in the human II, Experimental study during lactation. Am J Obstet Gynecol 97:519–529
28. McNeilly AS, Robinson ICAF, Houston MJ, Howie PW (1983) Release of oxytocin and prolactin in response to suckling. Brit Med J 286:257–259
29. Newton M, Newton N (1978) The let-down reflex in human lactation. Pediatrics 33:698–704
30. Dewey KG (2001) Maternal and fetal stress are associated with impaired lactogenesis in humans. J Nutr 131:3012S–3015S

31. Feher S, Berger LR, Johnson JD, Wilde JB (1989) Increasing breast milk production for premature infants with a relaxation/imagery audiotape. Pediatrics 83:57–60
32. Patel AL, Meier PP, Engstrom JL (2007) The evidence for use of human milk in very low-birthweight preterm infants. NeoReviews 8:e459–e466
33. de Carvalho M, Robertson S, Klaus M (1984) Does the duration and frequency of early breastfeeding affect nipple pain? Birth 11:81–84
34. Furman L, Minich N, Hack M (2002) Correlates of lactation in mothers of very low birth weight infants. Pediatrics 109:e57
35. Jones E, Dimmock PW, Spencer SA (2001) A randomised controlled trial to compare methods of milk expression after preterm delivery. Arch Dis Child Fetal Neonatal Ed 85:F91–F95
36. Hill PD, Aldag JC, Chatterton RT (1996) The effect of sequential and simultaneous breast pumping on milk volume and prolactin levels: a pilot study. J Hum Lact 12:193–199
37. Prime DK, Garbin CP, Hartmann PE, Kent JC Simultaneous breast expression in breastfeeding women is more efficacious than sequential breast expression Breastfeed Med (in press)
38. Meier PP (2001) Breastfeeding in the special care nursery. Prematures and infants with medical problems. Pediatr Clin North Am 48:425–442
39. Hurst NM, Valentine CJ, Renfro L, Burns P, Ferlic L (1997) Skin-to-skin holding in the neonatal intensive care unit influences maternal milk volume. J Perinatol 17:213–217
40. Charpak N, Ruiz-Pelaez JG, Figueroa de CZ, Charpak Y (2001) A randomized, controlled trial of kangaroo mother care: results of follow-up at 1 year of corrected age. Pediatrics 108:1072–1079
41. Bier JA, Ferguson AE, Morales Y et al (1996) Comparison of skin-to-skin contact with standard contact in low-birth-weight infants who are breast-fed. Arch Pediatr Adolesc Med 150:1265–1269
42. Wight NE, Morton JA, Kim JH (2008) Best medicine: human milk in the NICU. Hale Publishing, Amarillo
43. Morton J, Hall JY, Wong RJ, Thairu L, Benitz WE, Rhine WD (2009) Combining hand techniques with electric pumping increases milk production in mothers of preterm infants. J Perinatol 29:757–764
44. Jones E, Dimmock PW, Spencer SA (2001) A randomised controlled trial to compare methods of milk expression after preterm delivery. Arch Dis Child Fetal Neonatal Ed 85:F91–F95
45. Morton J, Wong RJ, Hall JY et al (2012) Combining hand techniques with electric pumping increases the caloric content of milk in mothers of preterm infants. J, Perinatology
46. Barlow SM (2009) Oral and respiratory control for preterm feeding. Curr Opin Otolaryngol Head Neck Surg 17:179–186
47. Bagnall A (2005) Feeding problems. In: Jones E, King C (eds) Feeding and nutrition in the preterm infant. Elsevier Churchill Livingstone, Edinburgh, pp 165–183
48. Bertoncelli N, Cuomo G, Cattani S et al (2012) Oral feeding competences of healthy preterm infants. Rev Int J Pediatr 2012
49. Douglas JE, Bryon M (1996) Interview data on severe behavioural eating difficulties in young children. Arch Dis Child 75:304–308
50. Burklow KA, McGrath AM, Valerius KS, Rudolph C (2002) Relationship between feeding difficulties, medical complexity, and gestational age. Nutr Clin Pract 17:373–378
51. Kliethermes PA, Cross ML, Lanese MG, Johnson KM, Simon SD (1999) Transitioning preterm infants with nasogastric tube supplementation: increased likelihood of breastfeeding. J Obstet Gynecol Neonatal Nurs 28:264–273
52. Shiao SY, Brooker J, DiFiore T (1996) Desaturation events during oral feedings with and without a nasogastric tube in very low birth weight infants. Heart Lung 25:236–245
53. Shiao SY, Youngblut JM, Anderson GC, DiFiore JM, Martin RJ (1995) Nasogastric tube placement: effects on breathing and sucking in very-low-birth-weight infants. Nurs Res 44:82
54. Bernbaum JC, Pereira GR, Watkins JB, Peckham GJ (1983) Nonnutritive sucking during gavage feeding enhances growth and maturation in premature infants. Pediatrics 71:41–45
55. DiPietro JA, Caughy MO, Cusson R, Fox NA (1994) Cardiorespiratory functioning of preterm infants: stability and risk associations for measures of heart rate variability and oxygen saturation. Dev Psychobiol 27:137–152

56. Bier JB, Ferguson A, Anderson L et al (1993) Breast-feeding of very low birth weight infants. J Pediatr 123:773–778
57. Hawdon JM, Beauregard N, Slattery J, Kennedy G (2000) Identification of neonates at risk of developing feeding problems in infancy. Dev Med Child Neurol 42:235–239
58. Bier JA, Ferguson A, Cho C, Oh W, Vohr BR (1993) The oral motor development of low-birth-weight infants who underwent orotracheal intubation during the neonatal period. Am J Dis Child 147:858–862
59. Nyqvist KH (2008) Breastfeeding preterm infants. In: Watson Genna C (ed) Supporting sucking skills in breastfeeding infants. Jones and Bartlett Publishers Sudbury, Massachusetts, pp 153–180
60. Siddell EP, Froman RD (1994) A national survey of neonatal intensive-care units: criteria used to determine readiness for oral feedings. J Obstet Gynecol Neonatal Nurs 23:783–789
61. Als H, Lawhon G, Duffy FH, McAnulty GB, Gibes-Grossman R, Blickman JG (1994) Individualized developmental care for the very low-birth-weight preterm infant. Medical and neurofunctional effects. JAMA 272:853–858
62. Als H, Gilkerson L, Duffy FH et al (2003) A three-center, randomized, controlled trial of individualized developmental care for very low birth weight preterm infants: medical, neurodevelopmental, parenting, and caregiving effects. J Dev Behav Pediatr 24:399–408
63. White-Traut RC, Berbaum ML, Lessen B, McFarlin B, Cardenas L (2005) Feeding readiness in preterm infants: the relationship between preterm behavioral state and feeding readiness behaviors and efficiency during transition from gavage to oral feeding. MCN 30:52–59
64. Nyqvist KH, Rubertsson C, Ewald U, Sjoden PO (1996) Development of the preterm infant breastfeeding behavior scale (PIBBS): a study of nurse-mother agreement. J Hum Lact 12:207–219
65. Thoyre SM, Holditch-Davis D, Schwartz TA, Melendez Roman CR, Nix W (2012) Coregulated approach to feeding preterm infants with lung disease: effects during feeding. Nurs Res 61:242–251
66. Fenwick J, Barclay L, Schmied V (2008) Craving closeness: a grounded theory analysis of women's experiences of mothering in the special care nursery. Women Birth 21:71–85
67. Buckley KM, Charles GE (2006) Benefits and challenges of transitioning preterm infants to at-breast feedings Int Breastfeed. J 1:13
68. Renfrew MJ, Craig D, Dyson L et al (2009) Breastfeeding promotion for infants in neonatal units: a systematic review and economic analysis Health Technol Assess 13(40)
69. Arvedson J (2006) Swallowing and feeding in infants and young children. GI, Motility online
70. Tamura YY, Matsushita SS, Shinoda KK, Yoshida SS (1998) Development of perioral muscle activity during suckling in infants: a cross-sectional and follow-up study. Dev Med Child Neurol 40:344–348
71. Smith W, Erenberg A, Nowak A, Franken E (1985) Physiology of sucking in the normal term infant using real-time US. Radiology 156:379–381
72. Ardan G, Kemp F, Lind J (1958) A cineradiographic study of breastfeeding. Br J Radiograph XXX1:156–162
73. Woolridge M (1986) The anatomy of infant sucking. Midwifery 4:164–171
74. Waller H (1936) The force exerted by the baby. In: Clinical studies in lactation. William Heinemann Ltd, London
75. Ramsay DT, Kent JC, Hartmann RA, Hartmann PE (2005) Anatomy of the lactating human breast redefined with ultrasound imaging. J Anat 206:525–534
76. Gooding MJ, Finlay J, Shipley JA, Halliwell M, Duck FA (2010) Three-dimensional ultrasound imaging of mammary ducts in lactating women: a feasibility study. J Ultrasound Med 29:95–103
77. Geddes DT, Kent JC, Mitoulas LR, Hartmann PE (2008) Tongue movement and intra-oral vacuum in breastfeeding infants. Early Hum Dev 84:471–477
78. McClellan HL, Sakalidis VS, Hepworth AR, Hartmann PE, Geddes DT (2010) Validation of nipple diameter and tongue movement measurements with B-mode ultrasound during breastfeeding. Ultrasound Med Biol 36:1797–1807

79. Geddes DT, Sakalidis VS, Hepworth AR et al (2011) Tongue movement and intra-oral vacuum of term infants during breastfeeding and feeding from an experimental teat that released milk under vacuum only. Early Hum Dev 88:443–449
80. Meier PP, Brown LP, Hurst NM et al (2000) Nipple shields for preterm infants: effect on milk transfer and duration of breastfeeding. J Hum Lact 16:106–114; quiz 129–131
81. Iwayama K, Eishima M (1997) Neonatal sucking behaviour and its development until 14 months. Early Hum Dev 47:1–9
82. Nyqvist KH, Ewald U (2006) Surface electromyography of facial muscles during natural and artificial feeding of infants: identification of differences between breast-, cup- and bottle-feeding. J Pediatr (Rio J) 82:85–86
83. Furman L, Minich N (2004) Efficiency of breastfeeding as compared to bottle-feeding in very low birth weight (VLBW, < 1.5 kg) infants. J Perinatol 24:706–713
84. Scheel CE, Schanler RJ, Lau C (2005) Does the choice of bottle nipple affect the oral feeding performance of very-low-birthweight (VLBW) infants? Acta Paediatr 94:1266–1272
85. Fucile S, Gisel E, Schanler RJ, Lau C (2009) A controlled-flow vacuum-free bottle system enhances preterm infants' nutritive sucking skills. Dysphagia 24(2):145–151
86. Dowling DA, Meier PP, DiFiore JM, Blatz M, Martin RJ (2002) Cup-feeding for preterm infants: mechanics and safety. J Hum Lact 18:13–20; quiz 46–19, 72
87. Rocha NM, Martinez FE, Jorge SM (2002) Cup or bottle for preterm infants: effects on oxygen saturation, weight gain, and breastfeeding. J Hum Lact 18:132–138
88. Oddy WH, Glenn K (2003) Implementing the Baby Friendly Hospital Initiative: the role of finger feeding. Breastfeed Rev 11:5–10
89. Mizuno K, Ueda A (2003) The maturation and coordination of sucking, swallowing, and respiration in preterm infants. J Pediatr 142:36–40
90. Lau C (2006) Oral feeding in the preterm infant. Neoreviews 7:e19–e27
91. Fucile S, Gisel EG, McFarland DH, Lau C (2011) Oral and non-oral sensorimotor interventions enhance oral feeding performance in preterm infants. Dev Med Child Neurol 53:829–835
92. Fucile S, Gisel E, Lau C (2002) Oral stimulation accelerates the transition from tube to oral feeding in preterm infants. J Pediatr 141:230–236
93. Field TM, Schanberg SM, Scafidi F et al (1986) Tactile/kinesthetic stimulation effects on preterm neonates. Pediatrics 77:654–658
94. Fucile S, McFarland DH, Gisel EG, Lau C (2012) Oral and nonoral sensorimotor interventions facilitate suck-swallow-respiration functions and their coordination in preterm infants. Early Hum Dev 88:345–350
95. Harding C (2009) An evaluation of the benefits of non-nutritive sucking for premature infants as described in the literature. Arch Dis Child 94:636–640
96. Poore M, Zimmerman E, Barlow SM, Wang J, Gu F (2008) Patterned orocutaneous therapy improves sucking and oral feeding in preterm infants. Acta Paediatr 97:920–927
97. Barlow SM, Finan DS, Lee J, Chu S (2008) Synthetic orocutaneous stimulation entrains preterm infants with feeding difficulties to suck. J Perinatol 28:541–548
98. Penn AA, Shatz CJ (1999) Brain waves and brain wiring: the role of endogenous and sensory-driven neural activity in development. Pediatr Res 45:447–458
99. Hensch TK (2004) Critical period regulation. Annu Rev Neurosci 27:549–579
100. Barlow SM (2009) Oral and respiratory control for preterm feeding. Curr Opin Otolaryngol Head Neck Surg 17:179–186
101. Pickler RH, Best AM, Reyna BA, Gutcher G, Wetzel PA (2006) Predictors of nutritive sucking in preterm infants. J Perinatol 26:693–699
102. Pickler RH, Chiaranai C, Reyna BA (2006) Relationship of the first suck burst to feeding outcomes in preterm infants. J Perinat Neonat Nur 20:157–162
103. Pickler RH, Best A, Crosson D (2009) The effect of feeding experience on clinical outcomes in preterm infants. J Perinatol 29:124–129
104. Bromiker R, Arad I, Loughran B, Netzer D, Kaplan M, Medoff-Cooper B (2005) Comparison of sucking patterns at the introduction of oral feeding and at term in Israeli and American preterm infants. Acta Paediatr 94:201–204

105. McCain GC, Gartside PS, Greenberg JM, Lott JW (2001) A feeding protocol for healthy preterm infants that shortens time to oral feeding. J Pediatr 139:374–379
106. Meier P (1988) Bottle- and breast-feeding: effects on transcutaneous oxygen pressure and temperature in preterm infants. Nurs Res 37:36–41
107. Meier PP (2003) Supporting lactation in mothers with very low birth weight infants. Pediatr Ann 32:317–325
108. Nyqvist KH (2008) Early attainment of breastfeeding competence in very preterm infants. Acta Paediatr 97:776–781
109. Nyqvist KH, Sjoden PO, Ewald U (1999) The development of preterm infants' breastfeeding behavior. Early Hum Dev 55:247–264
110. Nyqvist KH, Anderson GC, Bergman N et al (2010) Towards universal Kangaroo Mother Care: recommendations and report from the First European conference and Seventh International Workshop on Kangaroo Mother Care. Acta Paediatr 99:820–826
111. Bingham PM, Ashikaga T, Abbasi S (2012) Relationship of neonatal oral motor assessment scale to feeding performance of premature infants. J Neonat Nur 18:30–36
112. da Costa SP, van der Schans CP, Boelema SR, van der Meij E, Boerman MA, Bos AF (2010) Sucking patterns in fullterm infants between birth and 10 weeks of age. Infant Behav Dev 33:61–67
113. da Costa SP, van der Schans CP, Zweens MJ et al (2010) The development of sucking patterns in preterm, small-for-gestational age infants. J Pediatr 157:603–609, e601–e603
114. da Costa SP, van der Schans CP, Zweens MJ et al (2010) Development of sucking patterns in pre-term infants with bronchopulmonary dysplasia. Neonatology 98:268–277
115. Matthews MK (1993) Assessments and suggested interventions to assist newborn breastfeeding behavior. J Hum Lact 9:243–248
116. Matthews MK (1988) Developing an instrument to assess infant breastfeeding behaviour in the early neonatal period. Midwifery 4:154–165
117. Furman L, Minich NM (2006) Evaluation of breastfeeding of very low birth weight infants: can we use the infant breastfeeding assessment tool? J Hum Lact 22:175–181
118. Thoyre SM, Shaker CS, Pridham KF (2005) The early feeding skills assessment for preterm infants. Neonatal Netw 24:7–16
119. Haase B, Barreira J, Murphy PK, Mueller M, Rhodes J (2009) The development of an accurate test weighing technique for preterm and high-risk hospitalized infants. Breastfeed Med 4:151–156
120. Meier PP, Engstrom JL, Fleming BA, Streeter PL, Lawrence PB (1996) Estimating milk intake of hospitalized preterm infants who breastfeed. J Hum Lact 12:21–26
121. Funkquist EL, Tuvemo T, Jonsson B, Serenius F, Nyqvist KH (2010) Influence of test weighing before/after nursing on breastfeeding in preterm infants. Adv Neonatal Care 10:33–39
122. Hurst NM, Meier PP, Engstrom JL, Myatt A (2004) Mothers performing in-home measurement of milk intake during breastfeeding of their preterm infants: maternal reactions and feeding outcomes. J Hum Lact 20:178–187
123. Li R, Fein S, Chen J, Grummer-Strawn L (2008) Why mothers stop breastfeeding: mothers' self-reported reasons for stopping during the first year. Pediatrics 122:S69–S76
124. Australian, National, Breastfeeding, Strategy. Australian Government Departmant of Health and Ageing. Commonwealth of Australia:Canberra. 2010–2015
125. Ohyama M, Watabe H, Hayasaka Y (2010) Manual expression and electric breast pumping in the first 48 h after delivery. Pediatr Int 52:39–43
126. Flaherman VJ, Gay B, Scott C, Avins A, Lee KA, Newman TB (2012) Randomised trial comparing hand expression with breast pumping for mothers of term newborns feeding poorly. Arch Dis Child Fetal Neonatal Ed 97:F18–F23
127. Meier PP, Engstrom JL, Hurst NM et al (2008) A comparison of the efficiency, efficacy, comfort, and convenience of two hospital-grade electric breast pumps for mothers of very low birthweight infants. Breastfeed Med 3:141–150
128. Jones E (2005) Milk expression. In: jones E, King C (eds) Feeding and nutrition in the preterm infant. Elsevier Churchill Livingstone, Edinburgh, pp 69–85

129. Kent JC, Mitoulas LR, Cregan MD et al (2008) Importance of vacuum for breastmilk expression. Breastfeed Med 3:11–19
130. Berens P (2009) ABM clinical protocol #20: Engorgement. Breastfeed Med 4:111–113
131. Geddes DT (2009) Ultrasound imaging of the lactating breast. Methodology and application. Int Breastfeed J 4:4
132. Eglash A, Montgomery A, Wood J. Breastfeeding (2008) Disease-A-Month 54:343–411
133. Thomsen AC, Espersen T, Maigaard S (1984) Course and treatment of milk stasis, noninfectious inflammation of the breast, and infectious mastitis in nursing women. Am J Obstet Gynecol 149:492–495
134. Campbell SH (2006) Recurrent plugged ducts. J Hum Lact 22:340–343
135. Abou-Dakn M, Richardt A, Schaefer-Graf U, Wockel A (2010) Inflammatory breast diseases during lactation: milk stasis, puerperal mastitis, abscesses of the breast, and malignant tumors—current and evidence-based strategies for diagnosis and therapy. Breast Care (Basel) 5:33–37
136. ABM clinical protocol #4: mastitis (2008) Revision May 2008. Breastfeed Med 3:177–180
137. Fetherston CM, Lai CT, Hartmann PE (2006) Relationships between symptoms and changes in breast physiology during lactation mastitis. Breastfeed Med 1:136–145
138. Fetherston C (1998) Risk factors for lactation mastitis. J Hum Lact 14:101–109
139. Fetherston C (1997) Management of lactation mastitis in a Western Australian cohort. Breastfeed Rev 5:13–19
140. ABM clinical protocol #4: mastitis (2008) Revision, May 2008. Breastfeed Med 3:177–180
141. Morton JA (2003) The role of the pediatrician in extended breastfeeding of the preterm infant. Pediatr Ann 32:308–316
142. Berens P, Swaim L, Peterson B (2010) Incidence of methicillin-resistant Staphylococcus aureus in postpartum breast abscesses. Breastfeed Med 5:113–115
143. Northway WH Jr, Rosan RC, Porter DY (1967) Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia N Engl J Med 276: 357–368
144. Fanaroff AA, Stoll BJ, Wright LL et al (2007) Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol 196:147 e141–148
145. Mizuno K, Nishida Y, Taki M et al (2007) Infants with bronchopulmonary dysplasia suckle with weak pressures to maintain breathing during feeding. Pediatrics 120:e1035–e1042
146. Gewolb I, Vice F (2006) Abnormalities in the coordination of respiration and swallow in preterm infants with bronchopulmonary dysplasia. Dev Med Child Neurol 48:595–599
147. Wang LY, Luo HJ, Hsieh WS et al (2010) Severity of bronchopulmonary dysplasia and increased risk of feeding desaturation and growth delay in very low birth weight preterm infants. Pediatr Pulmonol 45:165–173
148. Daniels H, Casaer P, Devlieger H, Eggermont E (1986) Mechanisms of feeding efficiency in preterm infants. J Pediatr Gastroenterol Nutr 5:593–596
149. Ellsbury DL, Acarregui MJ, McGuinness GA, Eastman DL, Klein JM (2004) Controversy surrounding the use of home oxygen for premature infants with bronchopulmonary dysplasia. J Perinatol 24:36–40
150. Ballard JL, Auer CE, Khoury JC (2002) Ankyloglossia: assessment, incidence, and effect of frenuloplasty on the breastfeeding dyad. Pediatrics 110:e63
151. Hogan M, Westcott C, Griffiths M (2005) Randomized, controlled trial of division of tongue-tie in infants with feeding problems. J Paediatr Child Health 41:246–250
152. Hong P, Lago D, Seargeant J, Pellman L, Magit AE, Pransky SM (2010) Defining ankyloglossia: a case series of anterior and posterior tongue ties. Int J Pediatr Otorhinolaryngol 74:1003–1006
153. Geddes DT, Langton DB, Gollow I, Jacobs LA, Hartmann PE, Simmer K (2008) Frenulotomy for breastfeeding infants with ankyloglossia: effect on milk removal and sucking mechanism as imaged by ultrasound. Pediatrics 122:e188–194
154. Srinivasan A, Dobrich C, Mitnick H, Feldman P (2006) Ankyloglossia in breastfeeding infants: the effect of frenotomy on maternal nipple pain and latch. Breastfeed Med 1:216–224

155. Geddes DT, Sakalidis VS, Hepworth AR, McClellan HL, Kent JC, Hartmann PE (2010) Tongue movement and vacuum in infants feeding from the breast and an experimental teat that releases milk only with vacuum. In: International society for research in human milk and lactation. Lima, Peru
156. Buryk M, Bloom D, Shope T (2011) Efficacy of neonatal release of ankyloglossia: a randomized trial. Pediatrics 128:280–288
157. Berry J, Griffiths M, Westcott C (2012) A double-blind, randomized, controlled trial of tongue-tie division and its immediate effect on breastfeeding. Breastfeed Med 7(3):189–193
158. Amir LH, James JP, Donath SM (2006) Reliability of the hazelbaker assessment tool for lingual frenulum function. Int Breastfeed J 1:3
159. Vandenplas Y, Rudolph CD, Di Lorenzo C et al (2009) Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). J Pediatr Gastroenterol Nutr 49:498–547
160. Dhillon AS, Ewer AK (2004) Diagnosis and management of gastro-oesophageal reflux in preterm infants in neonatal intensive care units. Acta Paediatr 93:88–93
161. Birch JL, Newell SJ (2009) Gastrooesophageal reflux disease in preterm infants: current management and diagnostic dilemmas. Arch Dis Child Fetal Neonatal Ed 94:F379–F383
162. Herbst JJ, Minton SD, Book LS (1979) Gastroesophageal reflux causing respiratory distress and apnea in newborn infants. J Pediatr 95:763–768
163. Newell SJ, Booth IW, Morgan ME, Durbin GM, McNeish AS (1989) Gastro-oesophageal reflux in preterm infants. Arch Dis Child 64:780–786
164. Sifrim D, Castell D, Dent J, Kahrilas PJ (2004) Gastro-oesophageal reflux monitoring: review and consensus report on detection and definitions of acid, non-acid, and gas reflux. Gut 53:1024–1031
165. Omari TI, Rommel N, Staunton E et al (2004) Paradoxical impact of body positioning on gastroesophageal reflux and gastric emptying in the premature neonate. J Pediatr 145:194–200
166. Blumenthal I, Lealman GT (1982) Effect of posture on gastro-oesophageal reflux in the newborn. Arch Dis Child Fetal Neonatal Ed 57:555–556
167. Ewer AK, James ME, Tobin JM (1999) Prone and left lateral positioning reduce gastro-oesophageal reflux in preterm infants. Arch Dis Child Fetal Neonatal Ed 81:F201–F205
168. Chen IL, Gao WY, Johnson AP et al (2012) Proton pump inhibitor use in infants: FDA reviewer experience. J Pediatr Gastroenterol Nutr 54:8–14
169. Terrin G, Passariello A, De Curtis M et al (2012) Ranitidine is associated with infections, necrotizing enterocolitis, and fatal outcome in newborns. Pediatrics 129:e40–e45
170. Watson Genna C (2008) The influence of anatomical and structural issues on sucking skills. In: Watson Genna C (ed) Supporting sucking skills in breastfeeding infants. Jones and Bartlett Publishers. Sudbury, Massachusetts, pp 181–223
171. Eglash A, Montgomery A, Wood J (2008) Breastfeeding 54(6):343–411. Disease-A-Month 54:343–411
172. Thulier D, Mercer J (2009) Variables associated with breastfeeding duration. J Obstet Gynaecol Neonat Nur 38:259–268

Chapter 18
Donor Human Milk Banking in Neonatal Intensive Care

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Abstract Donor human milk banking has been practiced for over 100 years and is used where a mother's own milk is unavailable for her infant. With this historical practice has come evidence for the clinical use of pasteurised donor human milk (PDHM) primarily to reduce the risk of necrotising enterocolitis (NEC) in the preterm very low birth weight infant. However, clinicians are not universal in their support for the use of donor human milk in these at risk patients. Some remain unconvinced at the evidence for benefit and some may remain concerned regarding the safety of the product. These safety concerns can only be addressed through the proper management of donor human milk banking. This chapter reviews the current evidence for the use of pasteurised donor human milk and examines how recent developments in management practice in human milk banking are addressing these concerns. When a mother's own milk is unavailable, PDHM remains a viable feeding option where an infant is at risk of NEC. With an ongoing focus on safety in practice and demonstration of benefits through research, donor human milk banking may remain relevant for another 100 years.

Key Points

- Donor human milk banking has a +100 year history in clinical practice
- Current evidence supports the use of pasteurised donor human milk in preterm very-low birth weight infants to reduce the risk of necrotising enterocolitis when

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_18,

mother's milk is unavailable. In this context donor human milk banking is cost effective
- Donor human milk banks should be managed to minimise viral/microbiological/chemical risks to the recipient population
- Practice of donor human milk banking is currently not standardised internationally
- International practice may benefit with the development of 'quality principles' and standard risk assessment methodologies under which these principles can be managed

1 Introduction

For the human mammal it is biologically normal for the infant to receive milk from its own mother as its first nutrition and as an integral part of its acquired immune system. This incredibly simple biological statement of fact is complicated by the availability of artificial formula milks offering a 'choice' of feeding for parents and in situations where a mother is unable to initiate and sustain lactation for a variety of reasons. It is further complicated during the clinical nutritional management of the preterm infant, where enteral feeding itself is not the biological norm.

