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Supplementary Digital Content no.1

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Introduction, methods and limitations. (February 2022)

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Introduction

The Committee of Nutrition (CoN) of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recognised the need to provide an update of the previous position paper on enteral nutrition for preterm infants (1) and this was approved by the ESPGHAN council in 2019. The working group was coordinated by members of CoN but recognised the benefit of including additional experts in neonatal nutrition who were invited to reflect a range of research and topic expertise, geographical location and gender. An initial planning meeting was held as part of the CoN meeting in Oslo in March 2019, at which potential topics were discussed and a provisional list of sections were developed. A lead writer was assigned to each chapter, supported initially by an additional 2-3 co-writers to initiate literature reviews, and write the first drafts. All listed authors reviewed, contributed, and voted on every section including the published summary document.

We held one initial face-to-face meeting in Amsterdam in February 2020 at which broad consensus was achieved for most topics. Literature searching and review commenced in 2019 and continued until December 2020. However, all subsequent processes were affected by the COVID 19 pandemic, and paper development was completed using online meetings and correspondence.

Aims and methods

The working group agreed to avoid excessively long sections and to only include sufficient data on basic physiology in order that readers can interpret the conclusions and recommendations in different contexts and settings. ESPGHAN provided funding for one face-to-face meeting, but we did not obtain any additional funding to conduct the literature searching, online meetings and writing, and there was no involvement of any industry representatives at any stage. A full list of potential conflicts of interest are provided for each author.

The aim of the project was to provide a single published position paper that would be useful to clinicians, supported by supplementary digital content (SDC) for each section. The focus

of the position paper is enteral nutrition of stable preterm infants (< 1800 g birth weight) until around 35-36 weeks corrected gestation, and does not include larger or more mature infants, critically ill infants, or the management of infants after hospital discharge. Conclusions and recommendations are the same for both appropriate for gestational age (AGA) and small for gestational age (SGA) infants, because data are lacking to propose specific recommendations for SGA infants although all intakes must be adapted to individual circumstances. We recently provided our position on nutrition in late and moderately preterm infants and nutrition in critically ill infants (2)(3).

We aim to provide nutrient intake recommendations that appear most likely to support adequate growth and development of stable, growing preterm infants, that also aim to optimise both short- and long-term functional outcomes. Quality and quantity of nutrient intakes have major impacts on the risks of all common neonatal diseases especially diseases such as sepsis, necrotising enterocolitis (NEC), retinopathy of prematurity (ROP) and broncho-pulmonary dysplasia (BPD), as well as having direct impacts on biochemical homeostasis and organ function. Optimal nutritional management improves brain growth over the life course and reduces the risk of death. Therefore, the overall aim of nutrient intake recommendations is to provide intake ranges that optimise nutritional status and minimise risk, rather than simply promote a specific rate of growth. Defining and assessing optimal nutritional status is a complex topic and beyond the scope of this paper, although we allude to some of these concepts in the section on growth. However, we wish to highlight the importance of paying attention to certain key aspects of nutritional assessment:

1. The quality of the nutrients provided is critical. Nutrients (for example protein and fat) provided as human milk are likely to be superior to those in formula milk. Replacing milk volume intake with formula milk instead of mother's own milk (MOM) to support more rapid growth may not improve overall nutritional status even if weight gain is greater. Furthermore, there are increasing data highlighting the benefits of non-nutritional factors (so called immuno- or bionutrients) such as hormones, growth factors, cells and enzymes present in human milk that are typically not present in formula milk.
2. Assessment of growth would ideally be supported by measures of body composition, but currently available techniques are not validated for use in routine clinical practice. However, at the very least, interpretation of growth adequacy requires linear growth, head growth and weight gain to be considered alongside each other so that growth is 'proportional'. There are no agreed definitions to determine the proportionality of growth, but we emphasise the importance of using a validated growth reference and visually comparing trajectories for weight, head and linear gain.
3. Biochemical measures of nutritional adequacy can be problematic especially when preterm infants are considered. Serum concentrations may not reflect total body status. Normal serum concentrations of sodium, calcium and many other nutrients are frequently seen in situations where body stores are low and nutrient intakes are inadequate. However, whilst there are no reliable serum measures of macronutrient sufficiency, measurement of serum concentrations of certain nutrients and compounds are an essential part of nutritional care including electrolytes, glucose, minerals and perhaps urea, haemoglobin, ferritin and other compounds.
4. Nutrition is everyone's responsibility. Efforts to optimise nutritional status require a team approach. Families must be part of the process from the beginning, helped to

understand the approach to nutritional management and involved in decision making. Supporting mothers to provide MOM, providing an adequate NICU (Newborn Intensive Care Unit) environment that avoids separating parents from their babies, and considers sensory aspects of care (skin to skin, light exposure, touch, and other sensory aspects) are core components of a holistic assessment of nutritional status.