2 History of Donor Human Milk Banking

Historically, the use of alternative feeding options for infants has been required when a mother's own milk is not available. In 1886 Professor Theodor Escherich published his post-doctoral thesis on the intestinal bacteria in sucklings [1]. As well as the resulting identification of the bacterium that now bears his name, *Escherichia coli*, these studies led to his strong views on the value of breastfeeding [2] and in 1902 he was appointed the Chair of Pediatrics at the University of Vienna and Director of the St Anna Children's Hospital. Professor Escherich demonstrated a dedication to the social welfare of children through the establishment of an infant welfare society (Säuglingsschutz) and modernised the St Anna Children's Hospital through the design and construction of clinics, laboratories and in the hospital basement, a milk kitchen and milk depot [2]. Although records are not clear on this matter, it is suggested that this milk depot and milk kitchen represents the establishment of the first 'formal' donor human milk (DHM) bank. This first bank began operation in Vienna in 1909 [3].

In North America, donor human milk banking began in 1910 in the Boston Floating Hospital and was established by Francis Parkman Denny, a physician at the Massachusetts Infant Asylum [4]. In contrast to the contemporary practice of wet-nursing, a method was developed for the collection of milk from mothers in the community. Similarly to the process in Vienna, this separation of the product from its producer meant that more than one infant could be fed from the same donor's milk [4]. Mothers donating to the milk bank underwent a physical exam were screened for tuberculosis, syphilis and other contagious diseases. Once collected, milk was strained, pooled, boiled for one minute before being cooled and rebottled [4].

With what could be considered reasonably minor modifications, milk banking has continued in a similar manner until today. In more recent history, the focus of milk banking has become the very low birth weight (VLBW) preterm infant. Neonatologists continue to make considerable progress in life supporting techniques for preterm infants, particularly in the treatment and prevention of respiratory disorders leading to higher neonatal survival rates at the extremes of preterm delivery [5]. These infants may be considered a 'nutritional emergency' and have necessitated the provision of nutrition that is adequate and as 'natural' as possible [6]. This has resulted in the re-emergence of donor human milk banking in evidence based clinical practice in neonatal intensive care. However, in a broader context this re-emergence has occurred at a time when it is known that there is a low risk that HIV and some other viruses may be transmitted via human milk. The discovery of HIV in human milk in the mid 1980's resulted in a dramatic decline in milk banking around the world and in some countries, such as Australia, human milk banking completely ceased [7]. As contemporary human milk banking has been re-established in this context there has been an increasing focus on appropriate quality management practices aligned with practices in the food industry and donor tissue and blood banking [5]. This chapter will focus on the practice of human milk banking in contemporary neonatal intensive care in this country (Australia). Descriptions of practice that are included are illustrative of what is recommended in Australia, but not provided as an example of the only appropriate milk banking practice. This remains a discussion that is relevant internationally as this chapter does not seek to define a single practice of milk banking, but does endeavour to begin to define quality outcomes that could form the basis for international guidelines for human milk banking. It should be the goal of contemporary human milk banking to define internationally consistent quality assurance objectives or quality principles, even where practice may vary.

3 Clinical Indications for Using Pasteurised Donor Human Milk (PDHM) for the Preterm Infant

When a mother's own milk is unavailable for her preterm infant there are currently only two enteral feeding options available to clinicians. The infant may be fed donor human milk (which in contemporary human milk banking is usually pasteurised) or artificial formula milks which are derived from a variety of plant and animal sources but primarily of bovine origin.

4 Benefits of PDHM

4.1 Necrotising Enterocolitis (NEC)

NEC is the most common gastrointestinal emergency in preterm infants [8]. Although the pathophysiology is not well understood, it is clear that prematurity is the single most important risk factor for the illness. However, a wide range of risk factors

including hypoxia, artificial infant formula feeding, sepsis and ischemic-reperfusion injury contribute to the inflammatory cascade that leads to NEC [8]. The overall mortality related to NEC in VLBW infants remains around 20–30 %, but approaches 50 % in extremely preterm infants and those requiring surgery for the illness [8, 9]. As such, NEC remains a significant cause of morbidity and mortality in extremely preterm infants. It has been reported that NEC significantly prolongs the length of hospital stay (by as long as 6 months) and is associated with an increase in significant long-term neurodevelopmental impairment [8, 10]. Recent systematic reviews also suggest that the risk of cerebral palsy, visual, cognitive, and psychomotor impairment are all significantly increased in infants surviving NEC when compared to infants of a similar age and gestation without the disease [10, 11]. Need for surgery further increases the risk of adverse outcomes. An American study published in 2002 quantified the economic impact of NEC on the community as a whole [12]. On average a survivor of medical NEC spent an extra 22 days in hospital, incurring extra charges of US$73,700, while a survivor of surgical NEC spent an additional 2 months in hospital, incurring extra charges of US$186,200. The authors concluded that the yearly additional hospital charges were US$6.5 million in total, or US$216,666 per survivor. These costs may differ internationally, but clearly NEC has a major cost impact on health care.

A recent systematic review, including a small number of trials (five trials including 816 participants), has indicated that providing formula to preterm, particularly VLBW (Birth weight $\leq$ 1,500 g) infants, when compared to donor breast milk increases their risk of NEC, Relative risk: 2.5 (95 % Confidence interval: 1.2, 5.1) [13]. It was concluded that providing PDHM reduces the risk of NEC [13, 14]. The reviewers suggested that one extra case of NEC will occur in every 33 infants who receive artificial infant formula [13]. The most recent randomised control trial (RCT) was not included in this meta-analysis [15]. It assessed the effect of an exclusively human milk based diet on clinical outcomes including NEC. In this multi-centre trial preterm infants (birth weight 500–1,250 g) receiving their mothers' milk were randomised to one of three groups (HM100, HM40 and BOV). Groups HM100 and HM40 received a PDHM based fortifier (commercially available in the US) when enteral intakes reached 100 ml/kg/day and 40 ml/kg/day, respectively and both groups received PDHM if mothers milk was unavailable. The BOV group received a cow's milk based human milk fortifier when the enteral intake was 100 ml/kg/day and preterm infant formula was given if mothers milk was unavailable. The groups receiving an exclusively human milk diet showed a significantly lower rate of NEC and NEC requiring surgical intervention compared with the BOV group. The rates of NEC in the HM40, HM100 and BOV groups were 1.7 %, 3.2 % and 15.3 %, respectively. It is important to note that the BOV group is most representative of standard clinical practice in Australian neonatal intensive care units (NICU), the majority of which do not have access to PDHM. However, it should also be noted that the rate of NEC (15.3 %) in the BOV group was almost 4 times higher than rates of NEC (VLBW: 3.8 %) observed in a retrospective analysis in an Australian NICU without a donor human milk bank at the time [9]. It is unclear why the rates reported in the US study are substantially higher, but historically the US does have higher incidence of

NEC particularly when compared to Japan and many Northern European countries [16]. Despite the limitations, it is clear that the current evidence supports the use of PDHM as an alternative to artificial infant formula to reduce the incidence of NEC, an illness with substantial public health burden.

4.2 Infection

NEC remains the most commonly examined clinical indication for the use of PDHM however studies have demonstrated other benefits. A recent systematic review [17] has reported there was a protective effect of human milk against infection in preterm infants. However, it should be noted that although many of these studies included the use of DHM, they were not designed to allow its effect to be distinguished from the overall benefits of human milk. There are however, sound physiological and clinical reasons to suggest there may be a reduction in the incidence of infection when DHM is provided as an alternative to artificial infant formula. PDHM retains most of the immunological components which provide benefits to the acquired immune system of a neonate [18]. As such, these components are thought to provide some protection against infection in the neonatal period.

4.3 Chronic Lung Disease

A recent study has reported that DHM reduces the risk of chronic lung disease in preterm infants, compared to preterm artificial infant formula when used as a substitute for mothers own milk [19]. Preterm infants of mothers who intended to breastfeed were randomised to receive either PDHM or artificial infant formula if there was insufficient mothers' own milk. Unfortunately, analysis of the study was confounded as 21 % of the infants in the donor milk group were switched to artificial formula during the course of the study (at the clinician's discretion for reasons of poor growth). But these infants remained classified as 'donor milk' in the subsequent analysis.

4.4 Long Term Follow-Up

In addition to the short-term clinical benefits of PDHM, long-term follow-up of RCTs conducted in the 1980's in the UK has shown benefits in adolescence of providing PDHM versus artificial infant formula to preterm infants. This large study randomised infants born at less than 32 weeks' gestation to two trials, one (Trial 1) comparing PDHM to preterm infant formula and one (Trial 2) comparing standard term formula to preterm formula when each feed type was provided as a sole diet or

as supplements when a mother's own milk was unavailable [20]. Of 926 infants, 216 have been followed to 13–16 years of age. Adolescents who had received PDHM have shown lower mean arterial blood pressure, a lipoprotein profile demonstrating a lower likelihood of atherosclerosis and better insulin resistance than those who had received preterm infant formula [20–22]. Due to the changes in practice since these trials were conducted there are questions regarding the relevance of this study. However, it does represent clear evidence that early nutrition has long lasting benefits to infants who are born preterm. Such long-term follow-up studies are important considering the current focus on optimising nutrition in the first weeks and months of life. The potential for donor human milk banking to contribute to this area should not be discounted.

4.5 Breastfeeding Support

Anecdotally, it has been suggested that, when a mother's own milk is insufficient for her preterm infant, supplementing her supply with DHM while she works to increase her supply, is supportive of her breastfeeding in the longer term. The recent establishment of a donor human milk bank at King Edward Memorial Hospital (KEMH) in Perth, Western Australia has allowed an examination of this issue. Due to the well-documented benefits to preterm infants of receiving their own mother's milk, clinicians are extremely supportive and encouraging of mothers to provide their own breast milk. At KEMH NICU, this has resulted in very high breastfeeding initiation rates (close to 98 %—from recent clinical audit data). Although many mothers of preterm infants are successful at providing enough breast milk for their infant, many still struggle to reach a full lactation. Many of these mothers will continue to provide some breast milk for their infant and supplement the remaining requirement. In 2006, prior to the establishment of the donor human milk bank at KEMH, exclusive breastfeeding rate at discharge, and the breast milk feeding rate (any or all breast milk feeds) at discharge, were examined from a review of medical records in those infants born less than 30 weeks (and subsequently at high risk of NEC). The exclusive breastfeeding rate in these women was 53 %. In a similar time period, once the donor milk bank had been established (2008) the exclusive breastfeeding rate was 65 %. Although showing a slight trend to an increase, this difference was not statistically significant. When data was excluded from mothers who were able to provide sufficient breast milk to entirely meet their infants needs during hospitalisation, and those who did not provide any breast milk (i.e., the subgroup who intended to breastfeed but required some supplementation), there was a striking increase in the percentage of mothers providing any breast milk at discharge during the period when DHM was available as a feeding option. Prior to the donor milk bank, when their milk was supplemented with artificial infant formula, only 30 % of these women were still providing breast milk at discharge. When PDHM was available and used as a supplemental feed, 70 % of these women were providing some or all of their

infant's breast milk needs at discharge. This outcome is certainly beneficial in terms of meeting breastfeeding outcomes after preterm birth. It is also likely that there are short and long term clinical benefits to increasing the amount of mother's own milk provided to extremely premature infants.

5 Concerns Regarding the Use of PDHM in the Preterm Infant

5.1 Poor Growth

'Poor growth' is an outcome that is associated with the use of PDHM (and in fact mothers own milk) in preterm infants when compared to artificial infant formula. In addition to the finding that PDHM reduces the risk of NEC, the recent systematic review [13] also concludes that decreased short term growth is associated with DHM use when compared to artificial infant formula, but there is no evidence to suggest there is an adverse effect on long-term growth or development. The significance of this outcome is hard to discern as only one of the studies included in the meta-analysis compared DHM with additional nutrient fortification, as is routinely conducted in current clinical practice. It has also only recently become technically and practically possible to routinely measure human milk composition in the neonatal unit [23]. It is reasonable to suggest that current practice and recent advances may allow clinicians to better manage human milk in general, when managing the nutritional needs of preterm infants.

5.2 Evidence for Benefit and Changing Clinical Practice

Current practice of donor milk banking is guided by systematic reviews of clinical trials conducted over a long time period (in this case over 20 years). There are valid concerns that conclusions drawn from these trials are no longer relevant considering the changes in clinical practice over such a long period. For example, the formulation of preterm-specific infant formulas has developed significantly since many of these trials took place. It may well be that the risk of NEC associated with formula is reduced with the newer preparations. However, it should also be noted that improved standards of care across this time period have resulted in increased survival of extremely preterm infants who are at high risk for NEC [8]. Considering that it is unethical to truly randomise a study population to feeding with breast milk or an artificial formula irrespective of the maternal intention to breastfeed, conclusive high quality evidence (RCTs) in support of PDHM use in preterm infants is unlikely and it may be necessary to focus on collecting other physiological measures [7]. Although many questions remain, on balance, the available evidence supports the use of

PDHM in preterm infants when mothers' own milk is unavailable. It must be noted that outside this high risk group of infants there is currently little evidence of the benefit of routinely providing PDHM when there is insufficient maternal breast milk.

6 Contemporary Human Milk Banking

6.1 Definition

It is necessary to clarify that current discussions of milk banking often confuse two public health issues: the clinical use of PDHM in neonatal intensive care where there is an evidence based need as discussed above; and various forms of informal/formal milk sharing that are gaining prominence in the media and the society in general. In the Australian context, where the re-establishment of contemporary human milk banking as a clinical service in NICUs is a recent development, we advocate the following definition of DHM banking as distinct from wet nursing, informal milk sharing, commercial milk sharing and non-evidence based use of PDHM, which are separate practices.*'A DHM bank collects, stores, processes and dispenses donated human milk. DHM is excess human milk provided by a mother for use by a recipient that is not the mother's own baby. This recipient is a hospitalised, preterm or ill infant. The human milk is donated on a voluntary, non-remunerated basis. DHM should only be provided based on the clinical need of the recipient, and it is an alternative to infant formula for special needs infants, and not a substitute for the mother's own milk'*. This definition then enables the development and description of a service and process that manages the inherent clinical risks in the context of the described recipient. It is important to note that this description of milk banking is relevant in the Australian context but may differ internationally where the recipient and risk environment may vary.

7 Quality Management in Donor Human Milk Banking

Given the documented risks of potential viral transmission through breast milk, contemporary DHM banking practice bears similarities to both donor tissue and blood banking. Also given the risk that improper handling and storage may result in bacterial risks similar to other food products, it is now common to see risk management practices that are common in the food industry employed in milk banking [5]. During the recent re-establishment of DHM banking in Australia it became evident that hazard management methodologies used commonly in the food industry such as those meeting the Codex HACCP (Hazard Analysis Critical Control Point) requirements, provided a sound framework for the development of a quality assurance programme for DHM banking. These methodologies have also been used by other milk banks and are now a requirement in many human milk banking guidelines [24, 25].

Table 18.1 The 12 steps of CODEX HACCP (hazard analysis critical control point)

1. Assemble a multidisciplinary HACCP team
2. Describe product/process
3. Identify the intended use/consumer
4. Construct flow diagram of process steps
5. On-site verification of flow diagram
6. List potential hazards for each process step, conduct hazard analysis and determine control measures
7. Determine Critical Control Points (CCP's)
8. Establish Critical Limits for each CCP
9. Establish a monitoring system for each CCP
10. Establish corrective actions for deviations from critical limits
11. Establish verification procedures
12. Establish record keeping and documentation system

7.1 Hazard Analysis Critical Control Point

HACCP aims to ensure food safety for the consumer and is a system that was developed by NASA in the 1950's to ensure the safety of food for the US space programme [25]. It is a system to identify, evaluate and control hazards that are significant for food safety [5]. The 12 steps in the application of a HACCP programme are shown in Table 18.1 [5, 25]. The scope of a HACCP plan usually only covers the receipt of raw product from the donor through to dispensing to the nursery (see Fig. 18.1 for an example milk bank workflow modified from that used in the PREM Bank). It is therefore necessary for milk banks to develop appropriate guidelines for the screening of donors, hygienic collection of milk, prioritisation of recipients and obtaining informed consent for recipients. We have previously published a description of our evidence-based guidelines for milk banking in Australia [5] and there are many other published guidelines describing practice in other countries (see [24–26] for recent examples). The application of the stepwise approach of HACCP combined with formal risk assessment methodologies adapted from AS/NZS 4360:2004 (Australian risk assessment standard) provided a systematic approach to the safe development of a milk banking process in Australia.

8 Donor Selection and Recruitment

Since the first establishment of DHM banking, descriptions of the service provided demonstrate that selection of the donor to ensure 'quality' of the donated milk has been a fundamental premise of milk banking [4]. This has not changed and it has recently been summarised in the statement 'Donor selection has the aim of identifying the conditions contraindicating the donation, not only in the interest of the receiver but also in the interest of the donor herself and her own infant' [25]. This statement broadly demonstrates the quality management principle that most milk banks apply to

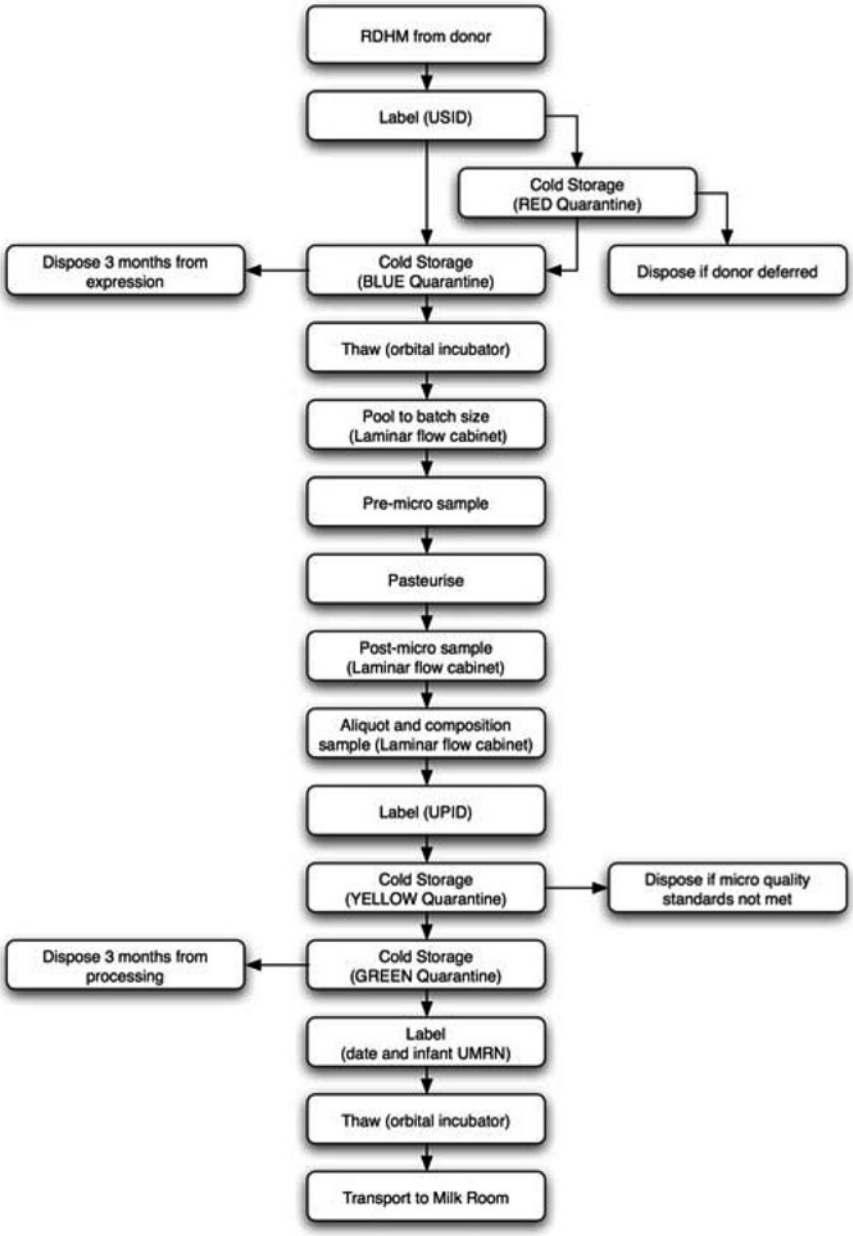

Fig. 18.1 An example workflow (PREM Bank)

the development of donor selection criteria. The risks that may be managed by these procedures include: (1) the potential transmission of infectious agents through human milk (e.g., viral, prion, bacterial) (2) the potential presence of pharmacologically active compounds in human milk at a level that may present risk to the recipient (e.g., medications, illegal drugs, alcohol, caffeine, herbal therapies) (3) lifestyle choices that may result in undesirable compositional changes in human milk (e.g., a strict vegan diet may cause vitamin B-12 deficiency) [27]. (4) That a donor's own milk supply may not be sufficient to meet her infants need in addition to donation.

In most cases, the response to managing these risks internationally has been for milk banks to adopt medical history collection and serology screening requirements that meet the local standards required during human tissue and blood donation [5, 24–26], although with additional requirements specific to human milk banking. DHM banks will also provide equipment, appropriate instruction and ongoing advice and support to donors for the proper collection of their expressed milk. However, it should be noted that where collection occurs in an uncontrolled environment (for example in the donor's home) it is not sufficient to rely on collection instruction to ensure hygienic collection and storage. Procedures (testing and processing) must be conducted by the milk bank to ensure appropriate safety of the product. When comparing human milk banking practices internationally it is clearly unlikely that donor screening and recruitment guidelines can be developed that are universally applicable in every milk bank. In current milk banking practice it is the responsibility of the milk bank itself, national associations of milk banks or regulators to identify local risks and appropriate management policies.

9 Effects of Processing and Storage on Human Milk

Although the risks associated with the donor population and the raw product vary internationally, the identifiable risks and effects of the storage and processing steps in milk banking are universally identifiable and have been the subject of research. Donated human milk will potentially experience a number of different storage temperatures when stored by the donor mother, stored at the milk bank, during processing and during dispensing and feeding. The storage conditions and thermal processing steps may affect bacterial content of human milk and change may be observed in some milk constituents. A milk bank process must be designed to minimise detrimental thermally induced changes to human milk components and ensure bacterial growth is minimised.

9.1 Storage at Room Temperature (20–25 °C)

The protein β-casein is hydrolysed to just 35 % when human milk is stored at 25 °C for 72 h. Stored with the same conditions the immuno-proteins, sIgA and lactoferrin do not change significantly. Bile salt stimulated lipase, which is responsible for

hydrolysis of triacylglycerols, decreases to 65 % activity in 4 h but after that remains stable on that level until 72 h of storage [28]. Human milk remains stable for 3–4 h at room temperature. After that, a significant decrease in protein, lactose and pH occurs. Additionally, the microbial content increases significantly after 3 h of storage [29, 30] and therefore immediate refrigeration is usually recommended when collecting for human milk banks.

9.2 Storage in the Refrigerator (4 °C)

No significant changes have been observed in osmolality, concentration of serum IgA, lactoferrin and fat during refrigerated storage for 96 h [31]. White blood cell count was observed to decrease by 20 % and total protein by 5 %. Total free fatty acid concentration increased threefold during that time [31]. It has been demonstrated that bacterial growth in expressed human milk does not significantly occur when stored at 4 °C for up to 48 h [32].

9.3 Storage in the Freezer (−20 °C)

Lawrence [33] has reported that human milk can be stored safely at −20 °C for 12 months. Immuno-protective constituents, lipase, amylase and lactose were stable and no bacterial growth was observed with those storage conditions. The only changes were in the cell count and activity, loss of lactoperoxidase activity and some alteration in the milk lipid composition, i.e., decrease in triacylglycerol with a free fatty acid increase [33, 34]. For this reason, many milk banks limit frozen storage of unpasteurised human milk to 3 months during which time the action of milk lipases is minimal [25].

9.4 Storage at Increased Temperature (∼38 °C)

The optimal temperature (37 °C) for multiplication of most bacteria found in human milk. Hamosh et al. [30] have therefore suggested that it is inappropriate to store human milk at 38 °C for even 4 h. However, just a minimal reduction of total protein was observed when human milk was stored at 38 °C for 24 h. These authors also suggest the activity of lipase and amylase was unaffected under these storage conditions [30]. However, other researchers have reported that lipase activity is decreased within 4 h of storage at this temperature [28]. Nonetheless, to maintain cold chain during milk banking most international recommendations minimise the time human milk is stored above −20 °C, and milk is only exposed to higher temperatures where processing steps are specifically designed to reduce bacterial content to acceptable levels, for example, where DHM is pasteurised [5, 24–26].

9.5 Selection of Storage Container

The storage container utilised by the milk bank must allow ease of handling (aseptic transfers, integrity during freeze/thaw cycle etc.) and should not pose chemical or physical risk to the product. Glass is often avoided within a food process due to its fragility and ability to chip, however it is chemically inert (with respect to milk) and is easily reused with sterilisation by thermal means. Plastic containers are often utilised during milk banking. The adherent properties of human milk fat and protein are well known, and selection of appropriate materials is required to ensure milk macronutrient composition is not altered and that chemicals utilised during plastic manufacture do not leach into product.

9.6 The Effect of Pasteurisation on DHM

Pasteurisation is a process where food, usually a liquid, is treated making the product safe for consumption and increasing its shelf life. Heat treatments are the most common pasteurisation methods but alternative treatments such as ultrasound or irradiation are emerging in the food industry and could improve the quality of pasteurised donor human milk.

10 Thermal Pasteurisation

The most common forms of pasteurisation are low-temperature, long-time (LTLT) and high-temperature, short time (HTST) methods. Both treatments are equivalent in destroying the most heat resistant of the non-spore-forming pathogenic organisms *Mycobacterium tuberculosis* and *Coxiella burnetii* [35]. Treatments at ultrahigh temperatures (UHT) where milk is treated higher than 135 °C for very short time (1–2 s) must be distinguished from pasteurisation.

Holder pasteurisation is the heat preservation method that is most widely employed in human milk banks around the world. Human milk is heated in a water bath and held at 62.5 °C for 30 min [5, 24–26]. This treatment is capable of a 5-$\log_{10}$ reduction of bacteria including *Escherichia coli*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Bacillus cereus* and *Staphylococcus aureus* [18]. However, bioactive proteins are only partially preserved during this process. For example, 72 % of sIgA, 22 % of lactoferrin and 39 % of lysozyme were retained after Holder pasteurisation. Furthermore, bile salt stimulated lipase (BSSL) was reported to be lost completely [18, 36, 37].