We describe intakes that best meet physiological demands in order to maintain health and development without producing excessive metabolic stress (disturbed homeostasis) or other adverse effect in the short and long-term. These recommendations apply to stable infants but may not be optimal for infants recovering from severe illness, or those with additional nutrient losses or demands. They are relevant for infants receiving enteral nutrition and encompass estimates for absorption and assimilation and are assumed to support growth and body composition (i.e. weight, linear and head growth) like a fetus of the same post-menstrual age. However, we highlight that growth in the ex-utero environment will never be the same as in-utero, that optimal proportions of fat and lean mass accretion will differ, and that optimal nutrient intakes and growth trajectory for an individual infant are impossible to determine. We strongly support individual units to develop these general recommendations and ensure the clinical practice on their units is consistent.

We provide nutrient ranges, and, in most cases, we rounded data to one decimal point in part to emphasise the imprecision of much of the data. In addition, for some nutrients we provide in parentheses an additional amount e.g., for sodium 3-5 (-8) mmol. The intention of this is to highlight that whilst most infants will require an intake of 3-5mmol there are common situations when a higher intake (up to 8mmol) might be needed, for example when infants are fed at higher intakes of protein and/or have higher anticipated growth rates.

Evidence base and grading of recommendations

We followed the methodology and guidelines recommended by similar groups producing clinical guidelines in nutrition as recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN) and ESPGHAN and acknowledge the work of these groups in establishing a workable methodology (4)(5). A systematic literature search was used and is included for each section, and all relevant identified publications were reviewed. Authors reviewed the ESPGHAN 2010 position paper in its entirety including re-evaluating data used in that paper. For all sections provided as SDCs, searching was conducted using online databases as described and there was no additional manual searching. The writers of each section were asked to produce 3-5 evidence-based conclusions which were individually graded according to the level of evidence (LOE). LOE is provided according to the Scottish Intercollegiate Guidelines Network (SIGN) with levels 1-4, and levels 1 and 2 further sub-classified as 1++, 1+, 1-, 2++, 2+, and 2- depending on the quality of the meta-analysis, RCTs (randomized controlled trials), case-control and cohort studies. Each section also includes 3-5 recommendations which are all individually graded according to the consensus

of the working group as Grade of Recommendation (GOR) A (strong recommendation), B (recommended), C (conditional recommendation) or GPP (Good Practice Points) (expert consensus). Where we did not achieve full consensus through online voting, we conducted further discussion and review followed by further rounds of voting until we achieved strong consensus of >95% for each of the conclusions and recommendations. We elected to summarise some of the conclusions and recommendations in the published summary, whereas the individual sections provided as SDCs may provide a more detailed list and grading.

Limitations

There are a number of common limitations for nutritional studies in preterm infants including study size and quality, a general lack of long term follow up, poor design, lack of blinding etc. These are reflected in the LOE and GOR. In addition, we highlight the complexity of producing a position paper of individual nutrient intakes when all nutrients and processes are co-dependant on other factors such as:

- nutrient ratios, for example the need to provide optimal protein:energy ratios at each level of protein intake, the variation of water requirements depending on protein and electrolyte intakes
- the importance of co-factors that affect nutrient absorption, for example lipase activity impacts on fat absorption
- differences in absorption depending on milk type, for example there are important differences in calcium absorption between unfortified and fortified human milk, and formula milk etc.

The conclusions and recommendations are considered in the broad context of health and social care conditions and resources for preterm infants in high income setting such as Europe and may not be directly applicable in other settings e.g., low- and middle-income countries.

We recognise the complexity created by population heterogeneity when providing recommendations for ‘preterm infants’ who are an extremely heterogenous population group. There are likely to be significant differences in benefits and risks between very immature infants weighing <750g who have recovered from critical illness, those born more mature and/or larger and those who were affected by fetal growth restriction. These factors are likely to affect gastrointestinal function, nutrient absorption and assimilation and metabolic capacity for example, effects on glucose or lipid homeostasis. The potential long-term impacts on metabolic and cognitive outcomes of more rapid catch-up growth may also differ between individual infants. Whilst we highlight these issues in brief, a detailed consideration of all relevant factors for each nutrient and nutritional strategies in all contexts is clearly impossible.

The strongest data come from well conducted randomised controlled trials that include long-term functional outcomes, but few such studies exist for most nutrients or nutritional strategies. We recognise the limitations of studies with short term follow up, those based on basic physiology or a factorial approach, or observational studies that simply reflect current intakes in the population. We noted specific difficulties for many micronutrients, trace

elements and vitamins where few good studies exist and where recommendations reflect estimates from organisations such as the European Food Standards Authority (EFSA). Many such organisations derived estimates from intakes in healthy breastfed term infants or amounts that have been provided in formula designed for preterm infants. Whilst these intakes appear broadly safe, they may not be optimal. All these methods and recommendations derived from them pose potential dangers and there are risks that intakes may have been over-estimated. It is not clear if these could result in harm.

Finally, whilst this paper provides evidenced based conclusions and recommendations for clinicians, EPSGHAN CoN consider this to be a position paper derived by expert consensus. The lack of strong and robust data in many areas imply we do not consider it to be a robust guideline to be adopted without consideration for local contexts and individual infants. We recognise multiple situations where variation in clinical practice is likely to be appropriate and strongly support the need for further research that might test or study nutrient intakes or nutritional strategies that differ from our current position. Neonatal nutrition research is also extremely active, and it is likely that alternative approaches and recommendations may be preferable as our knowledge expands over the next 10 years.