Various attempts have been made to optimise heat treatment in human milk banking. Wills et al. [38] have suggested reducing the hold time to 5 min at 62.5 °C to significantly increase the retention of immunological proteins while still maintaining an adequate reduction of bacteria commonly found in human milk. These authors also suggest the possibility of reducing the treatment temperature to 56 °C for a 15 min hold time. This treatment method would increase the retention of immunological proteins to above 90 % while still reducing inoculated organisms by 99 % [38].

Czank et al. [18] provided a similar 'optimisation' of the heat treatment of DHM. It was found that treatment at 57 °C for 30 min resulted in a retention of the major human milk immunological proteins greater than 90 % while maintaining a reduction in the common bacteria found in human milk of 99.9 % [18]. However, treatment at 62.5 °C for 30 min is still recommended by most international milk banking guidelines. As it is the most common method employed, Table 18.2 summarises the effect of holder pasteurisation and frozen storage on various human milk components and properties.

Holder pasteurisation has also been demonstrated to inactivate viruses of relevance due to their possible presence in human milk and their transmission risk to the preterm infant. This has been demonstrated for HIV 1 [39], HTLV1 [40] and CMV [41]. In most milk banks a Holder pasteurisation step is also retained to ensure that, should donor screening fail and a potentially infectious donation enter the milk bank product stream, the pasteurisation step would protect recipients from risk of exposure.

There has been recent interest in HTST or Flash pasteurisation of human milk, where the product is heated to 72 °C for 15 s. This is the preferred heat treatment in the dairy industry due to its lower energy consumption and due to the better colour and flavour preservation characteristics when compared to LTLT pasteurisation. However reported results are mixed. Goldblum et al. [42] have reported a reduction of microbial contamination without destroying the unique nutritional and immunologic qualities of human milk with the HTST method. Bile salt stimulated lipase was inactivated completely, but lactoferrin, secretory IgA and serum IgA antibody activity were not changed [42]. Other studies found a significant reduction of immunological proteins with the HTST method [43, 44]. Interestingly, Dhar et al. [45] have demonstrated retention of immunoglobulins when milk was treated at different flow rates. It was concluded that the difference in immunological protein retention in various studies may be due to differing thermal transfer conditions in different types of the heating apparatus, and/or differing sample volume (presumably also altering thermal exchange kinetics) [45].

The above thermal methods define both temperature and hold time parameters. Milk banking processes must therefore be developed to ensure that product is exposed to these specified conditions. Reference to the earlier description of HACCP methodology (refer to steps 9–12 Table 18.1) will demonstrate how the HACCP process can be used to ensure that a selected pasteurisation method is applied consistently to all product handled by the milk bank. Appropriate record keeping, calibration of equipment and quarantining of product through various process stages (see Fig. 18.1) must be part of this process, and in its proper application, HACCP can provide confidence to clinicians that consistently and demonstrably safe product can be provided by a human milk bank.

11 Ultrasound

Treatments with power-ultrasound (20–100 kHz) are an emerging technology for the preservation of food [46–48]. Power-ultrasound creates cavitation i.e., the formation, growth and implosive collapse of bubbles in liquids. During the collapse of these bubbles, localised hot spots occur with temperatures approaching 5,000 °C,

Table 18.2 Breast milk components and the effect of holder pasteurisation and storage. (Adapted from [63, 64])

Component	Finding
Immunological proteins	
IgA, sIgA	0–48 % reduction
IgG	34 % reduction
IgM	100 % reduction
Lactoferrin	57–80 % reduction
Lysozyme	0–60 % reduction
Enzymes	
Amylase	15 % reduced activity
Bile salt stimulated lipase	100 % reduction
Lipoprotein lipase	100 % reduction
Fats	
Total fats	No effects
C14:1–C24:1	No effects
C8:0–C24:0	No effects
n−3, n−6 PUFA	No effects
AA, DHA	No effects
Linoleic, linolenic	Reduced
Free fatty acids	80 % increase
Vitamins/Cytokines	
Vitamin A	No effect
Vitamin C	36 % reduced
Vitamin B_2	No effect
Vitamin B_6	15 % reduced
Vitamin B_9	31 % reduced
Vitamin B_{12}	No effect
Vitamin D	No effect
Vitamin E	No effect
Epidermal growth factor	No effect
Erythropoeitin	Significantly reduced
IGF-1, IGF-2, IGF-BP2, 3	7–39 % reduction
IL-10	Significantly reduced (maintained effect on T-cell proliferation)
TGF-α, TGF-β	No effect
Cells	
B-cells, T-cells	100 % reduction
Lymphocytes	100 % reduction
Trace elements	
Calcium	No effects
Copper	0–9 % reduction
Iron	0–15 % reduction
Magnesium	No effect
Phosphorus	No effect
Potassium	No effect
Sodium	No effect
Zinc	No effect
Other components/ Properties	
Lactose	No effect

Table 18.2 (continued)

Component	Finding
Oligosaccharides	No effect
Mannose-binding lectin	No effect
Lactate	7 % reduced
Lysine	Significantly reduced
CD14 (soluble)	88 % reduction
Escherichia coli inhibition	26 % reduced

pressures of about 50 MPa but a lifetime of a few microseconds [49]. The pressure changes resulting from these implosions create shock waves that disrupt the cellular membranes of bacteria resulting in cell lysis [50, 51].

Studies on bovine milk and fruit juices have shown that ultrasound treatment can eliminate various food-borne pathogens in a similar manner to thermal pasteurisation methods [52]. The synergistic effects of power-ultrasound in combination with other processing technologies have also been used to optimise food quality or to reduce the treatment time and energy [53–55]. There are few studies of these methods using human milk, however, ultrasound combined with temperature treatment (thermo-ultrasonic treatment) of human milk has been shown to inactivate *Escherichia coli* and *Staphylococcus epidermidis* with a greater retention of sIgA, lysozyme, lactoferrin and BSSL than with Holder pasteurisation [56]. However, some loss of these proteins appears unavoidable with any thermally based method.

12 Ultraviolet Irradiation

There are very few non-thermal pasteurisation methodologies that may be useful in human milk banking. However, one possibility that has been used by the food industry is ultraviolet light. Ultraviolet (UV) is part of the electromagnetic spectrum and subdivided by wavelength into UV-A (320–400 nm), UV-B (280–320 nm), UV-C (200–280 nm) and Vacuum-UV (100–200 nm). UV-C in the range of 250 and 270 nm has the most germicidal effect and is capable of destroying micro-organisms such as bacteria, viruses, protozoa, yeasts, moulds and algae [35, 57]. At this wavelength the DNA bases, mainly pyrimidine and purine, absorb the UV-C energy promoting chemical reactions. Common products of these reactions are pyrimidine dimers, other pyrimidine adducts, pyrimidine hydrates, and in addition, this may involve cross-linkages with proteins and on rare occasions breakage of the micro-organism's DNA [58]. In the food industry, UV-C is commonly used in surface sterilisation of fruits and vegetables and in the treatment of drinking water. The penetration depth of UV-C in liquid depends on solubility, density and turbidity of the liquid [59–61]. UV-C treatment of opaque liquids such as human milk is therefore difficult due to its macronutrient content. Keyser et al. [62] have showed in a study that with a turbulent flow of fruit juice around a UV-C source the penetration problem can be solved and the UV-C treatment can be applied to opaque liquid. Validation of the

Table 18.3 Composition of PDHM dispensed from PREM Bank [all values mean (SD)]

	Fat (g/100 ml)	Protein (g/100 ml)	Lactose (g/100 ml)	Calories (kcal/100 ml)
Audit 1	4.16 (0.90)	1.35 (0.33)	6.71 (0.60)	69.7
Audit 2	3.71 (0.90)	1.03 (0.22)	7.17 (0.22)	66.7

potential of this method is underway, and it may provide a novel method for removing microorganisms from human milk at ambient temperature reducing the potential for damage to the bioactive proteins in human milk.

13 Composition of DHM

The human milk banking process itself (selection and pooling of donors, freeze/thaw cycles, storage container selection etc.) may cause minor changes in milk composition. This will result in variable composition of the product supplied to the preterm infant and may be at least partly responsible for the poor growth observed and discussed previously.

During the operation of the PREM Bank it has not been practically feasible to routinely measure macronutrient composition of donated milk. However, two audits have been conducted (Table 18.3). The first monitored the composition of the first 50 batches of DHM processed by the PREM Bank when it was first established. The second monitored composition during a 3 month period in 2011 when the PREM Bank was operating at a much higher capacity. Most DHM in audit 1 was donated by mothers of preterm infants early in lactation, whereas during audit 2 there was a higher proportion of mothers of term infants or with established lactation. This may have been responsible for changes in the mean composition. However, the standard deviations, particularly with respect to fat and protein composition during both audits, demonstrate the broader issue that composition between batches of DHM is extremely variable. Relatively simple techniques (mid infrared) for the determination of macronutrient composition of human milk utilising small sample volume and rapid turnaround are becoming available [23] and are being applied to examination of these nutritional changes resulting from human milk banking practices. Future research may elucidate other technologies that allow the manipulation of the macronutrient composition of DHM to reduce batch variability and target the nutritional needs of preterm infants. However, in the short term, measurement of composition and appropriate fortification allow more appropriate nutritional management of donor human milk for the preterm infant.

14 Conclusions

The current evidence, on balance, supports the use of PDHM to reduce the risk of NEC in the preterm, VLBW, when a mother's own milk is unavailable [13]. However, universal support for donor human milk banking from clinicians is not evident. This may be in part to concerns about the safety of the process and the ability of milk banks to manage the low but real risks associated with milk donation. There has been a recent focus in contemporary human milk banking on addressing these concerns through the application of appropriate management practices used in similar industries, as discussed at length in this chapter, and considerable progress has been made in this regard. Milk bank adherence to consistent international practice may also serve to give clinicians confidence in the safety of the process and the quality of the product for their patient. However, the differing risk environments, particularly related to donor screening, probably make this goal unachievable. It may however be possible for donor human milk banks to define uniform quality principles, so that product quality can be assured, while local practices vary to meet local needs and manage local risks. With the emergence of donor human milk banks in the developing world this is also a relevant consideration. In these projects the definition of milk banking is broader than simply in the NICU, and milk banking is used to support and encourage breastfeeding (where local breastfeeding rates are low), to provide a safer alternative to infant formula (where infant mortality due to gastrointestinal disorders is endemically high) and even to provide a processing (pasteurisation) of an HIV positive mothers' own milk to be safely provided to her own infant. However, milk banking should still provide the safe provision of appropriate nutrition in these circumstances and development of these projects is recognising this need.

In addition to quality principles, a systematic methodology will be required to assess the risk of various milk bank processes in their local context. HACCP is now commonly used in milk banking and it would seem that this stepwise and systematic methodology, which is internationally recognised, could be modified by milk banks to assess other milk banking quality assurance decisions that fall outside its usual scope. This may also provide a risk assessment methodology that can be uniformly applied to milk banking, both in NICU and in projects in the developing world.

It is reasonable to suggest, when examining descriptions of the first establishment of milk banks that practice and technologies have not progressed far in its 100 plus year history. There are enormous opportunities to progress milk banking practices and technologies, and it will be the responsibility of human milk banks to validate the safety and efficacy of these technologies. An additional benefit of the development of a risk-based approach to milk banking could also be to provide a mechanism to introduce changes in practice in a systematically and safely managed manner.

Acknowledgement We acknowledge Dr Pieter Koorts for his review and comments on the section "Clinical indications for the use of PDHM for the preterm infant".

References

1. Death of Professor Escherich (1911) Lancet 1:626
2. Shulman ST, Friedmann HC, Sims RH (2007) Theodor Escherich: the first pediatric infectious diseases physician? Clin Infect Dis 45:1025–1029
3. Weaver G (2005) Human Milk Banking. In: Jones E, King C (eds) Feeding and nutrition in the preterm infant. Elsevier, London, p 87–102
4. Arnold LDW (2005) Donor human milk banking: creating public health policy in the 21st century. Union Institute and University of Cincinnati, Cincinnati
5. Hartmann BT, Pang WW, Keil AD, Hartmann PE, Simmer K (2007) Best practice guidelines for the operation of a donor human milk bank in an Australian NICU. Early Hum Dev 83:667–673
6. Aguayo J (2001) Maternal lactation for preterm newborn infants. Early Hum Dev 65:S19–S29
7. Simmer K, Hartmann BT (2009) The knowns and unknowns of human milk banking. Early Hum Dev 85:701–704
8. Patole S (2007) Prevention and treatment of necrotising enterocolitis in preterm neonates. Early Hum Dev 83:635–642
9. Srinivasjois R, Nathan E, Doherty D, Patole S (2010) Prediction of progression of definate necrotising enterocolitis to need for surgery or death in preterm neonates. J Matern Fetal Neonatal Med 23(7):695–700
10. Schulzke S, Despande GC, Patole SK (2007) Neurodevelopmental outcomes of very-low-birthweight infants with necrotising enterocolitis. Arch Pediat Adol Med 161:583–590
11. Rees CM, Pierro A, Eaton S (2007) Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. Arch Dis Child Fetal Neonatal Ed 92:F193–F198
12. Bisquera JA, Cooper TR, Berseth CL (2002) Impact of nectrotising enterocolitis on length of stay and hospital charges in very low birthweight infants. Pediatrics 109:423–428
13. Quigley MA, Henderson G, Anthony MY, McGuire W (2007) Formula milk versus donor breast milk for feeding preterm or low birthweight infants. Cochrane Database Systematic Reviews, Issue 4 Art. No.:CD002971. doi:10.1002/14651858.CD002971.pub2
14. Boyd CA, Quigley MA, Brockelhurst P (2007) Donor breast milk versus infant formula for preterm infants: a systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed 92:F169–F175
15. Sullivan S, Schanler RJ, Kim JH, Patel AL, Trawöger R, Kiechl-Kohlendorfer U et al (2010) An exclusively human milk-based diet is associated with a lower rate of nectrotizing enterocolitis than a diet of human milk and bovine milk-based products. J Pediatr 156:562–567
16. Kosloske AM (1997) The epidemiology and pathogenisis of necrotizing enterocolitis. Semin Neonatal 2:231–238
17. de Silva A, Jones PW, Spencer SA (2004) Does human milk reduce infection rates in preterm infants? A systematic review. Arch Dis Child Fetal Neonat Ed 89:F509–F513
18. Czank C, Prime DK, Hartmann BT, Simmer K, Hartmann PE (2009) Retention of the immunological proteins of pasteurized human milk in relation to pasteurizer design and practice. Pediatr Res 66(4):374–379
19. Schanler RJ, Lau C, Hurst NM, Smith EOB (2005) Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. Pediatrics 116(2):400–406
20. Singhal A, Cole TJ, Fewtrell M, Lucas A (2004) Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. Lancet 363:1571–1578
21. Singhal A, Cole TJ, Lucas A (2001) Early nutrition in preterm infants and later blood pressure: two cohirts after randomised trials. Lancet 2001:413–419
22. Singhal A, Fewtrell M, Cole TJ, Lucas A (2003) Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 361:1089–1097
23. Casadio YS, Williams TM, Lai CT, Olsson SE, Hepworth AR, Hartmann PE (2010) Evaluation of a mid-infrared analyser for the determination of the macronutrient composition of human milk. J Hum Lact 26:376–383

24. National Institute for Health and Clinical Excellence (2010) Donor Breast Milk Banks: The Operation of Donor Milk Bank Services
25. Arslanoglu S, Bertino E, Tonetto P, Nisi GD, Ambruzzi AM, Biasini A et al (2010) Guidelines for the establishment and operation of a donor human milk bank. J Matern Fetal Neonat Med 23(S2):1–20
26. HMBANA (2005) Guidelines for the establishment and operation of a human milk bank, 11th edn. p 39
27. Specker B, Black A, Allen L, Morrow F (1990) Vitamin B-12: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. Am J Clin Nutr 52(6):1073–1076
28. Molinari C, Casadio YS, Arthur P, Hartmann PE (2011) The effect of storage at 25 °C on proteins in human milk. Int Dairy J 21:286–293
29. Eteng M, Ebong P, Eyong E, Ettarh R (2001) Storage beyond three hours at ambient temperature alters the biochemical and nutritional qualities of breast milk. Afr J Reprod Health 5:130–134
30. Hamosh M, Ellis LA, Pollock D, Henderson T, Hamosh P (1996) Breastfeeding and the working mother: effect of time and temperature of short-term storage on proteolysis, lipolysis, and bacterial growth in milk. Pediatrics 97:492–498
31. Slutzah M, Codipilly CN, Potak D, Clark RM, Schanler RJ (2010) Refrigerator storage of expressed human milk in the neonatal intensive care unit. J Pediatr 156:26–28
32. Larson E, Zuill R, Zier V, Berg B (1984) Storage of human breast milk. Infect Cont 5:127–130
33. Lawrence RA (1999) Storage of human milk and the influence of procedures on immunological components of human milk. Acta Paediatr 88:14–18
34. Jensen R (1995) Miscellaneous factors affecting composition and volume of human and bovine milks. In: Jensen R (ed) Handbook of milk composition. Academic Press, San Diego, p 237–271
35. Jay J (2000) Modern food microbiology, 6th edn. Springer-Verlag
36. Henderson TR, Fay T, Hamosh M (1998) Effect of pasteurization on long chain polyunsaturated fatty acids and enzyme activities of human milk. J Pediatr 132:876–878
37. Tully D, Jones F, Tully MR (2001) Donor milk: what's in it and what's not. J Hum Lact 17:152–155
38. Wills ME, Han VEM, Harris D, Baum JD (1982) Short-time low-temperature pasteurisation of human milk. Early Hum Dev 7:71–80
39. Orloff S, Wallingford J, McDougal J (1993) Inactivation of human immunodeficiency virus type I in human milk: effects of intrinsic factors in human milk and of pasteurisation. J Hum Lact 9:13–17
40. Yamato K, Taguchi H, Yoshimoto S, Fujishita M, Yamashita M, Ohtsuki Y et al (1986) Inactivation of lymphocyte-transforming activity of human T-cell leukemia virus type 1 by heat. Jpn J Cancer Res 77:13–15
41. Hamprecht K, Maschmann J, Muller D, Dietz K, Besenthal I, Goelz R et al (2004) Cytomegalovirus (CMV) inactivation in breast milk: reassessment of pasteurization and freeze-thawing. Pediatr Res 56(4):529–535
42. Goldblum R, Dill C, Albrecht T, Alford E, Garza C, Goldman A (1984) Rapid high-temperature treatment of human milk. J Pediatr 104:380–385
43. Chantry C, Israel-Ballard K, Moldoveanu Z, Peerson J, Coutsoudis A, Sibeko L et al (2009) Effect of flash heat-treatment on immunoglobulins in breast milk. J Acquir Immune Defic Syndr 51:264–267
44. Goldsmith S, Dickson J, Barnhart H, Toledo R, Eitenmiller R (1983) IgA, IgG, IgM and lactoferrin contents of human milk during early lactation and the effect of processing and storage. J Food Protect 46:4–7
45. Dhar J, Fichtali J, Skura B, Nakai S, Davidson A (1996) Efficiency of a HTST system for human milk. J Food Sci 61:569–573
46. Chouliara E, Georgogianni K, Kanellopoulou N, Kontominas M (2010) Effect of ultrasonication on microbiological, chemical and sensory properties of raw, thermized and pasteurized milk. Int Dairy J 20:307–313

47. D'Amico D, Silk T, Wu J, Guo M (2006) Inactivation of microorganisms in milk and apple cider treated with ultrasound. J Food Protect 69:556–563
48. Wang J, Hu X, Wang Z (2010) Kinetics models for the inactivation of *Alicyclobacillus acidophilus* DSM14558(T) and *Alicyclobacillus acidoterrestris* DSM 3922(T) in apple juice by ultrasound. Int J Food Microbiol 139:177–181
49. Suslick K (1990) Sonochemistry. Science 247:1439–1445
50. Allison D, D'Emanuele A, Eginton P, Williams A (1996) The effect of ultrasound on *Escherichia coli* viability. J Basic Microbiol 36:3–11
51. Cameron M, McMaster L, Britz T (2008) Electron microscopic analysis of dairy microbes inactivated by ultrasound. Ultrason Sonochem 15:960–964
52. Piyasena P, Mohareb E, McKellar R (2003) Inactivation of microbes using ultrasound: A review. Int J Food Microbiol 87:207–216
53. Arroyo C, Cebrián G, Pagán R, Condón S (2011) Inactivation of *Cronobacter sakazakii* by ultrasonic waves under pressure in buffer and foods. Int J Food Microbiol 144:446–454
54. Walkling-Ribeiro M, Noci F, Cronin D, Lyng J, Morgan D (2009) Shelf life and sensory evaluation of orange juice after exposure to thermosonication and pulsed electric fields. Foods Bioprod Process 87:102–107
55. Walkling-Ribeiro M, Noci F, Riener J, Cronin D, Lyng J, Morgan D (2009) The impact of thermosonication and pulsed electric fields on *Staphylococcus aureus* inactivation and selected quality parameters in orange juice. Food Bioprocess Technol 2:422–430
56. Czank C, Simmer K, Hartmann PE (2010) Simultaneous pasteurisation and homogenisation of human milk by combining heat and ultrasound. J Dairy Res 77:183–189
57. Bintsis T, Litopoulou-Tzanetaki E, Robinson R (2000) Existing and potential applications of ultraviolet light in the food industry: A critical review. J Sci Food Agric 80:637–645
58. Shama G (1999) Ultraviolet light. Academic Press (Elsevier)
59. Falguera V, Pagán J, Garza S, Garvín A, Ibarz A (2011) Ultraviolet processing of liquid food: a review: Part 2: Effects on microorganisms and on food components and properties. Food Res Int 44:1580–1588
60. Guerrero-Beltran J, Barbosa-Canovas GV (2004) Advantages and limitations on processing foods by UV light. Food Sci Technol Int 10:137–147
61. Koutchma T (2008) UV light for processing foods. Ozone: Sci Eng 30:93–98
62. Keyser M, Muller I, Cilliers F, Nel W, Gouws P (2008) Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. Innovative Food Sci Emerg Technol 9:348–354
63. Ewaschuck J, Unger S, Harvey S, O'Connor D, Field CJ (2011) Effect of pasteurization on immune components of milk: Implications for feeding preterm infants. Appl Physiol Nutr Metab 36:175–182
64. Zoeren-Grobben D, Schrijver J, den Berg H, Berger H (1987) Human milk vitamin content after pastuerisation, storage, or tube feeding. Arch Dis Child 62:161–165

Part VII
Nutrition in Specific Conditions

Chapter 19
Feeding the Preterm Neonate with Intrauterine Growth Restriction

Flavia Indrio, Luca Maggio and Francesco Raimondi

Abstract Intrauterine growth restriction (IUGR) is the failure to achieve the genetically predetermined growth potential and may be caused by fetal, maternal, placental, and external factors. IUGR is associated with significant perinatal mortality and morbidity and adverse long-term outcomes, in preterm, especially extremely preterm infants with gestation under 28 weeks at birth. Optimal enteral feeding is crucial in this population as suboptimal nutrition during a critical phase of postnatal life is associated with a negative impact on long term neurodevelopment. However, enteral nutrition is a difficult issue in these infants considering the adverse effects of chronic hypoxia on the fetal gastrointestinal tract, and the inherent susceptibility of this high-risk population to a potentially devastating illness such as necrotising enterocolitis (NEC). Signs of feeding intolerance such as abdominal distension, large/bile stained gastric residuals are almost universal in the first week or two in extremely preterm IUGR infants and are difficult to differentiate from early NEC. This is also the period when suboptimal nutrition constitutes a nutritional emergency. Animal data associate IUGR with reduced intestinal weight (proportionate to body weight), length and wall thickness, and reduced villous height and crypt depth at the microscopic level. Initial observations on a distinct gut colonization pattern may also be relevant to the specific health hazards in this population. This chapter reviews the current strategies for enteral feeding, and the potential long term adverse effects of catch up growth (e.g., increased risk of obesity, hypertension and diabetes mellitus) in the preterm infant with IUGR.

Key points

- Intrauterine growth restriction (IUGR) is a common pregnancy outcome that carries significant mortality and morbidity in preterm infants

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3_19,

- IUGR is associated with structural and functional abnormalities of the gastrointestinal tract that may be relevant to stunted growth
- It is debated whether IUGR increases the risk of necrotizing enterocolitis in preterm infants
- Deficient nutrient uptake is associated to poor neurodevelopment in IUGR infants, and excessive catch up growth is linked to increased risk of metabolic syndrome
- Specific strategies are being evaluated for optimizing the nutrition of the preterm IUGR infant in the intensive care unit
- Future nutritional strategies will target the optimal growth window to avoid/minimise the negative outcomes associated with IUGR and catch up growth

1 Introduction

Intrauterine growth restriction (IUGR) is a failure to achieve the growth potential promised by interactions of genetic and epigenetic determinants functioning against an environment of maternal, fetal, and placental influences [1]. A common definition is a significant decrease in intrauterine fetal growth as assessed by ultrasound. IUGR manifests as a variable syndrome of suboptimal growth and body disproportions rather than a well-defined etiologic entity. Causes for IUGR are diverse and include aneuploidies, non-aneuploid syndromes, infections, metabolic factors and placental disorders. IUGR places the fetus and neonate at risk of death or disability in the perinatal period [2, 3] and predisposes the child to a lifelong increased risk for hypertension, cardiovascular disorders and renal disease, among others [4].

Reduced fetal growth and small size at birth remain a major cause of morbidity and mortality in early infancy and childhood throughout the world [5]. In addition, low birth weight has been associated with increased risk of developing metabolic syndrome or one of its components (insulin resistance, dyslipidemia, impaired glucose tolerance, type 2 diabetes -DM2- and hypertension), and cardiovascular disease in adulthood [6–8].

Infant with IUGR maybe small for gestational age (SGA), growing at a slower than normal rate due to a pathophysiological processes that either limit fetal nutrition or affect the capacity of the fetus to grow when provided with nutrients.

2 Intestinal Modification in the IUGR Newborn

Little is known about the postnatal effects of IUGR on structural and functional parameters of the gastrointestinal tract (GIT). However, this point appears to be crucial because the GIT is involved in the first steps of postnatal immune system maturation, in body protection against food allergens and environmental microorganisms and in nutrient assimilation. Several studies have been performed in animal models to evaluate the fetal intestine in presence of IUGR. Alterations in intestinal development such as impaired nutrient absorption and utilization [9, 10] and structure

Table 19.1 Intestinal morphological changes in IUGR

1	Reduced intestinal mass
2	Reduced intestinal weight and length
3	Reduced wall thickness
4	Reduced length of the intestinal villi
5	Reduced number of the intestinal villi

atrophy have been reported in the literature. The intestine of neonates with IUGR has reduced weight (proportionate to body weight), length, wall thickness, villous height, and crypt depth [11–14].

IUGR delays the normal developmental pattern of a series of intestinal parameters (increase in ileal mucosa density and villous area). IUGR also results in a relatively long and thin intestine in both preterm and term neonate. The important GIT changes in IUGR are summarized in Table 19.1.

IUGR affects intestinal development, regardless of the gestational age [15]. A recent study has shown that IUGR piglets have a reduced response to feeding probably due to the combination of prolonged exposure to IUGR stress in utero, higher energy expenditure to maintain body temperature postnatally, and the differences in gut microbiota implantation. These factors together contribute greatly to intestinal structural development. These modifications led to a reduction in the surface area of exchange of more than 60 % during the first days of life, as estimated by the combined reduction of small intestinal length, mucosa density, dry matter content and villous sizes. In contrast, intestinal enzyme activities were not markedly affected by IUGR from birth to day 5 in full-term IUGR piglets, while they were lower in preterm IUGR piglets at birth. From these data, the authors concluded that the effects of IUGR may indeed depend on delivery age (preterm or term). In the same study the authors have also demonstrated an elevated expression of PEPT1, which potentially enhanced intestinal transport of bacterial peptides, combined with the higher density of adherent bacteria and translocation. These changes together may lead to a decrease in gut barrier function and subsequently could play a role in the IUGR-related modulation of the immune system via the NF-kB pathway.

More elucidation regarding the molecular mechanisms underlying the intestinal impaired function in IUGR comes from an elegant study by D'Inca and co-workers [16]. This study also reports the role of microbiota colonization of the gut in this risk population. The authors demonstrated that IUGR affected weight and structure of the intestine and enhanced counts of adherent bacteria. Dynamic variations of intestinal genes involved in biological processes such as defense system and cell death were consistent with the IUGR-induced increase in postnatal bacterial challenge. Therefore, the lower intestinal trophic responses to enteral food introduction in piglets with IUGR during the early period of life could contribute to growth failure, a well-known morbidity associated with IUGR, and hamper digestive health in the slightly longer term. This study demonstrated that the proliferation-apoptosis homeostasis was affected in the intestine of piglets with IUGR, leading to a reduced surface of intestinal exchange. Piglets with IUGR possess a thinner intestine and a surface area of exchange reduced by 40 %, as estimated by the combined reduction of ileal weight: length ratio and villous sizes. This point appears to be crucial because of the

main role of the intestine in processing dietary molecules into available nutrients for the organism and in regulating the flux of antigenic materials that participate in the maturation of the gut-associated lymphoid tissue. Reducing the exchange surface of the ileum in piglets with IUGR may lead to compromised health [17].

Gut colonization by bacteria and the fermentation activity of the resulting intestinal microbiota may be altered in infants who have experienced IUGR compared with healthy infants because of the effect of IUGR on the small intestine. An altered initial colonization of the gut is likely to affect neonatal gut colonization by bacteria, which would explain why IUGR predisposes to NEC and induces intestinal microbial dysbiosis throughout adult life. This last assumption arises from the idea that an intestinal microbiota resembling that present in adults is definitively structured between birth and the first years of life, which is the time when the gut evolves from sterility to a highly populated bacterial ecosystem [18]. Another study by D'Inca [16] investigates whether IUGR alters the intestinal microbiota, especially the composition and activity of ceco-colonic microbiota from birth to adulthood in rats. The study considered both the immediate effects [soon after birth (day 5), before weaning (day 12), at early weaning (day 16), and on completing weaning (day 22)], and the long-term influences [sexual maturation (day 40) and adult life (day 100)]. The first effect observed was that, for all of the enumerated bacterial groups except lactobacilli, the number of bacteria either tended to be or was higher in pups with IUGR compared with control pups at day 5. Conversely, at day 12, the numbers of total and of some individual bacteria (Bifidobacterium spp, bacteria from clostridial clusters IV and XIVa) were lower in pups with IUGR than in control pups, indicating that the extent of bacterial colonization, which dramatically increased between day 5 and day 12 (i.e., from 8.9 to 11.2 log on average), was reduced in pups that had been subjected to IUGR. Interestingly, this influence particularly affected strict anaerobes, which are supposed to require lowered oxygen tension and oxidation-reduction potential before being capable of colonizing the gut [18]. Finally, the authors showed a different pattern of luminal short chain fatty acids (SCFA) probably related to the different pattern of microbiota and different pattern of orocecal transit time or of food digestibility in the small intestine. Bacteria metabolites such as SCFA may stimulate smooth muscle. In the colon they inhibit peristaltic activity and may stimulate tonic activity. Lastly, SCFA modify upper motility, inducing relaxation of the proximal stomach, lower esophageal sphincter and reducing gastric emptying via the involving of GI hormones as polypeptide YY 15. Crosstalk between the digestive nervous and motor activities, immune related mechanisms and microbiota have been considered as the main physiologic mechanisms involved. The authors of this study have also reported difference in the gene expression of SCFA. The expression of some genes involved in butyrate uptake was decreased at day 22 and luminal butyrate was decreased at day 40 in rats that had been subjected to IUGR. Such deficits in butyrate bioavailability could have a negative influence on the proliferation of colonocytes and the maintenance of colonic homeostasis by modulating the permeability [19], modulating the expression of some intestinal transcripts involved in barrier function [20], and reducing the secretion of mucins [21]. An alteration in the capability of the microbiota to produce SCFA may result from either the inherent characteristics of

Table 19.2 Intestinal biochemical and microbial changes in IUGR

1	Lactase activity maturation
2	Higher expression of the peptide transporter PEPT1
3	Expression of the proinflammatory cytokine IL-6
4	Different SCFA concentration in the intestinal lumen
5	Different intestinal microbial colonization

the microbiota or an insufficient or unbalanced provision of substrate. The major biochemical and microbial changes in the intestine in presence of IUGR are summarised in Table 19.2.

In conclusion, the intestine of IUGR neonates has reduced weight (proportionate to body weight), length, wall thickness, villous height, and crypt depth. Alterations in intestinal development such as impaired nutrient absorption and utilization and structure atrophy could be responsible for higher perinatal mortality and morbidity. They may also explain the predisposition of these infants to feeding intolerance, decreased fat absorption, and digestive diseases early in postnatal life.

In IUGR newborns the lower mucosal trophic responses to introduction of enteral feeds, indicated by the lower density and villous size, could contribute to growth failure, a well-known morbidity associated with IUGR, and hamper long-term digestive health. This issue appears to be important, because the intestinal barrier is involved in the first steps of postnatal immune system maturation, body protection against food allergens and environmental microorganisms, and nutrient assimilation.

The intestinal microbiota is now recognized as playing a key role in numerous physiological processes, including growth, angiogenesis, optimization of nutrition, and stimulation of various arms of the innate and adaptive immune systems. Therefore, any changes in microbiota density, composition, and/or activity are likely to affect the health of the host. The dysbiosis associated in these risk population could persist throughout life and drive the intestinal development during the neonatal period by dynamic modification of gene expression. All these differences in the intestine of infants with IUGR might be the starting point for investigation of nutritional strategies that will reduce the incidence of morbidity in these neonates.

3 Are IUGR Neonates at Increased Risk of Necrotizing Enterocolitis?

Necrotizing enterocolitis (NEC) is the most common surgical emergency affecting the gastrointestinal tract of infants in the neonatal intensive care unit (NICU). The incidence of NEC varies from 0.3 to 2.4 infants per 1,000 live births, with nearly 70 % of cases occurring in infants born at less than 36 weeks of gestation and up to 7 % of very low birth weight infants. The overall mortality for NEC ranges from 10 to 50 % and approaches 100 % for infants with the most severe form of the disease, characterized by extensive necrosis of the intestine leading to intestinal perforation, peritonitis, bacterial invasion, and sepsis. Despite optimal medical and

surgical management of NEC, infants that recover from disease may still require prolonged hospitalization for severe complications (intestinal obstruction, liver failure, short bowel syndrome), and are at greater risk for growth retardation and long term neurodevelopmental impairment.

The events that lead to NEC in preterm infants are multifactorial and complex; the only consistent epidemiologic risk factors for NEC are prematurity and a history of enteral feeding, which may include a rapid advancement in feeding or high osmotic strength formula feeding [22]. Recently much attention was focused on preterm infants with IUGR and abnormal blood flow on antenatal Doppler studies [23]. Increased placental resistance in the presence of placental failure leads to a reduction in end diastolic blood flow through the umbilical arteries, progressing to absent (AEDF) or reversed flow (AREDF) [24]. Fetal adaptation to chronic hypoxia involves preferential shunting of blood to the brain at the expense of the splanchnic circulation. It was shown that severe prenatal Doppler abnormalities are associated with poor fetal outcome [25, 26], but it is still debated if these infants are also at increased risk for developing NEC. Gut of the IUGR newborns has reduced weight, proportionate to body weight, length, wall thickness, villous weight, and crypt depth [10, 27]. Furthermore IUGR is associated with intestinal dysbiosis and alteration of the proliferation-apoptosis homeostasis which lead to a reduced surface of intestinal exchange [16]. Moreover, a combination of fetal hypoxia and increased mesenteric vascular resistance could produce hypoxic-ischaemic injury of the intestine or its mucosa before birth. Even if direct tissue injury does not occur, prolonged exposure to these conditions may modulate the development of motor, secretory, and mucosal function so that postnatally the intestine is more susceptible to stasis, abnormal colonisation, and bacterial invasion.

After birth, it might be expected that any circulatory redistribution would rapidly resolve because of the normal levels of circulating oxygen; however, investigators have shown persistent postnatal abnormalities in superior mesenteric artery (SMA) blood flow velocity in infants who experienced fetal AREDF [28]. Both SMA and coeliac axis blood flow velocity are considerably reduced on the first day of postnatal life and there is a slow recovery in baseline values during the first week of life [29]. Despite this recovery in baseline SMA blood flow velocity values, the dynamic response to the first enteral feed is still impaired in IUGR infants [30]. Moreover, in a prospective cohort study with analysis of Doppler flow velocity waveforms of splanchnic vessels on the first day of life, the end-diastolic velocity, mean velocity, and pulsatility index in the SMA, adjusted for gestational age at birth, were significantly predictive of the risk of NEC [31].

Experimental studies in animals show that hypoxia reduces intestinal blood flow and oxygen delivery through adrenergic vasoconstriction [32]. Increased oxygen extraction can compensate for a 30 % reduction in gut blood flow [33], but enteral feeding reduces the ability of oxygen extraction to compensate for the effects of hypoxia [34]. The metabolic demands of enteral feeding increase oxygen consumption by the intestine [35]. The combination of antenatal and persisting postnatal disturbances of gut perfusion, interacting with the metabolic demands of feeding, may adversely affect intestinal tissue oxygenation, combining with stasis and immunological factors to contribute to the development of NEC.

It is still to be confirmed whether SGA infants may be at higher risk for NEC. The case-control study by Beeby and Jeffrey of 82 infants with NEC revealed a different spectrum of associated factors for different gestational age groups: while for infants below 30 weeks gestation formula milk feeding was a significant risk factor (OR: 4, 95 % CI: 1.1–14.1), for babies of 30–36 weeks the growth retardation was a significant risk factor: OR: 6 (95 % CI: 1.3–26.8) for birth weight < 10th centile, and OR: 9 (95 % CI: 1.1–71) for birth weight < 3rd centile [36].

More recently, analysis of the effect of being SGA on outcome of 19.759 singleton infants born at 25–30 weeks' gestation and enrolled in the Vermont-Oxford Database revealed an increased risk of NEC when corrected for significant covariates (OR: 1.27, 95 % CI: 1.05–1.53) [3]. Some studies have demonstrated a closer association between AEDF or AREDF and NEC, which appears to be independent of other factors such as degree of growth retardation, prematurity and perinatal asphyxia [37, 38] while others have not confirmed these findings [39, 40].

A meta-analysis of 14 observational studies has demonstrated an increased incidence of NEC in preterm infants who had exhibited fetal AREDF compared with controls (OR: 2.13, 95 % CI 1.49–3.03) [41]. Nine of the included studies showed an excess of NEC in the AREDF infants; eight studies classified NEC using the stricter definition of radiological or surgical confirmation, of which six showed an excess of confirmed NEC in the AREDF group. Overall, confirmed NEC was not significantly increased in these studies (OR: 1.6, 95 % CI: 0.9–2.8), but the six studies examining confirmed NEC in preterm infants with IUGR showed greatly increased odds of confirmed NEC in infants with fetal AREDF (OR: 6.9, 95 % CI: 2.3–20). In many of the studies, fetuses with AREDF required earlier delivery than controls so it could be argued that the higher risk of NEC in these studies was primarily related to the lower gestational age and birth weight; nevertheless, the excess of confirmed NEC was also found in the two series that matched controls for gestation and weight (OR: 5.5, 95 % CI: 1.1–28) [37, 42]. A more recent study has confirmed the results of this meta-analysis demonstrating a strong relation between AREDF and subsequent development of NEC (OR: 5.88, 95 % CI: 2.41–14.34) also after adjustment for gestational age at birth (OR: 7.64, 95 % CI: 2.96–19.70) and after adjustment for birthweight for gestational age z score (OR: 6.72, 95 % CI: 2.23–20.25) [43].

It is important to note that all of the previous studies have examined the role of umbilical arteries Doppler flows alone. When Manogura et al. [44] investigated this topic using a more comprehensive fetal Doppler assessment that provided greater circulatory details (umbilical artery, middle cerebral artery, ductus venosus, and umbilical vein) the association between NEC and AREDF was lost. In this study 404 neonates with severe IUGR and elevated umbilical artery Doppler indices were evaluated: more than 40 % of the fetuses were under the 1st percentile corrected for gestational age at delivery; < 9 % of the fetuses were above the 5th percentile for birthweight. The multinomial logistic regression with NEC as dependent variable failed to demonstrate a relationship between placental resistance and the risk of NEC finding that birth weight and base deficit at birth were the independent risk factor for NEC. These results have raised some doubts on the reliability of all the evidences suggesting a causal relationship between NEC and abnormal placental resistance.

As discussed earlier Doppler investigations have been usually confined to single arterial beds (umbilical and mesenteric); considering the low frequency of NEC, most of the earlier studies have also been underpowered to detect a minimal but clinically significant rise in the risk for this illness. Furthermore, most of the worst AREDV cases would occur at below 28 weeks of gestation so use of multiple regression analysis is important if the overriding effect of gestational age as an important confounder is to be assessed. Finally, metabolic status at birth was not taken into consideration by any of these studies.

In conclusion, it is plausible that placental insufficiency predisposes to, but does not initiate, the cascade of events that lead to NEC and it is more likely that the limitations of prematurity define the origins of this disease. Future research about NEC should focus on the critical transition to neonatal life to identify relevant triggers in predisposed neonates.

4 Nutritional Strategies for the Clinically Unstable, Growth Restricted Infant

The idea that the gastrointestinal tract of newborns with IUGR is particularly vulnerable compared to their AGA controls is widespread among neonatologists although the evidence is debatable. Mihatsch et al. studied a population of 35 VLBW, growth restricted neonates compared to 89 AGA controls [45]. Human milk feeding was encouraged and standard preterm formula was used when breast milk was not available. They found no significant difference in the age at starting feeds (4 days with range 2–4 for SGA infants; 3 days with range 2–4 for AGA controls), the time (11 days with range 9–16 for SGA infants; 12 days with range 10–16 for AGA controls) or the age (14 days with range 12–21 for SGA infants; 15 days with range 12–21 for AGA controls), to achieve full enteral feeds (150 ml/kg). However, the relatively small study population included both symmetrical and asymmetrical growth restricted infants and data on the intrauterine hemodynamic adaptation were available only for half the enrolled patients. Also, the standardized feeding regimen in the study was particularly cautious. It dictated milk feedings with increments at a rather slow pace (16 ml/kg/day) only after bowel cleansing with enemas and initial oral tolerance of 5 % glucose solution. When, as in many NICUs worldwide, a less conservative protocol is used, the more severely IUGR infants might still show increased feeding intolerance. Indeed, the delayed introduction of enteral feeding might not be necessary as shown by a recent multicenter randomized controlled trial (ADEPT) on 404 preterm infants with birth weight below 10th centile and abnormal antenatal umbilical artery Doppler waveforms [46]. Infants who were started on enteral feeds on day of life 2 (“early introduction”) had a shorter duration of parenteral nutrition and high-dependency care, a lower incidence of cholestatic jaundice and an improved SD score for weight at discharge compared to the “late introduction” (i.e., day of life 6). The authors showed a significant advantage (with an average of 3 days) in reaching full enteral feeding of at least 150 ml/kg for the early introduction group; also there

was no difference in NEC occurrence between early and late feeds introduction with a relative risk of 1.20 (95 % CI: 0.37–3.37) although the study was powered only to detect a difference of 50 % with a 60 % power. Also, though the median gestation was 31 weeks, only 20 % approximately of the enrolled infants were below 29 weeks, when the risk of feed intolerance and NEC is expected to be highest. Indeed Gupta et al. have reported a subgroup analysis on the data from the ADEPT trial to study feeding tolerance in 82/404 growth restricted preterm infants with gestation < 29 weeks [47]. These infants were randomly allocated to early (commencing feeds at 24–48 h) or late introduction (commencing feeds at 120–144 h) of enteral feeding. Feeding intolerance was pre- defined and feed volume due to intolerance was altered or stopped at the clinician's discretion. Gestation and birth weight were comparable between the two groups. Both groups started total parenteral nutrition at a median age of 2 days and had central lines in place for an average of 18 days. Median number of days of feed intolerance was 7 days in both groups. The early feeding group had significantly more frequent episodes of intolerance compared to the late group. Birth weight < 600 g, late passage of meconium (> 72 h) and cholestasis were significantly associated with days of feeding intolerance. The median volume of feeds on first day of feeding intolerance was similar in both groups and was at volumes of 9 ml/kg/day. The median volume of feeds tolerated by infants in the first 10 days of life was much lower than the target volume in the trial. This feed intolerance in early days of life was present in both early and late feeding group. It was concluded that growth restricted infants of < 29 weeks' gestation with abnormal antenatal Dopplers failed to tolerate even the careful graded feeding regime used in the ADEPT trial. The authors suggested that this cohort of infants may require an increased duration of minimal enteral feeds and slower increments to decrease intolerance and establish full feeds [47].

Other nutritional interventions have been attempted to control feeding intolerance. A partially hydrolyzed protein formula (pHPF) has the theoretical advantage to be more readily digestible and might be a resource in the feeding intolerant preterm infant. Yet, concerns have been expressed on the nutritional adequacy of these special formulas in the premature baby. Rigo et al. have showed that preterm infants fed hydrolysed formula had significantly less nitrogen absorption and decreased protein efficiency compared to controls fed standard preterm formula [48]. A small randomized controlled trial has also demonstrated significant higher urinary excretion of essential amino acids in the group on pHPF for four weeks [49]. Results may be conditioned by several variables: source of protein (whey, casein or different whey:casein mixtures), the degree of hydrolysis with different amounts of free amino acids and oligopeptides that can greatly influence intestinal absorption.

The only study where protein formulas with varying degree of hydrolysis were tried for a prolonged period (12 weeks) in preterm infants (with no IUGR infant enrolled) did not show any significant nutritional disadvantage in comparison to standard preterm formula [50]. It was a randomized controlled trial where a total of 61 low birth weight infants were divided into four groups with different feeding strategies: extensively hydrolyzed protein formula (eHPF), pHPF, standard preterm formula and own mother's fortified breast milk. There were no differences in growth rate

(weight gain, increments in length and head circumference) or in main chemistries among eHPF, pHPF and standard preterm formula. The small number of infants enrolled makes it difficult to draw definite conclusions from this study. The data on actual nutritional intake and nitrogen absorption or retention during the trial was also not provided.

Mihatsch have reported that a pHPF formula accelerated the feeding advancement in a VLBW et al population of mixed SGA and AGA neonates when used for the initial four weeks of life [51]. The median time of parenteral nutrition was significantly reduced of three days in the hydrolysed formula group and body weight at day 28 was not affected. A subsequent double blind randomized controlled trial using a special pHPF tailored on the needs of the preterm infant (it was not specified if IUGR infants were included in the study) failed to confirm these results [52]. However, at the time of enrolment, the study population was relatively mature (corrected age 32.2 ± 3.2 weeks for control group and 32.2 ± 3.2 weeks for the treatment group; weight $1{,}376 \pm 200$ g for control group and 1419 ± 153 g for treatment group) and the low incidence of feeding intolerance in this population may contribute to explain the lack of effect of pHPF.

It is reasonable to conclude that partially, extensively and amino-acid based or elemental formulas do not have an indication in the routine nutrition of the preterm AGA or SGA infant. Special formulas might still have a role in the most clinically unstable populations of IUGR infants. Neonates with extreme degrees of formula intolerance are a good example.

An amino acid-based formula has been recently compared to standard preterm formula in a population of VLBW infants with IUGR and signs of marked intolerance that had led to withholding enteral feeds [53]. The introduction of the elemental formula was followed by a significant improvement in feeding tolerance as assessed by the frequency of voluminous (> 5 ml/kg) gastric residuals and mean gastric residual volume. No significant difference between groups was noted in growth, serum parameters and outcome at discharge, possibly due to the relatively short duration of the intervention (15.4 ± 12.3 days).

5 Growth of the Stabilized, Growth Restricted Infant: To Feed or Not to Feed

The observation in historical cohorts of an increased incidence of metabolic syndrome in formerly IUGR infants reaching adulthood has led to the theory of developmental programming or developmental origin of health and disease [54]. The insufficient provision during fetal life of protein and calories induces the fetal metabolism to adapt. However, the short-term advantage would have a long-term cost. In fact, fighting IUGR with high protein and energy diets postnatally, while granting faster growth, leads to greater accretion of fat body mass ultimately leading to the metabolic syndrome. Pylipow et al. have recently shown that excessive growth in the 16 weeks after prenatal growth failure is also associated not only with an increased BMI but also with decreased cognitive scores at the age of 7-years [55].

Still, a prolonged undernutrition after birth has been firmly linked to permanent neurological deficits [56]. Casey et al. have evaluated the impact of prenatal and/or postnatal growth problems in low birth weight preterm infants ($n = 985$) on school-age outcomes in an 8-year longitudinal evaluation [56]. All infants received standardized evaluations to age 8; 180 infants met the criteria for failure to thrive between 4 and 36 months' gestational corrected age. Children who failed to thrive had significant lower IQ scores than controls. Children who had both SGA and failure to thrive had the lowest cognitive and academic achievement scores [56].

Puzzled by the dilemma "to feed or not to feed", neonatologists will be looking for an ideal nutritional window for the catch up growth of the IUGR infant. However, in order to customize our interventions a we must understand the mechanisms behind developmental programming. Animal models show that maternal malnutrition or placental insufficiency can permanently alter the expression of genes involved in glucose homeostasis and glucocorticoid metabolism ultimately changing the phenotype [57]. This regulation involves the epigenetic level i.e., the chromatin's structure and its association with DNA transcription machinery. A good example comes from a rat maternal undernutrition model where offspring have selected DNA methylation changes on histone associated with hepatic IGF1gene. This leads to decreased hepatic IGF1-mRNA as well as a phenotype with reduced growth and altered glucose homeostasis [58].

In conclusion, although the condition of intrauterine growth failure has been known for a long time, the full appreciation of its postnatal consequences is relatively recent. We have learnt that both insufficient feeding and aggressive nutrition might have relevant pitfalls but it is unclear where to draw the line. From a nutritional standpoint, the optimal composition of parenteral and enteral feeds that meets the special requirements of infants with IUGR is still to be found. Also, future research should address specific clinical situations (e.g., the extremely low birth weight infant with IUGR; the severely feeding intolerant IUGR infant) that are not uncommon and particularly difficult to manage. Nutritional strategies during NICU admission and after discharge need to be supported by experimental as well as clinical research in the context of the current understanding of the pathophysiology of IUGR. Finally, since the prenatal and postnatal metabolic equilibrium is under genetic and epigenetic regulation, it will be essential to clarify the mechanisms by which environmental factors such as nutrition modulate gene transcription levels. Future research in this direction will likely grant us success in feeding the preterm IUGR infants in a "not too much, not too fast" fashion.

References

1. Gardosi J, Chang A, Kalyan B, Sahota D, Symonds EM (1992) Customised antenatal growth charts. Lancet 339:283–287
2. Baschat AA, Gembruch U, Gortner L, Reiss I, Weiner CP, Harman CR (2000) Coronary artery blood flow visualization signifies hemodynamic deterioration in growth-restricted fetuses. Ultrasound Obstet Gynecol 16:425–431

3. Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, Golan A (2000) Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. The Vermont Oxford Network. Am J Obstet Gynecol 182:198–206
4. Murphy VE, Smith R, Giles WB, Clifton VL (2006) Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. Endocrine Rev 27:141–169
5. Organiszation WH (2002) WHO report: reducing risks, promoting healthy life. World Health Organization, Geneva
6. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62–67
7. Hattersley AT, Tooke JE (1999) The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353:1789–1792
8. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C (1994) Thinness at birth and insulin resistance in adult life. Diabetologia 37:150–154
9. Wang T, Huo YJ, Shi F, Xu RJ, Hutz RJ (2005) Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. Biol Neonate 88:66–72
10. Xu RJ, Mellor DJ, Birtles MJ, Reynolds GW, Simpson HV (1994) Impact of intrauterine growth retardation on the gastrointestinal tract and the pancreas in newborn pigs. J Pediatr Gastroenterol Nutr 18:231–240
11. Bauer R, Walter B, Hoppe A et al (1998) Body weight distribution and organ size in newborn swine (sus scrofa domestica)—a study describing an animal model for asymmetrical intrauterine growth retardation. Exp Toxicol Pathol 50:59–65
12. Mostyn A, Litten JC, Perkins KS et al (2005) Influence of size at birth on the endocrine profiles and expression of uncoupling proteins in subcutaneous adipose tissue, lung, and muscle of neonatal pigs. Am J Physiol Regul Integr Comp Physiol 288:R1536–R1542
13. Widdowson EM (1971) Intra-uterine growth retardation in the pig. I. Organ size and cellular development at birth and after growth to maturity. Biol Neonate 19:329–340
14. Avila CG, Harding R, Rees S, Robinson PM (1989) Small intestinal development in growth-retarded fetal sheep. J Pediatr Gastroenterol Nutr 8:507–515
15. D'Inca R, Gras-Le Guen C, Che L, Sangild PT, Le Huerou-Luron I (2011) Intrauterine growth restriction delays feeding-induced gut adaptation in term newborn pigs. Neonatology 99:208–216
16. D'Inca R, Kloareg M, Gras-Le Guen C, Le Huerou-Luron I (2010) Intrauterine growth restriction modifies the developmental pattern of intestinal structure, transcriptomic profile, and bacterial colonization in neonatal pigs. J Nutr 140:925–931
17. Simmonds A, LaGamma EF (2006) Toward improving mucosal barrier defenses: rhG-CSF plus IgG antibody. Indian J Pediatr 73:1019–1026
18. Mackie RI, Sghir A, Gaskins HR (1999) Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr 69:1045
19. Mariadason JM, Kilias D, Catto-Smith A, Gibson PR (1999) Effect of butyrate on paracellular permeability in rat distal colonic mucosa ex vivo. J Gastroenterol Hepatol 14:873–879
20. Gaudier E, Jarry A, Blottiere HM et al (2004) Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. Am J Physiol Gastrointest Liver Physiol 287:1168–1174
21. Barcelo A, Claustre J, Moro F, Chayvialle JA, Cuber JC, Plaisancie P (2000) Mucin secretion is modulated by luminal factors in the isolated vascularly perfused rat colon. Gut 46:218–224
22. Berman L, Moss R (2011) Necrotizing enterocolitis: an update. Semin Fetal Neonat Med 16:145–150
23. Baschat AA, Hecher K (2004) Fetal growth restriction due to placental disease. Semin Perinatol 28:67–80
24. Baschat AA (2004) Fetal responses to placental insufficiency: an update. Br J Obstet Gynaecol 111:1031–1041
25. Gilbert WM, Danielsen B (2003) Pregnancy outcomes associated with intrauterine growth restriction. Am J Obstet Gynecol 188:1596–1599

26. Aucott SW, Donohue PK, Northington FJ (2004) Increased morbidity in severe early intrauterine growth restriction. J Perinatol 24:435–440
27. Baserga M, Bertolotto C, Maclennan NK et al (2004) Uteroplacental insufficiency decreases small intestine growth and alters apoptotic homeostasis in term intrauterine growth retarded rats. Early Hum Dev 79:93–105
28. Maruyama K, Koizumi T (2001) Superior mesenteric artery blood flow velocity in small for gestational age infants of very low birth weight during the early neonatal period. J Perinat Med 29:64–70
29. Gamsu HR, Kempley ST (1997) Enteral hypoxia/ischaemia and necrotizing enterocolitis. Semin Neonatol 2:245–254
30. Murdoch EM, Sinah AK, Kempley ST (2003) Impaired splanchnic haemodynamic responses to enteral feeding in preterm growth restricted infants. Early Hum Dev 73:93–109
31. Murdoch EM, Sinha AK, Shanmugalingam ST, Smith GCS, Kempley ST (2006) Doppler flow velocimetry in the superior mesenteric artery on the first day of life in preterm infants and the risk of neonatal necrotizing enterocolitis. Pediatrics 118:1999–2003
32. Nowicki PT, Miller CE (1988) Autoregulation in the developing postnatal intestinal circulation. Am J Physiol 254:G189–G193
33. Bulkley GB, Kvietys PR, Parks DA, Perry MA, Granger DN (1985) Relationship of blood flow and oxygen consumption to ischemic injury in the canine small intestine. Gastroenterology 89:852–857
34. Szabo JS, Mayfield SR, Oh W, Stonestreet BS (1987) Postprandial gastrointestinal blood flow and oxygen consumption: effects of hypoxemia in neonatal piglets. Pediatr Res 21:93–98
35. Nowicki PT, Stonestreet BS, Hansen NB, Yao AC, Oh W (1983) Gastrointestinal blood flow and oxygen consumption in awake newborn piglets: effect of feeding. Am J Physiol 245:697–802
36. Beeby PJ, Jeffery H (1992) Risk factors for necrotising enterocolitis: the influence of gestational age. Arch Dis Child 67:432–5
37. Malcolm G, Ellwood D, Devonald K, Beilby R, Henderson-Smart D (1991) Absent or reversed end diastolic flow velocity in the umbilical artery and necrotising enterocolitis. Arch Dis Child 66:805–807
38. Bhatt AB, Tank PD, Barmade KB, Damania KR (2002) Abnormal Doppler flow velocimetry in the growth restricted foetus as a predictor for necrotising enterocolitis. J Postgrad Med 48:182–185
39. Karsdorp VH, van Vugt JM, van Geijn HP et al (1994) Clinical significance of absent or reversed end diastolic velocity waveforms in umbilical artery. Lancet 344:1664–1668
40. Adiotomre PN, Johnstone FD, Laing IA (1997) Effect of absent end diastolic flow velocity in the fetal umbilical artery on subsequent outcome. Arch Dis Child Fetal Neonat Ed 76:35–38
41. Dorling J, Kempley S, Leaf A (2005) Feeding growth restricted preterm infants with abnormal antenatal Doppler results. Arch Dis Child Fetal Neonat Ed 90:359–363
42. Wilson DC, Harper A, McClure G (1991) Absent or reversed end diastolic flow velocity in the umbilical artery and necrotizing enterocolitis. Arch Dis Child 66:1467
43. Kamoji VM, Dorling JS, Manktelow B, Draper ES, Field DJ (2008) Antenatal umbilical Doppler abnormalities: an independent risk factor for early onset neonatal necrotizing enterocolitis in premature infants. Acta Paediatr 97:327–331
44. Manogura AC, Turan O, Kush ML et al (2008) Predictors of necrotizing enterocolitis in preterm growth-restricted neonates. Am J Obstet Gynecol 198:638. e1–e5
45. Mihatsch WA, Pohlandt F, Franz AR, Flock F (2002) Early feeding advancement in very low-birth-weight infants with intrauterine growth retardation and increased umbilical artery resistance. J Pediatr Gastroenterol Nutr 35:144–148
46. Leaf A, Dorling J, Kempley S et al (2012) Early or delayed enteral feeding for preterm growth-restricted infants: a randomized trial. Pediatrics 129:e1260–e1268
47. Gupta N, Kempley S (2010) on behalf of ADEPT study group. Analysis of feeding intolerance in growth restricted < 29 weeks infants. Presented at the Neonatal Society London, UK, Autumn Meeting. http://www.neonatalsociety.ac.uk/abstracts/guptan_2010_feedintolerance29weeks.shtml.

48. Rigo J, Salle BL, Picaud JC, Putet G, Senterre J (1995) Nutritional evaluation of protein hydrolysate formulas. Eur J Clin Nutri 49:S26–S38
49. Maggio L, Zuppa AA, Sawatzki G, Valsasina R, Schubert W, Tortorolo G (2005) Higher urinary excretion of essential amino acids in preterm infants fed protein hydrolysates. Acta paediatr 94:75–84
50. Szajewska H, Albrecht P, Stoitiska B, Prochowska A, Gawecka A, Laskowska-Klita T (2001) Extensive and partial protein hydrolysate preterm formulas: the effect on growth rate, protein metabolism indices, and plasma amino acid concentrations. J Pediatr Gastroenterol Nutr 32:303–309
51. Mihatsch WA, Franz AR, Hogel J, Pohlandt F (2002) Hydrolyzed protein accelerates feeding advancement in very low birth weight infants. Pediatrics 110:1199–1203
52. Florendo KN, Bellflower B, van Zwol A, Cooke RJ (2009) Growth in preterm infants fed either a partially hydrolyzed whey or an intact casein/whey preterm infant formula. J Perinatol 29:106–111
53. Raimondi F, Spera AM, Sellitto M, Landolfo F, Capasso L (2012) Amino acid-based formula as a rescue strategy in feeding very-low-birth-weight infants with intrauterine growth restriction. J Pediatr Gastroenterol Nutr 54:608–612
54. Painter RC, Roseboom TJ, Bleker OP (2005) Prenatal exposure to the Dutch famine and disease in later life: an overview. Reproductive Toxicol (Elmsford, NY) 20:345–352
55. Pylipow M, Spector LG, Puumala SE, Boys C, Cohen J, Georgieff MK (2009) Early postnatal weight gain, intellectual performance, and body mass index at 7 years of age in term infants with intrauterine growth restriction. J Pediatr 154:201–206
56. Casey PH, Whiteside-Mansell L, Barrett K, Bradley RH, Gargus R (2006) Impact of prenatal and/or postnatal growth problems in low birth weight preterm infants on school-age outcomes: an 8-year longitudinal evaluation. Pediatrics 118:1078–1086
57. Wiedmeier JE, Joss-Moore LA, Lane RH, Neu J (2011) Early postnatal nutrition and programming of the preterm neonate. Nutr Rev 69:76–82
58. Fu Q, Yu X, Callaway CW, Lane RH, McKnight RA (2009) Epigenetics: intrauterine growth retardation (IUGR) modifies the histone code along the rat hepatic IGF-1 gene. FASEB J 23:2438–2449

Chapter 20
Nutrition in Intestinal Failure/Short Bowel Syndrome

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Abstract Intestinal failure is the critical reduction of functional gut mass below the minimal amount necessary for adequate digestion and absorption to satisfy body nutrient and fluid requirements for maintenance and growth in children and adults. Short-bowel syndrome (SBS) is the most common cause of intestinal failure in infants, and results from surgical resection, congenital defect, or disease-associated loss of absorption capacity of the gut. It is characterized by the inability to maintain protein-energy, fluid, electrolyte, or micronutrient balances when on a conventionally accepted, normal diet, resulting in dependence on parenteral nutrition (PN). The duration of PN significantly correlates with the length of residual gut. The most common cause of SBS (35–50 % of cases) in the neonatal period is necrotizing enterocolitis. The other causes include abdominal wall defects such as gastroschisis, and omphalocele, midgut volvulus, and intestinal atresia. Approximately, 80 % of SBS in the paediatric population occurs in the neonatal period. The health burden of SBS is significant with high mortality (27.5–37.5 %), and morbidity including recurrent bouts of sepsis needing hospitalisation, prolonged hospital stay, impaired long term growth and development, and high cost of care. The pathophysiology, mechanisms of intestinal adaptation, and management of SBS are reviewed.

Key points

- Short-bowel syndrome (SBS) is the most common cause of intestinal failure in infants, and results from surgical resection, congenital defect, or disease-associated loss of absorption capacity of the gut.
- SBS is characterized by the inability to maintain protein-energy, fluid, electrolyte, or micronutrient balances when on a conventionally accepted, normal diet, resulting in dependence on parenteral nutrition (PN).
- The health burden of SBS is significant with high mortality (27.5–37.5 %), and morbidity including recurrent bouts of sepsis, impaired long term growth and development, prolonged hospital stay, and high cost of care.

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DOI 10.1007/978-94-007-6812-3_20,

- Management of SBS requires careful attention to the pathophysiology, type and length of the residual bowel, and the intestinal adaptations that follow. An individualised nutritional strategy to maintain and subsequently promote growth while minimizing or managing associated complications is the goal.

Short bowel syndrome [SBS] is a clinically complex disorder which results from a variety of causes leading to alterations in normal anatomic and physiologic functions and is associated with numerous complications including nutritional, infectious, metabolic and growth. It is difficult to define short bowel syndrome anatomically since the injury to the gastrointestinal tract can occur at different ages and different segments may be affected. Therefore, as suggested by Vanderhoof et al., short bowel syndrome is defined functionally by malabsorption in the presence of a shortened length of small intestine and subsequent inability to maintain an appropriate nutritional state on a conventional normal diet for age in conjunction with a shortened intestine [1]. SBS results in intestinal failure as manifested by an inability to maintain protein-energy, electrolyte and micronutrient balance. On the other hand, intestinal failure can occur independent of SBS as a result of intestinal obstruction, dysmotility, and congenital syndromes of malabsorption. Thus, an infant with SBS may have intestinal failure, but not all infants with intestinal failure have SBS [2, 3]. The provision of appropriate nutritional support, parenteral and enteral, and intestinal rehabilitation is an integral and essential part of gastrointestinal disorders in neonates and children.

1 Pathophysiology and Physiology

The small intestine is completely formed by 20 weeks gestation. Most of its growth occurs in the third trimester. In early prematurity [< 27 weeks gestation], the average length of the small intestine is 115 cm. This length increases to approximately 250 cm with a diameter of 1.5 cm after 35 weeks gestation. More specifically, for the preterm infant, total small and large bowel length is estimated to be 142 ± 22 cm at 19–27 weeks and 304 ± 44 cm in infants greater than 35 weeks [4]. In contrast, the adult intestine is 600–800 cm in length and 4 cm in diameter. The mucosal surface area increases with age allowing absorptive capacity to increase as well. Infants have 950 cm^2 of surface area for absorption whereas, adults have 7,500 cm^2 [4, 5]. As a consequence, the infant has a more favorable outcome compared to an adult because of the ability of the intestine to adapt and grow.

However, mucosal surface is not uniform throughout the small intestine as approximately one-half of the surface is contained within the proximal fourth of the small intestine. Absorption of fluids and nutrients occurs throughout the length of the small intestine, with more of it in the proximal bowel. The jejunum has a large absorptive surface area, long villi and a high concentration of enzymes and transport carrier proteins. In addition, the jejunum allows free flux of water and electrolytes from the vascular space to the intraluminal milieu. The duodenum and jejunum are the main sites for the absorption of carbohydrate, fat and protein. Iron, calcium, copper,

water and fat-soluble vitamins are also absorbed in the proximal small bowel. The distal small bowel is involved in the absorption of intrinsic factor bound B_{12} and the active transport of bile salts. The jejunum allows rapid flux of water and electrolytes, whereas the ileum acts as a functional reserve for substances not absorbed proximally, including efficient water absorption. The ileocecal valve acts to slow transit time of intestinal contents and bacteria into the ileum. The ileum also produces hormones that affect gut motility including enteroglucagon and peptide YY. It is therefore important to underscore the fact that loss of intestine due to disease or surgical resection will lead to specific losses of nutrients and expectations of absorption and choice of nutrition would depend on the area of loss. Moreover, bacterial colonization of the small bowel can reduce absorption of Vitamin B_{12}, deconjugate bile salts, reduce bile salt absorption and impair gut function [6, 7]. In SBS, however, small bowel bacterial overgrowth is common which is associated with malabsorption, and thus more dependence on parenteral nutrition and subsequent hepatic dysfunction [8].

As stated above, virtually all digestion and absorption is completed in the proximal small bowel. In the absence of an intact colon, the minimum length estimated to be required to avoid parenteral nutrition is approximately 100 cm and with a shorter segment of jejunum, significant malabsorption occurs [9]. Although the ileum is limited in its capacity to produce chylomicrons, studies have demonstrated that the ileum has a greater adaptive function to improve absorption in SBS. The intact colon becomes an important digestive organ with its ability to absorb fluids, electrolytes, medium-chain triglycerides, short-chain fatty acids [from carbohydrate salvage], amino acids and calcium [10–13]. Complex carbohydrates, starches and non-starch polysaccharides are poorly absorbed in the small intestine and pass into the colon where bacteria can ferment the short chain fatty acids which are then absorbed by the colonocyte and provide energy [14, 15].

Abnormalities in motility may also be observed after bowel resection. For example, gastric emptying is more rapid following ileal resection as is the jejunal transit time. Presence of the colon does allow some compensation in the transit time. In addition, motility may decrease with an increase in either small intestinal transit time or dilatation of the small intestine to allow for more nutrient contact time and increased surface area. However, this may be associated with increased bacterial growth as discussed previously.

2 Incidence

The incidence of SBS is approximately 0.02–0.1 % of all live births, 0.5–2 % among neonates and 0.7 % among very low birth weight infants with 80 % occurring in the neonatal period [16–18; http://www.uptodate.com/contents/management-of-the-short-bowel-syndrome-in-children/abstract/5]. Mortality rates depend on the age of the infant, the etiology of SBS, presence of infectious complications, hepatic dysfunction and/ or failure, and is estimated to be between 20 and 40 % [19–22].

Common causes of SBS are listed in Table 20.1 [2, 7].

Table 20.1 Common causes of Short Bowel Syndrome

Cause	Percent infants
Necrotizing enterocolitis	29
Malrotation with midgut volvulus	27
Multiple intestinal atresias	23
Gastroschisis	10
Aganglionosis	4
Other	7

3 Intestinal Adaptation

The degree to which the intestine adapts after the injury is the key to successful rehabilitation and survival. This process, adaptation, allows the intestine to functionally adapt to receive more nutrients in the face of a limited absorptive capacity [23]. Adaptation is characterized by cellular hyperplasia, villus hypertrophy, increased crypt depth and bowel dilatation [24]. The cell production rate in the crypt is governed by several factors including cell cycle time; in the human, jejunum cell cycle time is about 48 h [25]. Starvation, as occurs early in the adaptation process, reduces cell proliferation and increases cell cycle time [26]. Cell proliferation usually occurs in the bottom 2/3rd of the crypt and larger this area, the greater the cell production rate. These processes increase the absorptive surface and take variable periods of time, perhaps 1–2 years. The process of adaptation also includes changes in carrier proteins and enzymes on the remaining brush border, changes in motility and growth [24]. According to Jeppesen and Mortensen, therapies should be directed at improving adaptation to achieve a better state of intestinal function-"hyperadaptation", reduce time to reach this state-"accelerated adaptation", or both [15]. The increased absorptive surface area does not result in functional improvement immediately. There appears to be some functional immaturity in the epithelium as evidenced by measurements of lactase, maltase and surcrase. On the other hand, thymidine kinase is often increased and the functional immaturity gradually changes as absorptive function improves [27]. Functional adaptation can also occur independently of villus hyperplasia. Differences in absorption of glucose and amino acids may also be observed and changes in nutrient concentration can influence adaptive change. For example, increasing intake of carbohydrates may result in an increase in glucose transport without affecting the villus or mucosal mass. Thus, depending on the type of dietary substrate, the absorption of different substrates will improve.

Factors that influence intestinal adaptation include the residual bowel anatomy and site, nurtients, growth factors and hormones. For example, recovery from ileal resection is better than that from jejunal resection because glucagon-like peptide 2 [GLP-2], peptide YY and enetroglucagon promote adaptation and are produced in the ileum; in addition, they also influence intestinal transit time and gastric emptying [1, 24] In animal models of SBS, epithelial hyperplasia is observed within 24–48 h after bowel resection [28–33]. These changes are associated with the expression of several genes and it is thought that some of these changes may be mediated by microRNAs [34–37]. The mechanisms associated with intestinal adaptation are not

Table 20.2 Nutrients that influence intestinal adaptation

Arginine or Citrulline	Reduce intestinal permeability, can enhance intestinal adaptation
Glutamine	Reversed intestinal hypoplasia in an animal model [parenteral supplementation]; important fuel for the enterocyte [but enteral supplementation in animal models did not demonstrate any benefit]
Triglycerides	Enteral supplementation with long-chain triglycerides appears more beneficial than medium chain triglycerides
Omega-3 fatty acids	Beneficial in intestinal adaptation in the small intestine and colon; additional benefit may include reduction in hepatic dysfunction

completely understood, but the presence of nutrients in the lumen strongly influences adaptation. Further, intestinal angiogenesis and new blood vessel growth also occurs as a result of the adaptive process. Multiple growth factors – epidermal growth factor, transforming growth factor-α, insulin-like growth factor-1, keratinocyte growth factor and specific proteins- wnt proteins, intestinal oligopeptide transporter [Pept-1], ghrelin, are all expressed throughout the intestine and have been shown to enhance intestinal adaptation [38].

4 Nutrient Effects

Early establishment and maintenance of enteral feeding is an important part of intestinal rehabilitation. The effect is mediated by growth factors discussed above as well as by pancreatic and biliary secretions. Patients with SBS have a decreased bile acid pool as a result of an interruption in enterohepatic circulation; this in turn leads to fat malabsorption and associated vitamins and thus affects overall nutritional status. Increasing bile acid pool has been shown to have positive effects on intestinal adaptation [39, 40]. Studies in parenterally fed animals have demonstrated that adaptation requires enteral feeding and does not occur with parenteral nutrition alone [41]. Other nutrients that may influence intestinal adaptation are included in Table 20.2

5 Site of Intestinal Resection

As previously mentioned, the site of bowel resection and the physiology of the remaining bowel dictate not only function but also the feeding strategy.

5.1 Jejunal Resection

The jejunum plays an extremely important role because of its large absorptive surface; therefore, resection in this area results in a reduction in absorption. The jejunum

adapts to resection by changes in transport and enzyme activity [42]. Increases in mucosal weight, DNA and protein have been demonstrated in jejunal tissue [43]. The jejunum only responds in a modest fashion to enteral feeds and may require the concomitant presence of the ileum. Treatment with IGF-1 may also be beneficial [44]. Another way of looking at the evidence is presented by the Guidelines for management of patients with a short bowel [45]. These are:

- If less than 100 cm of jejunum remains, parenteral nutrition and fluids likely to be needed in the long term [Grade B]
- If less than 200 cm of jejunum remains, oral feedings may suffice with a supplement [Grade B]
- Hypomagnemia is common [Grade C]
- Jejunal output may be decreased by drugs that decrease motility or those that reduce gastric acid secretion [Grade B]

5.2 Ileal Resection

The ileum is responsible for Vitamin B_{12} absorption, bound to intrinsic factor. Therefore, resection can lead to malabsorption and clinical deficiency of Vitamin B_{12} in unsupplemented patients. If extensive ileal resection occurs, there is a disruption in enterohepatic circulation accompanied by bile salt deficiency and subsequent fat malabsorption. The length of ileum required to maintain or disrupt these functions are not clearly defined. The bile acid malabsorption leads to an increase in bile acid production, but increases bile acid passage may result in secretory diarrhea. Further, malabsorption of bile acids causes excess absorption of oxalate possibly leading to kidney stone formation. Unabsorbed lipids reaching the ileum can lead to a delay in gastric emptying possibly mediated by hormones such as peptide YY [46, 47]. Fluids and electrolytes can also be lost due to ileal resection and has implications for the type of carbohydrates that are subsequently fed. Lastly, the ileum has a much better adaptive capacity compared to the jeunum.

5.3 Ileocecal Valve

The ileocecal valve helps regulate passage of nutrients and fluid to the colon and its loss is associated with a longer duration of parenteral nutrition because of a reduction in intestinal transit time leading to decreased absorption and therefore a greater dependency on the parenteral route. Its loss is also associated with increased bacterial overgrowth [8]. Other risk factors for bacterial overgrowth include surgical blind loops, intestinal dysmotility, stasis, hypochlorhydria and malnutrition. Bacterial overgrowth also promotes bacterial translocation, lactic acidosis and infections with enteric organisms.

5.4 Colon

Given its role in absorption of water, electrolytes and short chain fatty acids, loss of the colon leads to fluid and electrolyte depletion. The colon does have an adaptive capacity, but evidence suggests that feeding of an elemental diet may slow the rate of colonic adaptation [48].

6 Management of SBS

Parenteral nutrition is the cornerstone for the initial management of infants with SBS and in turn, depending on its duration and dependence, can also contribute to both morbidity and mortality. Initially, however, the goal should be to maintain fluid and electrolyte balance and provision of adequate calories to maintain or promote growth. Patients tend to have large volume losses of fluids and electrolytes and careful measurement of loses and appropriate replacement is of paramount importance. Appropriate parenteral nutrition including electrolytes requires a multidisciplinary team approach since prolonged use of parenteral nutrition is associated with hepatic dysfunction ranging from cholestasis, steatosis, cholelithiasis to end stage liver disease. Another issue is the use of lipids as the currently available lipid emulsions are predominantly soy based with the phytosterols potentially displacing cholesterol from the lipid pool and increasing the oxidant load. Once liver disease has developed, "lipid reducing" strategies, providing lipid infusions only 2–3 times per week or limiting the quantity of infused lipid to 1–2 g/kg/day may be useful to reverse the disorder. Another potential treatment for PN cholestasis has been the use of fish-oil based emulsions which in limited, small studies, have been demonstrated to reverse TPN induced cholestasis when compared to historical controls. In animal models, a reduction in fat deposits in the liver was observed when intravenous omega-3 fatty acid emulsion was given [49, 50]. To date, however, no study has demonstrated prevention of hepatic dysfunction with the use of these fish-oil based emulsions in infants and children. Once enteral losses have decreased, enteral nutrition is started as outlined below.

Loss of ileum and a retained functional colon [45]

- May need parenteral nutrition if less than 50 cm small intestine remains [Grade B]
- Need a high carbohydrate low oxalate diet [not usually applicable in infants] [Grade A]

Enteral nutrition should be provided as soon as possible, for example, after an ostomy is deemed functional, although the mainstay of nutritional therapy during this phase of recovery will be parenteral. Enteral feeding promotes intestinal adaptation and as stated previously, the adaptive process depends on the presence and quality of enteral nutrients [51]. Enteral nutrition also plays a role in maintaining normal gut flora and decreasing the incidence of bacterial translocation [52]. Knowing the site of lesion and loss of intestinal segments involved will help the provider in planning

a strategy. For example, fluid replacement along with electrolytes would be a major initial goal in lesions involving the stomach or proximal small bowel as large volume losses are common. Infants with SBS may develop hypersecretion of gastric acid and fluids in variable amounts and for variable times. These in turn, may deactivate pancreatic enzymes, decrease pH, alter enteral absorption of fats and drugs and increase fluid losses [53]. Initially, a H2 blocker may be given intravenously to suppress gastric hypersecretion.

Enteral Nutrition can be provided either by small frequent feedings or by continuous infusion; the latter has the advantage of "exposing" the intestine to small quantities of nutrients and as the intestine adapts, the amounts can be increased [54]. A variety of products are available and a particular product should be selected based on the individual patients' needs. It should be emphasized that attention be given to oral feeding even if it is non-nutritive sucking or in very small amounts to avoid the development of oral aversion that frequently develops in chronically ill or tube fed infants.

The three categories of formulas commonly used are as follows:

1. Cow milk based formulas: Since there may be an increased risk of cow milk protein allergy or intolerance, it is not uncommon to begin feeds in infants with SBS with either human milk or semi-elemental formulas.
2. Semi-elemental formulas: The nitrogen source are proteins that have been hydrolyzed into oligopeptides of varying lengths, dipeptides and tripeptides. The latter have specific transport mechanisms and are thought to be absorbed better and more efficiently than individual amino acids or whole proteins [55, 56]. Casein hydrolysate formulas stimulate jejunal reabsorption of water and electrolytes.
3. Elemental Formulas: These formulas contain individual amino acids, are low in fat [especially long-chain triglycerides] and are thought to require minimal digestive function and cause less stimulation of pancreatic secretions. In many products, medium-chain triglycerides are the predominant fat source and can be absorbed in the absence of lipase or bile salts.

In general, malabsorption occurs from a defect in transport of nutrients across the mucosa as in some disease states [e.g., Crohn's disease] or due to intra-luminal defects of absorption as occurs with bile salt deficiency or bacterial overgrowth. Infants with SBS are considered ideal candidates for semi-elemental and elemental formulas because of the malabsorption associated with SBS and the benefit of more efficient absorption. In adults, results of efficacy between these two formulas are conflicting and will not be discussed here. In children with SBS, no significant difference in intestinal permeability, energy and nitrogen balance were found when comparing feedings with hydrolyzed protein vs non hydrolyzed protein [57].

6.1 Protein

The initial feeding of choice is human milk unless it is suspected that the carbohydrate sources [lactose] may not be tolerated. Once feedings are initiated and advanced,

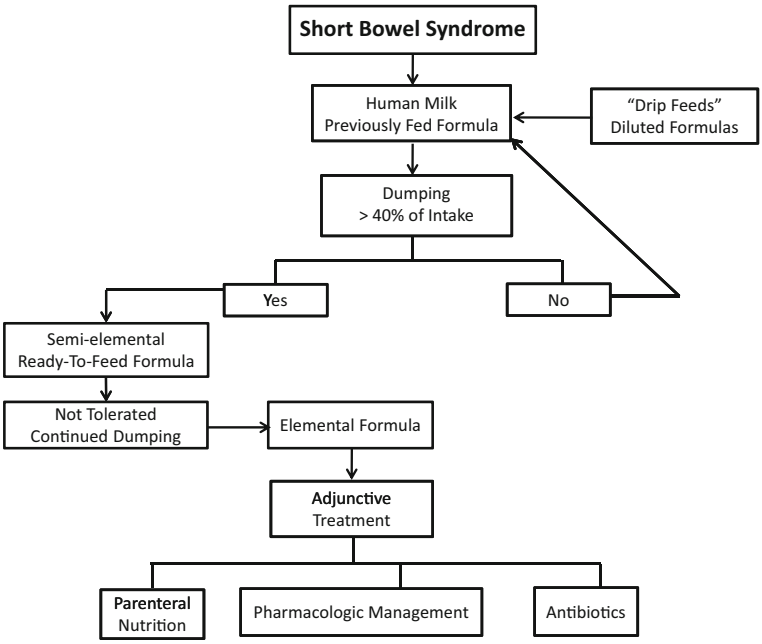

Fig. 20.1 Suggested algorithm in feeding infants with short bowels syndrome

careful assessment of output is important. If "dumping" [arbitrarily defined as output $> 40\,\%$ input], occurs, the next choice would be a semi-elemental formula which would be hydrolysates of casein or whey, rationale behind which is the increased risk of cow milk protein allergy or intolerance to intact cow-milk protein [58, 59]. If these formulas are not tolerated by the same criteria used above, the next step would be to use an elemental formula which contains free amino acids. A suggested algorithm is shown (Fig. 20.1). Some practitioners tend to use diluted formulas to assure tolerance but this practice is controversial since the feeds may become iso-osmolar when they reach the proximal bowel.

6.2 Fats

Infants and young children will benefit from a diet relatively high in fat [$\sim$40 %] given their higher energy needs for growth. In addition, higher fat containing formulas do not present a higher osmotic load and may decrease gut motility. Long chain triglycerides are not well absorbed in the small intestine making formulas with medium chain triglycerides a better choice. The latter are also water soluble and better absorbed in the presence of bile acid or pancreatic insufficiency. There is some evidence that high proportion of fat in the form of MCT stimulates mucosal

adaptation to a lesser degree than feedings that contain long-chain triglycerides [60]. Thus in infants where luminal bile acid concentrations may be decreased due to ileal resection, a combination of medium and long-chain triglycerides may be preferred [61]. Malabsorption due to fat will result in steatorrhea, whereas secretory diarrhea may occur with the loss of the distal ileum. Enterally administered triglycerides and omega-3 fatty acids as well as parenteral omega-3 fatty acids appear to be beneficial in inducing adaptation in the small intestine and colon in an animal model [62, 63].

6.3 Carbohydrates

Most infants with SBS do not tolerate high amounts of carbohydrates because of the increased osmotic load. Lactose may also be poorly absorbed due to relative lactase deficiency. In patients with an intact colon, a stool pH < 5.5 may indicate carbohydrate malabsorption with a resultant increase in osmotic diarrhea. In older infants, if diarrhea persists, addition of fiber to the formula may decrease stool quantity. Fiber may also play a role in intestinal adaptation by producing short-chain fatty acids [64]. Starches need to be avoided because they are hydrolyzed in the upper gastrointestinal tract and produce an osmotic load.

In general, a formula containing a protein hydrolysate, medium chain triglycerides and glucose polymers appears to be a good initial choice for infants with SBS in the absence of using human milk. In addition, careful attention needs to be paid to both fat and water soluble vitamins, vitamin B_{12} [ileal resection] and iron. The supplementation of iron is important in infants with the loss of proximal bowel. Supplements of copper, zinc and sodium may also be required with extensive diarrhea. Parenteral nutrition needs to be tapered as enteral nutrition advances.

Pharmacological therapy includes the use of antisecretory agents to reduce stomal and fecal losses in infants with SBS and antimotility agents and these include:

- H_2 blockers and proton pump inhibitors: excess gastrin levels
- Cholestyramine: a trial may be warranted if bile salt malabsorption is thought to be resulting in diarrhea. This resin binds bile acids; however, if bile acid deficiency is present as in the case of massive ileal resection, this agent may exacerbate steatorrhea
- Loperamide: may be useful in older infants and children, but also may promote bacterial overgrowth; this may be beneficial in patients with rapid bowel transit time
- Octreotide: increases small bowel transit time; limited evidence in infants; fat malabsorption may occur

6.4 Chronic Complications

As stated previously, careful management and evaluation and treatment of complications of SBS requires a multidisciplinary approach. Complications not only

are related to the delivery of parenteral nutrients [catheter related] but also to the development of infectious and metabolic consequences.

Bacterial overgrowth is often unrecognized in neonates. A high concentration of gastric acid normally limits the number of bacteria entering the small intestine. Thus, agents that reduce gastric acidity should be used with caution. This is of particular importance since some clinicians routinely use acid reducing agents after the placement of feeding gastrostomies. Since SBS involves both interruption in bowel anatomy and motility, when motility slows, the bowel gets dilated and it is not uncommon for bacterial content of the proximal small intestine to exceed 10^5. Reduction in gut-associated lymphatic tissue may also impair the immune response to the bacteria [65]. These bacteria deconjugate bile salts resulting in rapid reabsorption of bile acids thus depleting the bile acid pool; this in turn impairs micellar solubilization and results in malabsorption of fat and fat-soluble vitamins. Bacterial overgrowth also causes mucosal inflammation which exacerbates nutrient malabsorption in addition to competing for B_{12}; bacterial overgrowth should be considered when there is continued malabsorption. Although breath hydrogen determination, aspiration and testing of small bowel intestinal content and urine indican are used to diagnose bacterial overgrowth, these tests are impractical and not used in the neonatal population. The overgrowth can also be suspected, when in addition to diarrhea, infants manifest d-lactic acidosis or symptoms of colitis. D-lactic acidosis occurs because only the L-form is well metabolized in humans leaving behind the d-form as both are produced by the bacteria [66]. Bacterial overgrowth, once suspected is treated with broad spectrum antibiotics every 2–4 weeks; the usual agents are metronidazole in combination with trimethoprim-sulphamethoxazole or neomycin.

Diarrhea due to excessive fluid secretion also occurs simply as a result of excessive osmotic load in the small intestine due to excessive carbohydrates in the diet. This may also occur as a result of elevated serum gastrin levels and may respond to H_2 receptor antagonists.

Nutrient Deficiencies largely occur in the case of micronutrients such as minerals, trace elements and vitamins. Malabsorption of fat- soluble vitamins, A, D, and E is common and will require replacement especially in the face of hepatic dysfunction. Iron and zinc deficiencies have also been demonstrated in SBS. The latter is manifested as low serum zinc in the face of a low serum alkaline phosphatase level. Since zinc deficiency results in poor growth, it may delay intestinal adaptation and exogenous zinc may be needed [67]. As previously mentioned, hypomagnesemia, hypocalcemia, and selenium malabsorption may also be present. Adequate intakes of calcium, magnesium as well as Vitamin D will assure improvement in bone mineralization [68].

To improve gut function, ostomy re-feeding has been used with limited success. The idea is to use the unused portion of the distal gut and also to minimize losses of GI contents from the proximal gut.

Surgical options which are beyond the scope of this chapter include intestinal lengthening and/or tailoring [Bianchi or STEP procedure] or a liver-intestine transplant as these infants often develop severe hepatic dysfunction as well. Hepatic

dysfunction and associated complications and treatment strategies are discussed elsewhere in this text.

Management of SBS requires careful attention to the pathophysiology, residual bowel and type of bowel present, and the intestinal adaptations that follow. A nutritional strategy to individualize therapy to maintain and subsequently promote appropriate growth while minimizing or managing associated complications is the goal. Regardless of the feeding mode, attention to psychosocial factors including oral motor and overall development is of paramount importance. Early referral to speech, occupational and developmental therapists may be helpful for feeding and social support. Thus, with improvement in parenteral nutrition and nutrient delivery devices, improvements in enteral formulations, attention to associated complications and the advent of transplant technology, have all improved outcome in this group of patients.

Until a few decades ago, the prognosis after the development of short gut syndrome, was extremely poor for lack of available nutritional strategies. The onset of parenteral nutrition and its advancements, development of specialized formulas and adjunctive strategies have changed the prognosis to one of survival [83]. More than 90 % of infants and children survive after extensive bowel resection in the neonatal period. In a follow up study at 15 years, overall survival was nearly 90 % out of 87 infants followed [84]. It is anticipated that with the advent of small bowel and liver transplantation, the use of fish oil-containing lipid emulsions and advances in enteral formulations, that the survival rate could be increased further.

References

1. Vanderhoof JA, Young RJ (2001) Enteral nutrition in short bowel syndrome. Sem Pediatr Surg 10:65
2. Bhatia J, Gates A, Parish A (2010) Medical management of short gut syndrome. J Perinatol 30:S2–S5
3. O'Keefe SJ, Buchman AL, Fishbein TM et al (2006) Short bowel syndrome and intestinal failure: consensus definitions and overview. Clin gastroenterol hepatol 4:6–10
4. Touloukian RJ, Smith GJ (1983) Normal intestinal length in preterm infants. J pediatr surg 18:720–723
5. O'Neill (2003) The American pediatric surgical association. www.pediatricsurgerymd.org/AM, page 1–7. Principles of Pediatric Surgery, Elsevier
6. Ziegler MM (1986) Short bowel syndrome in infancy. Etiology and management Clin Perinatol 13:167
7. Taylor SF, Sokol RJ (1991) Infants with short bowel syndrome. In: Hay WM (ed) Neonatal nutrition and metabolism. Mosby, St. Louis, MO. pp 432–450
8. Cole CR, Ziegler TR (2007) Small bowel bacterial overgrowth a negative factor in gut adaptation in pediatric SBS. Curr gastroenterol Rep 9:456–462
9. Cuffari C Pediatric short bowel syndrome. Emedicine.medsacpe.com/article/931855-overview. Accessed 22 March 2012
10. Phillips SF, Giller J (1973) The contribution of the colon to electrolyte and water conservation in man. J Lab Clin Med 81:733–746
11. Debongie JC, Phillips SF (1978) Capacity of the colon to absorb fluid. Gastroenetrology 74:698–703

12. Hylander E, Ladefoged K, Jarnum S (1990) Calcium absorption after intestinal resection the importance of preserved colon. Scand J Gastroenetrol 25:705–710
13. Ruppin H, Bar MS, Soergela KH et al (1980) Absorption of short chain fatty acids by the colon. Gastroenterology 78:1500–1507
14. Buchman A, Kotlar D, Abu-Elmagd K (2004) Practical approach to the management of short bowel syndrome. Gastroenterology Endoscopy, News, gastroendonews.com
15. Jeppesen PB, Mortensen PB (2003) Experimental approaches dietary and hormone therapy. Best Pract Res Clin Gastroenetrol 17:1041–1054
16. Wales PW, de Silva N, Kim J et al (2004) Neonatal short bowel syndrome population-based estimates of incidence and mortality rates. J Pediatr Surg 39:690
17. Salvia G, Guarino A, Terrin G et al (2008) Neonatal onset intestinal failure: an Italian Multicenter Study. J Pediatr 153:674
18. Cole CR, Hansen NI, Higgins RD et al (2008) Very low birth weight preterm infants with surgical short bowel syndrome: incidence, morbidity and mortality, and growth outcomes at 18–22 months. Pediatrics 122(3):e573–e582
19. PediatricWales PW, de Silva N, Kim J et al (2004) Neonatal short bowel syndrome: population-based estimates of incidence and mortality rates. J Pediatr Surg 39:690
20. Quirós-Tejeira RE, Ament ME, Reyen L et al (2004) Long-term parenteral nutritional support and intestinal adaptation in children with short bowel syndrome: a 25-year experience. J Pediatr 145:157
21. Wales PW, de Silva N, Kim JH et al (2005) Neonatal short bowel syndrome: a cohort study. J Pediatr Surg 40:755
22. Grosfeld JL, Rescorla FJ, West KW (1986) Short bowel syndrome in infancy and childhood. Analysis of survival in 60 patients. Am J Surg 151:41.s 2008 122:e573
23. Williamson RCN, Chir M (1978) Intestinal adaptation: structural, functional and cytokinetic changes. N Engl J Med 298:1393–1402
24. Buchman AL, Scolapio J, Fryer S (2003) AGA technical review on short bowel syndrome and intestinal transplantation. Gastroenterology 124:1111–1134
25. Wright NA, Watson A, Morley A et al (1973) The cell cycle time in the flat [avillous] mucosa of the human small intestine. Gut 14:603
26. Al-Dewachi HS, Wright NA, Appleton DR, Watson AJ (1978) The effect of starvation and refeeding on cell population kinetics in the rat small bowel mucosa. J Anat 119:105
27. Bury KD (1972) Carbohydrate digestion and absorption after massive resection of the small intestine. Surg Gynecol Obstet 135:177–187
28. Dowling RH, Booth CC (1966) Functional compensation after small-bowel resection in man. Demonstration by direct measurement. Lancet 2:146
29. Dowling RH, Booth CC (1967) Structural and functional changes following small intestinal resection in the rat. Clin Sci 32:139
30. Hanson WR, Osborne JW (1971) Epithelial cell kinetics in the small intestine of the rat 60 days after resection of 70 % of the ileum and jejunum. Gastroenterology 60:1087
31. Hanson WR, Osborne JW, Sharp JG (1977) Compensation by the residual intestine after intestinal resection in the rat. I. Influence of amount of tissue removed. Gastroenterology 72:692
32. Hanson WR, Osborne JW, Sharp JG (1977) Compensation by the residual intestine after intestinal resection in the rat. II. Influence of postoperative time interval. Gastroenterology 72:701
33. Nygaard K (1967) Resection of the small intestine in rats. 3. Morphological changes in the intestinal tract. Acta Chir Scand 133:233
34. Juno RJ, Knott AW, Profitt SA et al (2004) Preventing enterocyte apoptosis after massive small bowel resection does not enhance adaptation of the intestinal mucosa. J Pediatr Surg 39:907
35. Stern LE, Erwin CR, Falcone RA et al (2001) CDNA microarray analysis of adapting bowel after intestinal resection. J Pediatr Surg 36:190
36. Erwin CR, Jarboe MD, Sartor MA et al (2006) Developmental characteristics of adapting mouse small intestine crypt cells. Gastroenterology 130:1324

37. Balakrishnan A, Stearns AT, Park PJ et al (2012) Upregulation of proapoptotic microRNA mir-125a after massive small bowel resection in rats. Ann Surg 255:747
38. Jackson C, Buchman AL (2005) Advances in the management of short bowel syndrome. Curr Gastroenerol Rep 7:373–378
39. Emmett M, Guirl MJ, Porter JL et al (2003) Conjugated bile acid replacement therapy reducing urinary oxalate excretion in short bowel syndrome. Am J Kidney Dis 41:230–237
40. Furst T, Bott C, Stein J, Dressman JB (2005) Enteric coated cholylsarcosine microgranules for the treatment of short bowel syndrome. J Pharm Pharmacol 57:53–60
41. Feldman EJ, Dowling RH, McNaughton J, Peters TJ (1976) Effects of oral versus intravenous nutrition on intestinal adaptation after small bowel resection in the dog. Gastroenterology 70:712
42. De Francesco A, Malfi G, Delsedime L et al (1994) Histological findings regarding jejunal mucosa in short bowel syndrome. Transplant Proc 26:1455
43. Vanderhoof JA, Kollman KA, Griffin S, Adrian TE (1997) Growth hormone and glutamine do not stimulate intestinal adaptation following massive small bowel resection in the rat. J Pediatr Gastroenterol Nutr 25:327
44. Gillingham MB, Dahly EM, Carey HV et al (2000) Differential jejunal and colonic adaptation due to resection and IGF-I in parenterally fed rats. Am J Physiol Gastrointest Liver Physiol 278:G700–G
45. Nightingale J, Woodward JM (2006) On behalf of the small bowel and nutrition committee of the British society of gastroenterology. Gut 55:iv1–iv12
46. Welch IM, Cunningham KM, Read NW (1988) Regulation of gastric emptying by ileal nutrients in humans. Gastroenterology 94:401
47. Van Citters GW, Lin HC (2006) Ileal brake: neuropeptidergic control of intestinal transit. Curr Gastroenterol Rep 8:367
48. Healey KL, Bines JE, Thomas SL et al (2010) Morphological and functional changes in the colon after massive small bowel resection. J Pediatr Surg 45:1581
49. Alwayn IP, Gura K, Nose V, Zausche B, Javid P, Garza J, Verbesey J, Voss S, Ollero M, Andersson C, Bistrian B, Folkman J, Puder M (2005) Omega-3 fatty acid supplementation prevents hepatic steatosis in a murine model of nonalcoholic fatty liver disease. Pediatr Res 57:445
50. Van Aerde JE, Duerksen DR, Gramlich L, Meddings JB, Chan G, Thomson AB, Clandinin MT (1999) Intravenous fish oil emulsion attenuates total parenteral nutrition-induced cholestasis in newborn piglets. Pediatr Res 45:202
51. Warner BW, Vanderhoof JA, Reyes JD (2000) What's new in the management of short gut syndrome in children. Am J Coll Surg 190:275
52. Pfau PR, Rombeau JL (2000) Advances in gastroenterology: nutrition. Med Clin North Am 84:1209
53. Severijnen R, Bayat N, Bakker H et al (2004) Enteral drug absorption in patients with short small bowel: a review. Clin Pharmacokinet 43:951
54. Andorsky DJ, Lund DP, Lillehei CW et al (2001) Nutritional and other postoperative management of neonates with short bowel syndrome correlates with clinical outcomes. J Pediatr 139:27
55. Farrell JJ (2002) Digestion and absorption of nutrients and vitamins. In: Feldman M, Freidman LS, Sleisenger MH (eds) Sleisenger and Fordtrans's gastrointestinal and liver disease: pathophysiology, diagnosis, management. 7th edn. Saunders, Philadelphia 2v. xli, 2385, 98
56. Silk DB, Faircloth PD, Clark ML et al (1980) Use of a peptide rather than a free amino acid nitrogen source in chemically defined "elemental" diets. JPEN 4(6):548–553
57. Ksiazyk J, Piena M, Kierkus J et al (2002) Hydrolyzed versus nonhydrolyzed protein diet in short bowel syndrome in children. J Pediatr gastroenetrol Nutr 35:615–618
58. Mazon A, Solera E, Alentado N et al (2008) Frequent IgE sensitization to latex, cow's milk, and egg in children with short bowel syndrome. Pediatr Allergy Immunol 19:180
59. Andorsky DJ, Lund DP, Lillehei CW et al (2001) Nutritional and other postoperative management in neonates with short bowel syndrome correlates with clinical outcomes. J Pediatr 139:27

60. Vanderhoof JA, Grandeau C, Kaufman S et al (1984) Effect of high percentage medium chain triglyceride on mucosal adaptation during massive small bowel resection in rats. JPEN 8:685
61. Mu H, Hoy CE (2001) Intestinal absorption of structured triacylglycerols. J Lipid Res 42:792
62. Yang Q, Kock ND (2010) Effects of dietary fish oil on intestinal adaptation in 20-day-old weaning rats after massive ileocecal resection. Pediatr Res 68:183
63. Sukhotnik I, Shany A, Bashenko Y et al (2010) Parenteral but not enteral omega-3 fatty acids (Omegaven) modulate intestinal regrowth after massive small bowel resection in rats. JPEN 34:503
64. Frankel W, Zhang W, Singh A et al (1995) Fiber effect on bacterial translocation and intestinal mucin content. World J Surg 19:144
65. Dorney SFA, Ament ME, Berquist WE et al (1985) Improved survival in very short small bowel of infancy with use of long-term parenteral nutrition. J Pediatr 106:521
66. Mayne AJ, Handy DJ, Preece MA et al (1990) Dietary management of D-lactic acidosis in short bowel syndrome. Arch Dis Child 65:229–231
67. Vanderhoof JA, Park JHY, Grandjean CJ (1986) Effect of zinc deficiency on mucosal hyperplasia following 70 % bowel resection. Am J Clin Nutr 44:670–677
68. Ament ME (1998) Bone mineral content in patients with short bowel syndrome: the impact of parenteral nutrition. J Pediatr 132:386–388
69. Wakabayashi Y, Yamada E, Yoshida T, Takahashi N (1995) Effect of intestinal resection and arginine-free diet on rat physiology. Am J Physiol 269:G313–G
70. Osowska S, Neveux N, Nakib S et al (2008) Impairment of arginine metabolism in rats after massive intestinal resection: effect of parenteral nutrition supplemented with citrulline compared with arginine. Clin Sci (Lond) 115:159
71. Osowska S, Moinard C, Neveux N et al (2004) Citrulline increases arginine pools and restores nitrogen balance after massive intestinal resection. Gut 53:1781
72. Bailly-Botuha C, Colomb V, Thioulouse E et al (2009) Plasma citrulline concentration reflects enterocyte mass in children with short bowel syndrome. Pediatr Res 65:559
73. Hull MA, Jones BA, Zurakowski D et al (2011) Low serum citrulline concentration correlates with catheter-related bloodstream infections in children with intestinal failure. JPEN J Parenter Enteral Nutr 35:181
74. Rhoads JM, Plunkett E, Galanko J, et al (2005) Serum citrulline levels correlate with enteral tolerance and bowel length in infants with short bowel syndrome. J Pediatr 146:542
75. Tamada H, Nezu R, Matsuo Y et al (1993) Alanyl glutamine-enriched total parenteral nutrition restores intestinal adaptation after either proximal or distal massive resection in rats. JPEN J Parenter Enteral Nutr 17:236
76. Chen K, Nezu R, Sando K et al (1996) Influence of glutamine-supplemented parenteral nutrition on intestinal amino acid metabolism in rats after small bowel resection. Surg Today 26:618
77. Michail S, Mohammadpour H, Park JH, Vanderhoof JA (1995) Effect of glutamine-supplemented elemental diet on mucosal adaptation following bowel resection in rats. J Pediatr Gastroenterol Nutr 21:394
78. Byrne TA, Morrissey TB, Nattakom TV et al (1995) Growth hormone, glutamine, and a modified diet enhance nutrient absorption in patients with severe short bowel syndrome. JPEN J Parenter Enteral Nutr 19:296
79. Vanderhoof JA, Grandjean CJ, Kaufman SS et al (1984) Effect of high percentage medium-chain triglyceride diet on mucosal adaptation following massive bowel resection in rats. JPEN J parenter enteral nutr 8:685
80. Sukhotnik I, Hayari L, Bashenko Y et al (2008) Dietary palmitic acid modulates intestinal re-growth after massive small bowel resection in a rat. Pediatr Surg Int 24:1313
81. Yang Q, Kock ND (2010) Effects of dietary fish oil on intestinal adaptation in 20-day-old weanling rats after massive ileocecal resection. Pediatr Res 68:18382. Sukhotnik I, Shany A, Bashenko Y et al (2010) Parenteral but not enteral omega-3 fatty acids (Omegaven) modulate intestinal regrowth after massive small bowel resection in rats. JPEN J Parenter Enteral Nutr 34:503

82. Sukhotnik I, Shany A, Bashenko Y, et al (2010) Parenteral but not enteral omega-3 fatty acids (Omegaven) modulate intestinal regrowth after massive small bowel resection in rats. JPEN J Parenter Enteral Nutr 34:503.
83. Vanderhoof JA, Young RJ, Thompson JS (2003) New and emerging therapies for short bowel syndrome in children. Pediatr Drugs 5:525–531
84. Goulet O, Baglin-Gobert S, Talbotec C et al (2005) Outcome and long-term growth after extensive small bowel resection in the neonatal period: A survey of 87 children. Eur J Pediatr Surg 15:95–101

Chapter 21
Nutrition in Preterm Infants with Bronchopulmonary Dysplasia

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Abstract Bronchopulmonary dysplasia (BPD) is a common complication of preterm birth. Currently most infants developing BPD are extremely preterm infants who develop chronic lung inflammation despite being treated with antenatal steroids, early surfactant and gentle ventilation. This new form of BPD is caused mainly by premature arrest of alveolarization, and lung growth, and not predominantly by iatrogenic lung injury. Undernutrition, specifically insufficient protein intake, has been considered to play an important role in the pathogenesis of the new BPD. Infants with BPD have higher energy requirements and oxygen consumption than healthy preterm infants. Nutritional support for preterm infants with BPD includes early and aggressive parenteral nutrition, high protein and energy intakes, balancing of lipids and carbohydrates and fluid restriction. Protein intakes of 4.5 g/kg/day have been reported to be well tolerated by preterm infants. Glucose administration is limited by glucose oxidative capacity, above which glucose is converted into fat in an energy inefficient process that results in increased basal energy expenditure, oxygen consumption and CO_2 production. Lipids provide essential fatty acids, improve bioavailability of fat-soluble vitamins, provide energy, and limit conversion of carbohydrates to fat. Recent studies do not support an association between early lipid administration and BPD. Enteral feeding difficulties in infants with BPD include inability to tolerate higher volume of enteral feeds; limitations imposed by fluid restriction, gastroesophageal reflux, and importantly, delayed suck-swallow maturation, difficulties in feeding-breathing coordination, and fatigue. The current strategies for parenteral and enteral nutrition in infants with BPD are reviewed.

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*

DOI 10.1007/978-94-007-6812-3_21,

Key points

- Bronchopulmonary dysplasia (BPD) is the most common complication of extremely preterm infants, caused by premature arrest of lung growth and development
- Undernutrition, specifically protein deficiency may play an important role in the pathogenesis of BPD
- Basal metabolic rate and energy expenditure are higher in preterm infants with BPD compared with healthy preterm infants
- Early and aggressive nutrition, with fluid restriction, high protein and calories, and balanced glucose and lipid administration are the mainstay of parenteral and enteral nutrition in infants with BPD
- Difficulties in enteral feeding of preterm infants with BPD include low tolerance, immature suck-swallow mechanisms, immature feeding—breathing coordination, oral aversion and gastroesophageal reflux
- Postnatal and post discharge growth is generally delayed in preterm infants with BPD. However, there has been some improvement in the growth of these infants over the last decade following the appreciation of the role of nutrition in BPD

Bronchopulmonary dysplasia (BPD) is the most common complication of very preterm birth [1]. It was originally described by Northway and colleagues [2], as a lung injury resulting from exposure to high oxygen concentration and prolonged mechanical ventilation in preterm infants with respiratory distress syndrome (RDS). The mean gestation and weight at birth of these infants was 34 weeks and 2,400 g, respectively. BPD was diagnosed and graded by severity of changes such as atelectasis and hyperinflation on chest x-rays [3]. In 1988 it was defined [1, 4] as an ongoing requirement for oxygen or positive pressure beyond 36 weeks corrected GA. This disease is described today as the "old BPD" because the epidemiology and pathophysiology of BPD has changed over years with improved survival of extremely preterm infants following the advances in neonatal intensive care such as surfactant therapy, better ventilation techniques, and early preferential use of continuous positive airway pressure (CPAP) support. The old form of BPD is now infrequent among infants weighing $> 1{,}200$ g or born at > 30 weeks' gestation. Currently majority of those developing BPD are extremely preterm (gestation < 28 weeks) infants who develop chronic lung inflammation despite having been treated with antenatal steroids, early surfactant and gentle ventilation. Some of them have had minimal respiratory disease in the first few days of life [3]. This severity of this "new BPD" has been classified based on the level of oxygen requirement at 36 weeks (Mild: Nil, Moderate: < 30 %, Severe: > 30 % and/or Positive pressure ventilation), provided the infant has required oxygen beyond the first 28 days of life, and gestation at birth is under 32 weeks. Based on this classification, Stoll et al. [5] have reported that BPD occurred in 68 % of very low birth weight (VLBW) infants, with 27, 23 and 18 % having mild, moderate or severe BPD, respectively. Ehrenkranz et al. have reported a similar rate [6]. Considering 1.5 % live born infants are VLBW [7], the health burden of BPD is significant. Klinger et al. [8] reported an excess length of hospital stay in infants with

BPD ranging from 26.4 days in infants born < 750 g to 27.7 days in those weighing over > 1,250 g at birth. Infants with BPD tend to have more short and long term complications including neurodevelopmental impairment (NDI), lower pulmonary function, respiratory infections, growth retardation and poor academic achievements [9–14]. BPD has been reported as a better predictor than infection for late death and NDI in preterm infants [15]. A recent study has reported that of the four common morbidities; necrotizing enterocolitis (NEC), sepsis, brain injury and BPD, BPD had the highest economic burden, costing up to 43,312 USD for infants < 750 g [7].

1 Pathophysiology of BPD

RDS, or hyaline membrane disease (HMD) is the most commonly seen acute respiratory disorder in the preterm infant, with an incidence that is directly proportional to the degree of prematurity [16]. The pulmonary changes in conventional BPD relate to the volutrauma associated with mechanical ventilation, and free oxygen radical injury to the preterm lung, that results in chronic inflammation characterized by airway injury, and heterogeneous areas of atelectasis and hyper-inflation [1, 3, 17]. With improved survival of neonates at the limits of viability, a new variety of BPD has been noted. It occurs in extremely preterm neonates with mild, or no RDS, that require minimal respiratory support if any and develops despite antenatal steroids and gentle respiratory support including continuous positive airway pressure support (CPAP), and surfactant [17]. The pathological changes in this "new BPD" include simplified lungs, with larger and fewer alveoli and abnormal pulmonary microvasculature [3]. Considering that alveolarization in humans begins at 32–36 weeks of gestation and continues for several years, the new BPD relates mainly to premature arrest of lung development and growth and not predominantly to iatrogenic lung injury. It is important to note that both intra and extra uterine processes may contribute to the arrest of pulmonary alveolarization and growth [3, 4, 17]. Antenatal infection (chorioamnionitis) induces angiogenesis and an inflammatory profile similar to BPD in mice [18]. Furthermore, infants exposed to severe chorioamnionitis are reported to have a reduced response to surfactant and an increased risk of BPD [19]. However other studies have not reported this correlation [20]. The length of exposure and the gestation at the time of exposure may play a role in determining the extent of the effect on lung development. Acute or chronic exposure to oxygen may also contribute to the development of BPD [21–23]. Mechanical ventilation has been reported to interfere with lung development in animal models [24]. Furthermore, many have reported favourable respiratory outcomes with CPAP compared with various modes of mechanical ventilation [25, 26]. Both antenatal and postnatal corticosteroids inhibit alveolarization in animal models [27]. However preterm infants at high risk for severe BPD may benefit from corticosteroid treatment, despite interference with alveolarization [28].

2 Role of Nutrition in the Pathogenesis of BPD

The pathogenesis of BPD is a complex process, with multiple intra and extrauterine adverse effects that impair lung maturation and growth. Under nutrition was suggested as a key factor in the BPD pathogenesis as early as 1988 [29]. General under nutrition, specifically insufficient protein intake may interfere with lung growth and function by promoting lung growth and adversely affecting remodeling following injury, tolerance to oxidative stress and resistance to infection [29]. Perinatal malnutrition augments pre and postnatal lung injury and delays lung repair [30, 31]. A large trial from the NICHD demonstrated that preterm infants growing on the lowest percentiles are more likely to develop BPD [32]. Animal studies support the hypothesis that nutritional measurements have a crucial impact on the development, treatment, and prevention of BPD [33, 34]. In an adult rat model, calorie restriction to 33 % of the normal resulted in a 55 % reduction in alveoli number and a 25 % reduction in alveolar surface area. Within 72 h of refeeding, alveoli number and surface area returned to normal [33]. Mataloun et al. have reported that nutritional restriction resulted in reduced alveolarization in a preterm rabbit model [34], and a combination of nutritional restriction and hyperoxia further interfered in alveolarization [34]. In addition, undernourished newborn mice were more likely to die and had more microscopic evidence of lung injury following exposure to hyperoxia than the well fed controls [35]. More specifically, protein deficient rats had increased mortality with hyperoxia than protein sufficient rats. Supplementation with specific amino acids; cysteine, cystine and methionine prevented the increased susceptibility to oxygen [36]. This supports the "multi hit" pathogenesis theory of BPD. The significance of adequate nutrition for maintaining pulmonary structure and function in humans is further supported by the evidence of emphysema in CT's of patients with anorexia nervosa [37]. Ong et al. [38] have reported that the lung function (evaluated by spirometry and normalized for body size) was significantly related to nutritional indices in boys and girls in rural India. This may be related to early maldevelopment as it was independent of their stature [39]. Malnutrition can come in different forms; protein inadequacy, total caloric insufficiency and specific vitamin deficiencies [40]. VLBW infants are at risk for all of these inadequacies. Supplying adequate calories and protein is especially challenging in infants with BPD as they are fluid restricted [41]. In a retrospective study of 100 VLBW infants, Wemhoner et al. [42] reported a trend towards reduced total caloric and protein intake in infants who developed BPD. There was no difference in carbohydrate intake between babies who eventually developed BPD and those who did not [42]. In conclusion, nutritional strategies in preterm infants who are at risk for BPD have the potential to prevent or minimize the severity of lung injury and disease and to contribute to better lung healing. Understanding the crucial role of adequate nutrition in these infants, and practicing "lung minded nutrition" can promote adequate lung maturation and growth [43].

3 Metabolic Requirements of the Preterm Infant, in Sickness and in Health

The main goal of energy accumulation in the preterm infant is to achieve growth comparable with intra uterine growth rates [44]. A daily energy intake of 120–130 kcal/kg is estimated sufficient to meet the metabolic demands of a healthy premature infant [45, 46]. Energy expenditure (EE) is the sum of energy required for maintenance, growth, thermoregulation and activity, and it is affected by various factors including neonatal morbidities, treatments and medications including ventilation modes, thermal environment etc [44]. In preterm infants, EE is negatively related to GA, and positively related to intake, postnatal age and weight gain [47, 48]. Sick preterm infants tend to have increased EE [44]. Various studies have reported infants with BPD to have an up to 25 % increase in EE, which is directly related to their respiratory status [49–54]. In addition, studies report that treatment with theophylline or caffeine, which is a common treatment in BPD, can cause an additional rise in EE [55]. Other studies have reported that oxygen consumption was higher in infants with BPD, more so in non thriving infants [50, 56]. A systematic review has concluded that there were no RCTs comparing increased versus standard energy intakes for preterm infants with BPD [57]. In planning a nutritional program for a preterm infant with BPD, one should bear in mind the higher energy consumption and therefore metabolic demands of such infants.

4 Parenteral Nutrition (PN) in Infants with BPD

Extremely preterm infants may not reach full feeds until 2–4 weeks of age making PN essential for the first few weeks of life [58]. The principles of nutritional support for the preterm infant with BPD include aggressive PN initiation and advancement, protein and energy intakes to support the best possible growth, balancing of lipids and carbohydrates, careful fluid management and early initiation of enteral feeds [43].

4.1 Energy Intake

Adequate nutrition is crucial for reducing both the risk and severity of BPD and the healing of established BPD [42]. The recent European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGAN) recommendations suggest a calorie range of 110–135 kcal/day for a healthy preterm infant [46]. Considering the higher EE of infants with BPD [49–54], It would be reasonable to assume that these infants could benefit from a higher caloric intake. Results of a retrospective cohort study of extremely low birth weight (ELBW) infants ($n = 1{,}366$) indicate that as total daily energy intake during the first 7 days of life increased in critically ill infants, the odds of adverse outcomes (e.g., BPD, necrotising enterocolitis, late onset

sepsis, neurodevelopmental impairment) decreased by about 2 % for each 1 kcal/kg of total energy intake [59].

4.2 Fluid Intake

High fluid intakes may contribute to pulmonary oedema by the persistence of the ductus arteriosus, and accumulation of pulmonary interstitial fluid [60, 61], that decreases lung compliance, increases need for respiratory support, leading to the development of BPD [61]. A retrospective analysis of 1,383 ELBW infants, of which 797 developed BPD [62] has reported higher daily fluid intakes and less weight loss in the first 10 days of life in infants who died or developed BPD. Other studies do not support this association [63]. However, a recent systematic review of randomized controlled trials (RCTs) has concluded that restricted fluid intake reduced the risk of patent ductus arteriosus, necrotizing enterocolitis and there was a trend towards reduction of BPD [41]. When balancing the fluid intake of an infant with BPD, it is important to provide appropriate caloric and protein intake in minimum volume without causing dehydration [46, 61].

4.3 Protein Intake

The goal of protein administration is to match intra uterine growth and support protein accretion. Amino acids (AA) are essential in maintaining a positive nitrogen balance to prevent a catabolic state. Certain AA are known to decrease in the first 24 h in preterm infants (e.g., glutamate, cysteine, isoleucine, leucine, arginine, methionine) and it is also reported that infants receiving AA have better glucose tolerance [58]. In infants with BPD, protein is required to promote lung growth and development. Vadivel et al. have reported that L-citrulline and arginine levels were low in rats exposed to hyperoxia compared with the control rats. Provision of L-citrulline to these rats with BPD rats has prevented alveolar simplification, preserved lung growth and prevented pulmonary hypertension [23]. Early administration of intravenous AA is well tolerated by extremely preterm infants. Potential side effects of early AA administration include hyperamonemia, azotemia and metabolic acidosis [58]. However, studies [64] show that these don't occur even when AA intake is as high as 3 g/kg/day on the first day of life. Current recommendations for protein intake in VLBW infants are 3.5–4.5 g/kg/day [46, 65].

4.4 Carbohydrate Intake

Glucose is considered the main energy source in PN [43]. However, glucose intake that exceeds glucose oxidative capacity has been shown to increase basal energy

expenditure and to be converted into fat. The conversion of glucose to fat is an energy inefficient process that results in an increase in both oxygen consumption and carbon dioxide (CO_2) production [43, 66, 67]. This effect is particularly undesirable in preterm infants with lung disease as it increase their work of breathing. This effect was reported by Yunis et al. who administrated 4 and 12 mg/kg/min intravenous glucose on consecutive days to 6 subjects with BPD and 6 controls [66]. Infants with BPD had a significant increase in basal energy expenditure, oxygen consumption and CO_2 production during the glucose loading, while control infants remained unaffected [66]. The dose of glucose that exceeds glucose oxidative capacity has not been precisely defined in preterm neonates, but it is most probably above 11–13 mg/kg/day [68]. Furthermore, hyperlgycemia is common in extremely preterm infants and is associated with an increase in morbidity and mortality [69]. A pilot study has indicated that preterm infants who received early insulin treatment have improved growth outcomes [70]. In a multicenter trial, Beardsall et al. [71] have assigned 195 infants to receive early insulin therapy combined with 20 % intravenous glucose drip. These were compared to a group of control infants. The early insulin group had less weight loss during the first day of life, but more episodes of hypoglycaemia and a significantly increased mortality at 28 days of age. This trial was discontinued early due to concerns regarding the increase mortality [71].

Various studies show that using either glucose or lipids as the sole source of energy results in a metabolic stress and increased oxygen consumption and CO_2 production. However, a combination of the two does not result in this adverse effect and is therefore the recommended regimen for parenteral nutrition [72].

4.5 Lipid Intake

Lipids are an essential component of PN for preterm infants [73]. They provide essential fatty acids, improve the bioavailability of fat soluble vitamins, help meet energy goals and limit the conversion of carbohydrates to fat thereby reducing oxygen consumption and CO_2 production [61, 73]. There is substantial evidence supporting a well balanced early fatty acid supply is essential for growth and visual and cognitive development [74–76]. The minimal amount of lipids required to avoid essential fatty acid deficiency, is starting with 0.5–1 g/kg/day and progressing up to 4.8–6.6 g/kg/g (40–50 % of energy intake) [46].

The role of lipid administration in the development of BPD remains controversial, with concerns regarding potential adverse effects such as increased pulmonary vascular resistance, impaired pulmonary gas diffusion and free radical injury [58, 61]. Some studies have indicated early administration of parenteral lipids may increase the risk of BPD and mortality [77–79]. Nevertheless, other studies [80] as well as a systematic review of studies of early lipid administration [73] have reported that there was no difference in outcome between early and late lipid administration.

Most commonly used soy bean based lipid emulsions contain high amounts of linoleic acid, low arachidonic acid and no long chain polyunsaturated fatty acids

(LC-PUFA), a combination that is not ideal for neonatal care [81]. In an effort develop a more appropriate lipid emulsion, Rayyan et al. [82] conducted a prospective double blind RCT, evaluating the safety, tolerability and efficacy of a lipid emulsion containing a mixture of medium chain triglycerides and soybean, olive and fish oil compared to a standard soybean emulsion. They reported that in their study of 53 preterm infants the combined lipid emulsion was both safe and well tolerated and improved the infant's fatty acid profile [82]. Similar results were reported by others [83–85].

5 Enteral Nutrition in Infants with BPD

When infants with BPD reach the convalescent phase of the illness, they continue to require an extra 20–40 % of energy and optimal protein intake to grow at an appropriate rate [58, 86]. The caloric requirements of these infants range from 120–150 kcal/kg/day and may exceed 150 kcal/kg/day in those with severe BPD [58]. Enteral feeding difficulties are common in preterm infants, particularly in those with BPD. These include inability to tolerate even low volume of enteral feeds, need for fluid restriction, gastroesophageal reflux (GER) , delayed maturation of sucking and swallowing mechanisms, difficulties in the coordination of feeding and breathing, fatigue with enteral feeds, and oral aversion [43]. Achieving a high calorie, high protein and low volume feeding regimen for optimal growth, is a challenging aspect of caring for the preterm infant with BPD [58]. Higher protein intake has been reported to increase growth in formula fed VLBW infants, but there was a concern regarding the association of higher protein intake and lower IQ scores [87]. Hicks et al. have compared the growth and calcium absorption in 16 VLBW preterm infants with BPD, fed 130–150 ml/kg preterm formula (> 24 kcal/oz) or fortified human milk (FHM) with that in 25 control VLBW infants, fed 150–170 ml/kg 24 kcal/oz preterm formula or FHM [88]. They reported comparable growth and mineral absorption between the groups.

Singer et al. [89] have compared feeding behaviours of 141 mother-infant pairs: 55 VLBW infants with BPD, 34 VLBW infants without BPD, and 52 term infants. Despite increased maternal efforts, infants with BPD took less volume, spent less time sucking, and spent a greater proportion of time not feeding. VLBW infants without BPD were equivalent to term infants in percentage of time sucking and in volume ingested and were more likely to take in higher calories than infants with BPD. In addition, bolus feeding was reported to influence respiratory function in preterm infants [90]. A study comparing the effects of intermittent and continuous feedings on pulmonary function, has reported that in 24 VLBW infants at 2–4 weeks of age with a previous diagnosis of RDS, there was a significant decrease in tidal volume, minute ventilation and dynamic compliance and an increase in pulmonary resistance after bolus feeds. Pulmonary function remained unchanged after continuous feedings [90]. However, a recent systematic review [91] did not report any significant outcome differences in VLBW infants when comparing bolus and continuous feeding regimens.

Fatigue resistant muscle fibers are poorly developed in preterm infants [92]. Intercostal and diaphragmatic muscles have been shown to have less type I (slow twitch, high oxidative) muscle fibers in preterm compared with full term and two year old infants. Similar findings have also been associated with malnutrition [93]. This has been hypothesised to contribute to the fatigue in BPD infants trying to concur both increased work of breathing and feeding [92].

A rapidly accumulating body of evidence suggests that mechanical ventilation, with its attendant diaphragm muscle inactivity and unloading, is an important cause of diaphragmatic Dysfunction [94–96].

Animal studies have consistently found that conventional mechanical ventilation decreases the force-generating capacity of the diaphragm in a time dependent manner [94, 95]. Although most of this data comes from animal studies and the information in humans, particularly in the context of current modes of ventilation, is scarce, this could be an additional factor in fatigue during oral feeds in preterm BPD infants. Malnutrition also affects ventilatory drive [96]. The interaction of nutrition and ventilatory drive appears to be a direct function of the influence of nutrition on metabolic rate [97]. A 58 % reduction in the ventilatory response to hypoxia was found in adult volunteers placed on a balanced 550 kcal/day diet for 10 days. It returned to normal with refeeding. Furthermore, After a 7 day protein-free diet, a blunted ventilatory response to carbon dioxide was noted [98].

GER is common (up to 10 %) in preterm infants with BPD [99]. Reasons for this include transient relaxation of the lower oesophageal sphincter in response to gastric distension (commonly seen in infants with CPAP), inflammation and bolus feeds [100]. In a study of 46 VLBW infants with BPD, 50 % (23/46) were reported to have GER diagnosed by pH monitoring, with the main risk factors for GER as prolonged tube feeding and feeding intolerance [101]. Other studies have not supported the association between BPD and GER [102].

The transition from gavage to oral feeds takes an average of 5–10 days [103] in healthy preterm infants, and an average of 15 days for preterm infants with BPD [104]. An important aspect of nutritive sucking is coordination of suck, swallow and respiration [105]. Sucking patterns can be categorized as immature, transitional and mature, based on rhythm and pauses between sucking bursts [106]. In a retrospective study comparing 41 preterm infants with BPD with 99 control infants, Tsu-Hsin et al. have reported that infants with BPD have delayed maturation of sucking patterns compared to control infants, and take longer to achieve full oral feeds [105]. Other studies have supported the conclusion that anticipated maturational patterns of suckle and swallow do not occur in infants with BPD [107–109]. In addition to the immature suck-swallow pattern, Mizuno et al. [110] have reported that infants with BPD suck with a weak pressure, resulting in less swallowing. Furthermore, infants with BPD have a poor swallow-suck-breath coordination, and therefore have frequent desaturations during feeds [103, 107, 110, 111]. A recent study of 86 extremely preterm infants compared a standard gradual increase in oral feeds to a semi demand method where the feeding frequency was regulated by the infant's behavioural and cardio respiratory signs [112]. It was reported that the semi demand feeding significantly shortened the time to oral feeds. Barlow et al. have developed a "motorized pacifier

(dummy)" which delivers patterned orosensory events, which may train the suck central pattern generator in infants with disrupted suck-swallow pattern and improve nutritive sucking [113]. Oral aversion related to endotracheal and suctioning stimuli is also common in infants with BPD, and needs to be evaluated and treated by a feeding specialist [114].

6 Special Nutritional Considerations in Preterm Infants with BPD

6.1 Vitamin A

Vitamin A is necessary for normal pulmonary growth, development and repair [58]. Serum and tissue retinol levels are low in extremely premature infants, and have been associated with BPD [115]. In a recent systematic review [116] of studies evaluating the effect of vitamin A supplementation on BPD and death, the reviewers concluded that vitamin A reduced the odds of oxygen requirement or death at one month of age (RR 0.93, 95 % CI 0.88–0.99), and there was a trend towards reduction at 36 weeks (RR 0.91, 95 % CI 0.82–1.00).

6.2 Vitamin E

Vitamin E is an antioxidant that protects cell membranes from oxidative injuries. Although some studies have shown a protective effect of vitamin E on preterm lungs [117], other studies have not supported this conclusion [118]. Low vitamin E levels have been associated with a higher risk of BPD [119], and vitamin E supplementation is recommended in preterm infants [58].

6.3 Selenium (Se)

Se and vitamin E act synergistically in the prevention of oxidant injury, and Se deficiency in animal models has been reported to increase the susceptibility to oxidative lung injury [120]. However, a systematic review of studies evaluating the effect of Se supplementation reported that Se supplementation did not reduce oxygen dependency in preterm infants [121].

6.4 Inositol

Inositol is a naturally occurring nutrient required by human cells for growth, differentiation and survival. It promotes maturation of several components of surfactant

and may play a critical role as an antioxidant [58, 86]. Studies in the pre surfactant era have reported that supplementation with Inositol has reduced BPD [122]. However, a recent Meta analysis concludes that there was no reduction in the incidence of BPD at 28 days or 36 weeks GA [123]. This systematic review did however report reduction in neonatal and infant deaths; severe retinopathy of prematurity and severe intraventricular haemorrhage [123].

6.5 Glutamine

Glutamine is essential for rapidly dividing cells and is associated with increased levels of glutathione and may therefore have antioxidant affects [61]. However, a recent Meta analysis [124] has concluded that neither parenteral or enteral supplementation with glutamine reduced BPD or mortality in preterm infants.

6.6 N-acetylcysteine and Cysteine

While both of these amino acids posses antioxidant qualities, a Cochrane review of N-acetylcysteine supplementation [125] has reported no significant reduction in the risk of death, BPD, and death or BPD.

7 Post Natal Growth in Infants with BPD

Preterm infants with BPD are prone to postnatal growth failure, due to high basal energy expenditure, fluid restriction, feeding intolerance and medications including diuretics and post natal steroids[126, 127]. In a randomized blinded nutritional intervention trial, Brunton et al. [128] compared growth outcomes of 32 preterm infants with BPD fed with standard term formula or high energy (900 kcal/l), high protein, high mineral formula after 37 weeks corrected GA. They reported that at 3 months corrected GA the study group had greater length, greater lean mass and greater radial bone mineralization compared to the control group.

Madden et al. compared growth of infants with BPD over two time periods; 1996–1999 and 2000–2003. The authors concluded that although there was some improvement in growth outcomes and despite the advances in neonatal care, poor post natal growth remains a major problem in infants with BPD [129] However, in a retrospective study of 88 ELBW infants with BPD in 2006–2008, Theile et al. [130] reported that 73 % of the infants grew at, or above intrauterine rates. The authors concluded that improved nutritional strategies including the use of calorie and protein dense milk products (formula or human milk fortifiers), early and aggressive PN and early enteral feeds [130].

8 Metabolic Bone Disease (MBD) in BPD

MBD predominantly affects extremely preterm and VLBW infants [131, 132]. The risk factors include prolonged PN, sepsis, immobility and medications that increase mineral losses and bone catabolism. Infants with BPD are at high risk due to their prolonged ventilation and therefore less mobility, increased use of loop diuretics and steroid therapy and their tendency for enteral feeding intolerance. On the other hand, MBD may increase BPD severity by rib fractures, due to reduced bone mineralization and abnormal bone remodeling, that may prolong mechanical ventilation [133, 134]. Diagnostic and follow up biochemical markers include normal serum calcium; low serum phosphate and high serum alkaline phosphatase [135]. Neither standard PN solutions nor the enteral intake can safely deliver the amounts of calcium and phosphorus necessary to match intrauterine accretion [131, 136]. High concentrations of calcium and phosphate in PN can result in calcium phosphate precipitation [134]. Early enteral feeding, fortification of preterm human milk with calcium, phosphorus and supplementation of vitamin D have an important role in the prevention of MBD [137], particularly in high risk infants with BPD. In addition, physical activity has been reported to increase bone mineralization in preterm infants [138]. In cases of established MBP, additional oral supplementation of calcium and phosphate salts is recommended [139].

9 Post Discharge Nutrition of Infants with BPD

Up to 67 % of infants with BPD continue to have growth failure after discharge due to difficulties in ensuring sufficient protein and calorie intake [51, 58]. These include the infant's oromotor coordination, occurrence of gagging, GER and vomiting as well as increased metabolic demands. Even if these infants consume a calorie appropriate diet for age, they may have relative caloric deficiency [51, 58]. Additional factors contributing to malnutrition may include an infant's difficult temperament, lower socioeconomic status, and developmental problems [140]. Details regarding type and frequency of feeds, nutritional supplements, food allergies and/or intolerance, issues with swallowing, vomiting, GER diarrhoea or constipation, and behavioural problems, should be obtained at every follow-up visit [141]. In addition weight, length, head circumference and a body mass index should be recorded at every follow up visit [142]. The nutritional plan should be individualized before discharge and reviewed regularly. These infants should continue to receive 22 kcal/oz preterm formula for 6–8 months and low salt and limited volume, high calorie nutrition may be required. Feeding related hypoxia may also be an issue after discharge from the hospital [143]. Chronological age may not be a good predictor for initiation of solid food, as some infants with BPD may achieve these skills later. However, they often tolerate spoon feeding better than liquids, and foods with thicker consistency may be swallowed more easily [58]. An occupational therapist should be consulted for supervised feeding if there is food aversion or respiratory compromise. Many

feeding problems may be related to psychosocial issues and referral to behavioural psychologist may be beneficial [58].

10 Summary

BPD is the most common morbidity in extremely preterm infants. Current form of BPD mainly involves arrest of alveolarization, lung development and growth Malnutrition has an important role in both the pathogenesis and the prevention of BPD. Early and aggressive PN, providing high calories and high protein in a restricted volume, and early establishment of enteral feeds are the mainstay of the current nutritional approach to BPD. Establishing full enteral and full oral nutrition may be a prolonged process in BPD infants due to issues of intolerance, GER, oral aversion and delayed maturation of suck, swallow and coordination of breathing. Growth is an ongoing concern in infants with BPD and should be monitored closely during hospital stay and even after discharge.

References

1. Jobe AH, Bancalari E (2001) Bronchopulmonary dysplasia. Am J Resp Crit Care Med 163(7):1723–1729
2. Northway WH, Jr., Rosan RC, Porter DY (1967) Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. New Engl J Med 276(7):357–368
3. Jobe AH (2006) The New BPD. NeoReviews 7(10):e531–e544
4. Kair LR, Leonard DT, Anderson JDM (2012) Pediatr Rev 33(6):255–264
5. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC et al (2010) Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics 126(3):443–456
6. Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA et al (2005) Validation of the national institutes of health consensus definition of bronchopulmonary dysplasia. Pediatrics 116(6):1353–1360
7. Johnson TJ, Patel AL, Jegier BJ, Engstrom JL, Meier PP (2013) Cost of morbidities in very low birth weight infants. J Pediatr 162(2):243–249
8. Klinger G, Sirota L, Lusky A, Reichman B (2006) Bronchopulmonary dysplasia in very low birth weight infants is associated with prolonged hospital stay. J Perinat Off J Calif Perinat Assoc 26(10):640–644
9. Karagianni P, Tsakalidis C, Kyriakidou M, Mitsiakos G, Chatziioanidis H, Porpodi M et al (2011) Neuromotor outcomes in infants with bronchopulmonary dysplasia. Pediatr Neurol 44(1):40–46
10. Clark RH, Gerstmann DR, Jobe AH, Moffitt ST, Slutsky AS, Yoder BA (2001) Lung injury in neonates: causes, strategies for prevention, and long-term consequences. J Pediatr 139(4):478–486
11. Eber E, Zach MS (2001) Long term sequelae of bronchopulmonary dysplasia (chronic lung disease of infancy). Thorax 56(4):317–323
12. Anderson PJ, Doyle LW (2006) Neurodevelopmental outcome of bronchopulmonary dysplasia. Semin Perinatol 30(4):227–232

13. Hack M, Fanaroff AA (2000) Outcomes of children of extremely low birthweight and gestational age in the 1990s. Semin neonatol SN 5(2):89–106
14. Skidmore MD, Rivers A, Hack M (1990) Increased risk of cerebral palsy among very low-birthweight infants with chronic lung disease. Dev Med Child Neurol 32(4):325–332
15. Bassler D, Stoll BJ, Schmidt B, Asztalos EV, Roberts RS, Robertson CM et al (2009) Using a count of neonatal morbidities to predict poor outcome in extremely low birth weight infants: added role of neonatal infection. Pediatrics 123(1):313–318
16. Kamath BD, Macguire ER, McClure EM, Goldenberg RL, Jobe AH (2011) Neonatal mortality from respiratory distress syndrome: lessons for low-resource countries. Pediatrics 127(6):1139–1146
17. Jobe AH (2011) The new bronchopulmonary dysplasia. Curr Opin Pediatr 23(2):167–172
18. Miller JD, Benjamin JT, Kelly DR, Frank DB, Prince LS (2010) Chorioamnionitis stimulates angiogenesis in saccular stage fetal lungs via CC chemokines. Am J Physiol-Lung C 298(5):L637–L645
19. Been JV, Rours IG, Kornelisse RF, Jonkers F, de Krijger RR, Zimmermann LJ (2010) Chorioamnionitis alters the response to surfactant in preterm infants. J Pediatr 156(1):10–15 e11
20. Soraisham AS, Singhal N, McMillan DD, Sauve RS, Lee SK (2009) A multicenter study on the clinical outcome of chorioamnionitis in preterm infants. Am J Obstet Gynecol 200(4):372 e371–e376
21. Vento M, Moro M, Escrig R, Arruza L, Villar G, Izquierdo I et al (2009) Preterm resuscitation with low oxygen causes less oxidative stress, inflammation, and chronic lung disease. Pediatrics 124(3):e439–e449
22. Carlo WA, Finer NN, Walsh MC, Rich W, Gantz MG, Laptook AR et al (2010) Target ranges of oxygen saturation in extremely preterm infants. New Engl J Med 362(21):1959–1969
23. Vadivel A, Aschner JL, Rey-Parra GJ, Magarik J, Zeng H, Summar M et al (2010) L-citrulline attenuates arrested alveolar growth and pulmonary hypertension in oxygen-induced lung injury in newborn rats. Pediatr Res 68(6):519–525
24. Mokres LM, Parai K, Hilgendorff A, Ertsey R, Alvira CM, Rabinovitch M et al (2010) Prolonged mechanical ventilation with air induces apoptosis and causes failure of alveolar septation and angiogenesis in lungs of newborn mice. Am J Physiol-Lung C 298(1):L23–L35
25. Morley CJ, Davis PG, Doyle LW, Brion LP, Hascoet JM, Carlin JB (2008) Nasal CPAP or intubation at birth for very preterm infants. New Engl J Med 358(7):700–708
26. Kribs A, Hartel C, Kattner E, Vochem M, Kuster H, Moller J et al (2010) Surfactant without intubation in preterm infants with respiratory distress: first multi-center data. Klin Padiatr 222(1):13–17
27. Albertine KH, Jones GP, Starcher BC, Bohnsack JF, Davis PL, Cho SC et al (1999) Chronic lung injury in preterm lambs. Disordered respiratory tract development. Am J Resp Crit C 159(3):945–958
28. Doyle LW, Halliday HL, Ehrenkranz RA, Davis PG, Sinclair JC (2005) Impact of postnatal systemic corticosteroids on mortality and cerebral palsy in preterm infants: effect modification by risk for chronic lung disease. Pediatrics 115(3):655–661
29. Frank L, Sosenko IR (1988) Undernutrition as a major contributing factor in the pathogenesis of bronchopulmonary dysplasia. Am Rev Respir Dis 138(3):725–729
30. Frank L (1992) Antioxidants, nutrition, and bronchopulmonary dysplasia. Clin Perinatol 19(3):541–562
31. Bhatia J, Parish A (2009) Nutrition and the lung. Neonatology 95(4):362–367
32. Clark RH, Thomas P, Peabody J (2003) Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics 111(5 Pt 1):986–990
33. Massaro GD, Radaeva S, Clerch LB, Massaro D (2002) Lung alveoli: endogenous programmed destruction and regeneration. Am J Physiol-Lung C 283(2):L305–L309
34. Mataloun MM, Rebello CM, Mascaretti RS, Dohlnikoff M, Leone CR (2006) Pulmonary responses to nutritional restriction and hyperoxia in premature rabbits. J Pediatr 82(3):179–185

35. Polgar G, Antagnoli W, Ferrigan LW, Martin EA, Gregg WP (1966) The effect of chronic exposure to 100 % oxygen in newborn mice. Am J Med Sci 252(5):580–587
36. Deneke SM, Gershoff SN, Fanburg BL (1983) Potentiation of oxygen toxicity in rats by dietary protein or amino acid deficiency. J Appl Physiol 54(1):147–151
37. Coxson HO, Chan IH, Mayo JR, Hlynsky J, Nakano Y, Birmingham CL (2004) Early emphysema in patients with anorexia nervosa. Am J Resp Crit Care 170(7):748–752
38. Ong TJ, Mehta A, Ogston S, Mukhopadhyay S (1998) Prediction of lung function in the inadequately nourished. Arch Dis Child 79(1):18–21
39. Abrams SA (2001) Chronic pulmonary insufficiency in children and its effects on growth and development. J Nutr 131(3):938S–941S
40. Jobe AH (2006) Let's feed the preterm lung. J Pediatr 82(3):165–166
41. Bell EF, Acarregui MJ (2008) Restricted versus liberal water intake for preventing morbidity and mortality in preterm infants. Cochrane Database Syst Rev (1):CD000503
42. Wemhoner A, Ortner D, Tschirch E, Strasak A, Rudiger M (2011) Nutrition of preterm infants in relation to bronchopulmonary dysplasia. BMC Pulm Med 11:7
43. Reynolds RM, Thureen PJ (2007) Special circumstances: trophic feeds, necrotizing enterocolitis and bronchopulmonary dysplasia. Semin Fetal Neonat Med 12(1):64–70
44. Hulzebos CV, Sauer PJ (2007) Energy requirements. Semin Fetal Neonat Med 12(1):2–10
45. Leitch CA, Denne SC (2000) Energy expenditure in the extremely low-birth weight infant. Clin Perinatol 27(1):181–195, vii–viii
46. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T et al (2010) Enteral nutrient supply for preterm infants: commentary from the european society of paediatric gastroenterology, hepatology and nutrition committee on nutrition. J Pediatr Gastr Nutr 50(1):85–91
47. Bauer J, Maier K, Hellstern G, Linderkamp O (2003) Longitudinal evaluation of energy expenditure in preterm infants with birth weight less than 1,000 g. Br J Nutr 89(4):533–537
48. DeMarie MP, Hoffenberg A, Biggerstaff SL, Jeffers BW, Hay WW Jr., Thureen PJ (1999) Determinants of energy expenditure in ventilated preterm infants. J Perinat Med 27(6):465–472
49. Bauer J, Maier K, Muehlbauer B, Poeschl J, Linderkamp O (2003) Energy expenditure and plasma catecholamines in preterm infants with mild chronic lung disease. Early Hum Dev 72(2):147–157
50. Yeh TF, McClenan DA, Ajayi OA, Pildes RS (1989) Metabolic rate and energy balance in infants with bronchopulmonary dysplasia. J Pediatr 114(3):448–451
51. Kurzner SI, Garg M, Bautista DB, Sargent CW, Bowman CM, Keens TG (1988) Growth failure in bronchopulmonary dysplasia: elevated metabolic rates and pulmonary mechanics. J Pediatr 112(1):73–80
52. Kurzner SI, Garg M, Bautista DB, Bader D, Merritt RJ, Warburton D et al (1988) Growth failure in infants with bronchopulmonary dysplasia: nutrition and elevated resting metabolic expenditure. Pediatrics 81(3):379–384
53. Weinstein MR, Oh W (1981) Oxygen consumption in infants with bronchopulmonary dysplasia. J Pediatr 99(6):958–961
54. Kalhan SC, Denne SC (1990) Energy consumption in infants with bronchopulmonary dysplasia. J Pediatr 116(4):662–664
55. Carnielli VP, Verlato G, Benini F, Rossi K, Cavedagni M, Filippone M et al (2000) Metabolic and respiratory effects of theophylline in the preterm infant. Arch Dis Child Fetal Neonat Ed 83(1):F39–F43
56. Kao LC, Durand DJ, Nickerson BG (1988) Improving pulmonary function does not decrease oxygen consumption in infants with bronchopulmonary dysplasia. J Pediatr 112(4):616–621
57. Lai NM, Rajadurai SV, Tan KH (2006) Increased energy intake for preterm infants with (or developing) bronchopulmonary dysplasia/chronic lung disease. Cochrane Database Syst Rev 3:CD005093
58. Biniwale MA, Ehrenkranz RA (2006) The role of nutrition in the prevention and management of bronchopulmonary dysplasia. Semin Perinat 30(4):200–208

59. Ehrenkranz RA, Das A, Wrage LA, Poindexter BB, Higgins RD, Stoll BJ et al (2011) Early nutrition mediates the influence of severity of illness on extremely LBW infants. Pediatr Res 69(6):522–529
60. Bell EF, Warburton D, Stonestreet BS, Oh W (1980) Effect of fluid administration on the development of symptomatic patent ductus arteriosus and congestive heart failure in premature infants. New Engl J Med 302(11):598–604
61. Dani C, Poggi C (2012) Nutrition and bronchopulmonary dysplasia. J Matern-Fetal Neonat Med Off J Eur Assn Perinat Med, Fed Asia Oceania Perinat Soc, Int Soc Perinat Obstet 25(Suppl 3):37–40
62. Oh W, Poindexter BB, Perritt R, Lemons JA, Bauer CR, Ehrenkranz RA et al (2005) Association between fluid intake and weight loss during the first ten days of life and risk of bronchopulmonary dysplasia in extremely low birth weight infants. J Pediatr 147(6):786–790
63. Stephens BE, Gargus RA, Walden RV, Mance M, Nye J, McKinley L et al (2008) Fluid regimens in the first week of life may increase risk of patent ductus arteriosus in extremely low birth weight infants. J Perinat Off J California Perinat Assoc 28(2):123–128
64. Thureen PJ, Melara D, Fennessey PV, Hay WW Jr (2003) Effect of low versus high intravenous amino acid intake on very low birth weight infants in the early neonatal period. Pediatr Res 53(1):24–32
65. Hay WW Jr (2008) Strategies for feeding the preterm infant. Neonatology 94(4):245–254
66. Yunis KA, Oh W (1989) Effects of intravenous glucose loading on oxygen consumption, carbon dioxide production, and resting energy expenditure in infants with bronchopulmonary dysplasia. J Pediatr 115(1):127–132
67. Chessex P, Belanger S, Piedboeuf B, Pineault M (1995) Influence of energy substrates on respiratory gas exchange during conventional mechanical ventilation of preterm infants. J Pediatr 126(4):619–624
68. Sauer PJ, Van Aerde JE, Pencharz PB, Smith JM, Swyer PR (1986) Glucose oxidation rates in newborn infants measured with indirect calorimetry and [U-13C]glucose. Clin Sci (Lond) 70(6):587–593
69. van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M et al (2001) Intensive insulin therapy in critically ill patients. New Engl J Med 345(19):1359–1367
70. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart AL, Ahluwalia JS, Vanhole C, Palmer C et al (2007) A randomised controlled trial of early insulin therapy in very low birth weight infants, "NIRTURE" (neonatal insulin replacement therapy in Europe). BMC Pediatr 7:29
71. Beardsall K, Ogilvy-Stuart AL, Frystyk J, Chen JW, Thompson M, Ahluwalia J et al (2007) Early elective insulin therapy can reduce hyperglycemia and increase insulin-like growth factor-I levels in very low birth weight infants. J Pediatr 151(6):611–617, 617:e611
72. Van Aerde JE, Narvey M (2006) Acute respiratory failure. In: Thureen P, Hay Jr W.W (eds) Neobatal nutrition and metabolism. Cambridge University Press, Cambridge p 508–521
73. Simmer K, Rao SC (2005) Early introduction of lipids to parenterally-fed preterm infants. Cochrane Database Syst Rev 2:CD005256–C
74. Innis SM (2007) Fatty acids and early human development. Early Hum Dev 83(12):761–766
75. Innis SM (2007) Dietary ($n-3$) fatty acids and brain development. J Nutr 137(4):855–859
76. Fleith M, Clandinin MT (2005) Dietary PUFA for preterm and term infants: review of clinical studies. Crit Rev Food Sci 45(3):205–229
77. Sosenko IR, Rodriguez-Pierce M, Bancalari E (1993) Effect of early initiation of intravenous lipid administration on the incidence and severity of chronic lung disease in premature infants. J Pediatr 123(6):975–982
78. Periera GR, Fox WW, Stanley CA, Baker L, Schwartz JG (1980) Decreased oxygenation and hyperlipemia during intravenous fat infusions in premature infants. Pediatrics 66(1):26–30
79. Cooke RW (1991) Factors associated with chronic lung disease in preterm infants. Arch Dis Child 66(7 Spec No):776–779
80. Brownlee KG, Kelly EJ, Ng PC, Kendall-Smith SC, Dear PR (1993) Early or late parenteral nutrition for the sick preterm infant? Arch Dis Child 69(3 Spec No):281–283

81. Deckelbaum RJ (2003) Intravenous lipid emulsions in pediatrics: time for a change? J Pediatr Gastr Nutr 37(2):112–114
82. Rayyan M, Devlieger H, Jochum F, Allegaert K (2012) Short-term use of parenteral nutrition with a lipid emulsion containing a mixture of soybean oil, olive oil, medium-chain triglycerides, and fish oil: a randomized double-blind study in preterm infants. JPEN 36(1 Suppl):81S–94S
83. D'Ascenzo R, D'Egidio S, Angelini L, Bellagamba MP, Manna M, Pompilio A et al (2011) Parenteral nutrition of preterm infants with a lipid emulsion containing 10 % fish oil: effect on plasma lipids and long-chain polyunsaturated fatty acids. J Pediatr 159(1):33–38 e31
84. Sala-Vila A, Barbosa VM, Calder PC (2007) Olive oil in parenteral nutrition. Curr Opin Clin Nutri Metab Care 10(2):165–174
85. Skouroliakou M, Konstantinou D, Agakidis C, Delikou N, Koutri K, Antoniadi M et al (2012) Cholestasis, Bronchopulmonary Dysplasia, and Lipid Profile in Preterm Infants Receiving MCT/omega-3-PUFA-Containing or Soybean-Based Lipid Emulsions. Nutr Clin Pract Off Publ Am Soc Parenter Enter 27(6):817–824
86. Ganapathy V, Hay JW, Kim JH (2012) Costs of necrotizing enterocolitis and cost-effectiveness of exclusively human milk-based products in feeding extremely premature infants. Breastfeeding Med Off J Acad Breastfeeding Med 7(1):29–37
87. Deshpande G, Rao S, Patole S (2007) Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. Lancet 369(9573):1614–1620
88. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T (2011) Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 3:CD005496
89. Finlay ER, Subhedar NV (2000) Pulmonary haemorrhage in preterm infants. Eur J Pediatr 159(11):870–871
90. Blondheim O, Abbasi S, Fox WW, Bhutani VK (1993) Effect of enteral gavage feeding rate on pulmonary functions of very low birth weight infants. J Pediatr 122(5 Pt 1):751–755
91. Premji SS, Chessell L (2011) Continuous nasogastric milk feeding versus intermittent bolus milk feeding for premature infants less than 1,500 g. Cochrane Database Syst Rev 11:CD001819
92. Keens TG, Bryan AC, Levison H, Ianuzzo CD (1978) Developmental pattern of muscle fiber types in human ventilatory muscles. J Appl Physiol 44(6):909–913
93. Lewis MI, Sieck GC, Fournier M, Belman MJ (1986) Effect of nutritional deprivation on diaphragm contractility and muscle fiber size. J Appl Physiol 60(2):596–603
94. Vassilakopoulos T, Petrof BJ (2004) Ventilator-induced diaphragmatic dysfunction. Am J Resp Crit Care 169(3):336–341
95. Sassoon CS (2002) Ventilator-associated diaphragmatic dysfunction. Am J Resp Crit Care 166(8):1017–1018
96. Doekel RC, Jr., Zwillich CW, Scoggin CH, Kryger M, Weil JV (1976) Clinical semi-starvation: depression of hypoxic ventilatory response. New Engl J Med 295(7):358–361
97. Kinney JM, Askanazi J, Gump FE, Foster RJ, Hyman AI (1980) Use of the ventilatory equivalent to separate hypermetabolism from increased dead space ventilation in the injured or septic patient. J Trauma 20(2):111–119
98. Askanazi J, Weissman C, LaSala PA, Milic-Emili J, Kinney JM (1984) Effect of protein intake on ventilatory drive. Anesthesiology 60(2):106–110
99. Campfield LA, Smith FJ, Rosenbaum M (1992) Human hunger: is there a role for blood glucose dynamics? Appetite 18(3):244
100. Jadcherla SR (2012) Pathophysiology of aerodigestive pulmonary disorders in the neonate. Clin Perinatol 39(3):639–654
101. Mendes TB, Mezzacappa MA, Toro AA, Ribeiro JD (2008) Risk factors for gastroesophageal reflux disease in very low birth weight infants with bronchopulmonary dysplasia. J Pediatr 84(2):154–159
102. Akinola E, Rosenkrantz TS, Pappagallo M, McKay K, Hussain N (2004) Gastroesophageal reflux in infants < 32 weeks gestational age at birth: lack of relationship to chronic lung disease. Am J Perinat 21(2):57–62

103. McCain GC, Gartside PS, Greenberg JM, Lott JW (2001) A feeding protocol for healthy preterm infants that shortens time to oral feeding. J Pediatr 139(3):374–379
104. Pridham K, Brown R, Sondel S, Green C, Wedel NY, Lai HC (1998) Transition time to full nipple feeding for premature infants with a history of lung disease. J Obst, Gyn, Neonat Nur: JOGNN/NAACOG 27(5):533–545
105. Howe TH, Sheu CF, Holzman IR (2007) Bottle-feeding behaviors in preterm infants with and without bronchopulmonary dysplasia. Am J Occup Ther: Off Publ Am Occup Ther Assoc 61(4):378–383
106. Palmer MM, Crawley K, Blanco IA (1993) Neonatal Oral-Motor Assessment scale: a reliability study. J Perinat Off J Calif Perinat Assoc 13(1):28–35
107. Gewolb IH, Vice FL (2006) Abnormalities in the coordination of respiration and swallow in preterm infants with bronchopulmonary dysplasia. Dev Med Child Neurol 48(7):595–599
108. Gewolb IH, Vice FL (2006) Maturational changes in the rhythms, patterning, and coordination of respiration and swallow during feeding in preterm and term infants. Dev Med Child Neurol 48(7):589–594
109. Gewolb IH, Bosma JF, Taciak VL, Vice FL (2001) Abnormal developmental patterns of suck and swallow rhythms during feeding in preterm infants with bronchopulmonary dysplasia. Dev Med Child Neurol 43(7):454–459
110. Mizuno K, Nishida Y, Taki M, Hibino S, Murase M, Sakurai M et al (2007) Infants with bronchopulmonary dysplasia suckle with weak pressures to maintain breathing during feeding. Pediatrics 120(4):e1035–e1042
111. Craig CM, Lee DN, Freer YN, Laing IA (1999) Modulations in breathing patterns during intermittent feeding in term infants and preterm infants with bronchopulmonary dysplasia. Dev Med Child Neurol 41(9):616–624
112. McCain GC, Del Moral T, Duncan RC, Fontaine JL, Pino LD (2012) Transition From Gavage to Nipple Feeding for Preterm Infants With Bronchopulmonary Dysplasia. Nurs Res 61(6):380–387
113. Schmidt B, Davis P, Moddemann D, Ohlsson A, Roberts RS, Saigal S et al (2001) Long-term effects of indomethacin prophylaxis in extremely-low-birth-weight infants. New Engl J Med 344(26):1966–1972
114. Young TE, Marshall DD, Bose CL, O'Shea TM (2006) Early fluid intake and chronic lung disease. J Pediatr 149(5):732; author reply 732
115. Spears K, Cheney C, Zerzan J (2004) Low plasma retinol concentrations increase the risk of developing bronchopulmonary dysplasia and long-term respiratory disability in very-low-birth-weight infants. Am J Clin Nutr 80(6):1589–1594
116. Darlow BA, Graham PJ (2011) Vitamin A supplementation to prevent mortality and short- and long-term morbidity in very low birthweight infants. Cochrane Database Syst Rev 10:CD000501
117. Janvier A, Lantos J, Barrington K (2013) The politics of probiotics: probiotics, necrotizing enterocolitis and the ethics of neonatal research. Acta Paediatr 102(2):116–118
118. Claud EC, Walker WA (2001) Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. FASEB J: (Official publication of the Federation of American Societies for Experimental Biology) 15(8):1398–1403
119. Deshpande G, Rao S, Patole S, Bulsara M (2010) Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics 125(5):921–930
120. Forman HJ, Rotman EI, Fisher AB (1983) Roles of selenium and sulfur-containing amino acids in protection against oxygen toxicity. Lab Invest; J Tech Methods Pathol 49(2):148–153
121. Darlow BA, Austin NC (2003) Selenium supplementation to prevent short-term morbidity in preterm neonates Cochrane Database Syst Rev 4:CD003312
122. Howlett A, Ohlsson A (2003) Inositol for respiratory distress syndrome in preterm infants Cochrane Database Syst Rev 4:CD000366
123. Howlett A, Ohlsson A, Plakkal N (2012) Inositol for respiratory distress syndrome in preterm infants. Cochrane Database Syst Rev 3:CD000366

124. Moe-Byrne T, Wagner JV, McGuire W (2012) Glutamine supplementation to prevent morbidity and mortality in preterm infants. Cochrane Database Syst Rev 3:CD001457
125. Soghier LM, Brion LP (2006) Cysteine, cystine or N-acetylcysteine supplementation in parenterally fed neonates. Cochrane Database Syst Rev 4:CD004869
126. Marks KA, Reichman B, Lusky A, Zmora E (2006) Fetal growth and postnatal growth failure in very-low-birthweight infants. Acta Paediatr 95(2):236–242
127. Wood NS, Costeloe K, Gibson AT, Hennessy EM, Marlow N, Wilkinson AR (2003) The EPICure study: growth and associated problems in children born at 25 weeks of gestational age or less. Arch Dis Child Fetal Neonat Ed 88(6):F492–F500
128. Brunton JA, Saigal S, Atkinson SA (1998) Growth and body composition in infants with bronchopulmonary dysplasia up to 3 months corrected age: a randomized trial of a high-energy nutrient-enriched formula fed after hospital discharge. J Pediatr 133(3):340–345
129. Madden J, Kobaly K, Minich NM, Schluchter M, Wilson-Costello D, Hack M (2010) Improved weight attainment of extremely low-gestational-age infants with bronchopulmonary dysplasia. J Perinat Off J Calif Perinat Assoc 30(2):103–111
130. Theile AR, Radmacher PG, Anschutz TW, Davis DW, Adamkin DH (2012) Nutritional strategies and growth in extremely low birth weight infants with bronchopulmonary dysplasia over the past 10 years. J Perinat Off J Calif Perinat Assoc 32(2):117–122
131. Atkinson SA (1994) Calcium and phosporus needs of premature infants. Nutr (Burbank, Los Angeles County, Calif) 10:66–68
132. Glasgow JF, Reid M (1977) 1 alpha-hydroxyvitamin D in nutritional rickets. Lancet 2(8032):302
133. Koo W. W SJJ (1998) Osteopenia and rickets of prematurity. WB Saunders, Philadelphia
134. Stennett DJ, Gerwick WH, Egging PK, Christensen JM (1988) Precipitate analysis from an indwelling total parenteral nutrition catheter. JPEN 12(1):88–92
135. Hung YL, Chen PC, Jeng SF, Hsieh CJ, Peng SS, Yen RF et al (2011) Serial measurements of serum alkaline phosphatase for early prediction of osteopaenia in preterm infants. J Paediatr Child Health 47(3):134–139
136. Koo WW, Steichen JJ (1998) Osteopenia and rickets of prematurity. In: Polin RA, W.W F (ed) Fetal and neonatal physiology. WB Saunders, Philadelphia p 2235–2249
137. Atkinson SAaRT (2005) Recommended reasonable range for parenteral and enteral nutritio for preterm infants during hospitalisation. Digital Educational Publishing, Cincinati
138. Schulzke SM, Trachsel D, Patole SK (2007) Physical activity programs for promoting bone mineralization and growth in preterm infants. Cochrane Database Syst Rev (2):CD005387
139. Rigo J, Pieltain C, Salle B, Senterre J (2007) Enteral calcium, phosphate and vitamin D requirements and bone mineralization in preterm infants. Acta Paediatr 96(7):969–974
140. Johnson DB, Cheney C, Monsen ER (1998) Nutrition and feeding in infants with bronchopulmonary dysplasia after initial hospital discharge: risk factors for growth failure. J Am Diet Assoc 98(6):649–656
141. Allen J, Zwerdling R, Ehrenkranz R, Gaultier C, Geggel R, Greenough A et al (2003) Statement on the care of the child with chronic lung disease of infancy and childhood. Am J Resp Crit Care 168(3):356–396
142. Ogden CL, Kuczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R et al (2002) Centers for disease control and prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. Pediatrics 109(1):45–60
143. Singer L, Martin RJ, Hawkins SW, Benson-Szekely LJ, Yamashita TS, Carlo WA (1992) Oxygen desaturation complicates feeding in infants with bronchopulmonary dysplasia after discharge. Pediatrics 90(3):380–384

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S. Patole (ed.), *Nutrition for the Preterm Neonate,*
DOI 10.1007/978-94-007-6812-3, © Springer Science+Business Media Dordrecht 2013