ESPGHAN is not responsible for the practices of physicians or other healthcare professionals and provides position papers as indicators of best practice only. Diagnosis and treatment is at the discretion of the healthcare provider.

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Supplementary Digital content no. 2

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Human milk nutrient composition: Evidence base and justification (February 2022)

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Human milk composition

Human milk (HM) is considered the gold standard for infant nutrition, also in premature infants if adequately fortified (1-3) (SDC no. 19). HM is a fat emulsion that contains much more than just nutrients. In addition to the functional components that are described in SDC no 18, HM also contains growth factors, enzymes, microbes, cellular components, smell and taste. It is not within the scope of this chapter to discuss the significance of these different non-nutritional factors on growth and development in preterm infants, but we need to keep in mind that these factors and the mother-child interaction play an important role.

It is well recognized that the nutrient composition of HM is highly variable and changes with the duration of lactation, time of day, and during each breast feed (4, 5). Moreover, there does not seem to be any strict correlation between the different macronutrient components (6). However, the nutrient composition also depends on the nutritional status and diet of the mother, on genetic and environmental factors, as well as on how the milk is processed and provided (4, 7). Besides, energy content of human milk is most often reported as gross energy whereas not all energy is bioavailable (8). From a clinical perspective, bioavailable energy contents would probably be more informative. Roughly estimated, metabolizable energy content amounts 90-95% of the reported (and measured) gross energy intakes. Taken together, these factors make it very difficult to provide a reference table for the average macro- and micronutrient concentrations of HM. On the other hand, a reference table may serve as a crude basis for nutrient calculations when providing unfortified HM or standardized fortifier regimens using multi-component fortifier products.

With this in mind, we have tried to summarize the range of means/medians and the total range (or 95% CI) for the macro- and micronutrients in HM. For the macronutrients and the main electrolytes, with the largest differences during the first weeks of lactation, we

provide different ranges for colostrum, early HM (7-28 days postpartum) and "mature" HM (> 28 days postpartum). The reference table is not based on a formal systematic overview, but we have derived the numbers from systematic reviews, whenever available. However, many nutrients, especially if they came from multiple sources, were rounded off with a big rule of thumb.

If individual infants exhibit growth failure despite estimated nutrient intakes in the upper range of the recommendations given in this position paper, this might reflect selective malnutrition, and "adjustable" or "targeted" fortification may be indicated to compensate for variations in HM composition (SDC no 19).

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Table 1. Human milk nutrient composition. If available, we used literature reports in which milk was assessed from women that had delivered preterm. If not available, this was denoted in the last column with references.

Nutrient	Unit per 100 mL	Postnatal age						Reference
		Colostrum		7-28 d		>28 d		
		Range of means/medians	Total range/95%CI	Range of means/medians	Total range/95%CI	Range of means/medians	Total range/95%CI	
Energy *	kcal	50-65	40-75	65-75	45-90	66-68	45-95	(9-11)
Protein	g	1.8-3.0	1.0-4.0	1.2-1.5	1.0-2.5	0.9-1.2	0.6-1.5	(9-11)
Lactose	g	5.1-6.0	3.9-8.5	5.7-6.7	4.1-8.1	5.8-6.8	4.1-8.1	(9-11)
Fat	g	2.2-2.6	1.0-4.5	3.0-3.5	2.0-5.5	3.4-3.7	2.5-6.5	(9-11)
· *Linoleic acid (C18:2 n−6)*	*% of fat*	*15.0*	-	*12.5*	-	*13.3*	-	*(12)*
· *α-Linolenic acid (C18:3 n−3)*	*% of fat*	*0.89*	-	*0.86*	-	*0.99*	-	*(12)*
· *ARA (C20:4 n−6)*	*% of fat*	*0.79*	-	*0.61*	-	*0.53*	-	*(12)*
· *EPA (C20:5 n−3)*	*% of fat*	*0.08*	-	*0.13*	-	*0.12*	-	*(12)*
· *DHA (C22:6 n−3)*	*% of fat*	*0.57*	-	*0.57*	-	*0.40*	-	*(12)*
Sodium	mmol mg	1.7-2.4 40-55	1.7-2.6 40-60	0.9-1.1 20-25	0.7-2.0 15-45	0.8-0.9 18-20	0.7-1.3 15-30	(11, 13)
Potassium	mmol mg	1.7 65	1.4-1.8 55-70	1.2-1.4 45-55	1.1-1.6 41-62	1.2-1.4 45-55	1.1-1.6 41-62	(11, 13)
Chloride	mmol mg	2.1-3.1 75-110	1.7-3.1 60-110	1.0-1.8 35-65	0.8-2.1 30-75	1.0-1.4 35-50	0.8-1.7 30-60	(11, 13)
Calcium	mmol mg	0.60-0.75 25-30	0.40-1.0 15-40	0.60-0.75 25-30	0.40-1.0 15-40	0.60-0.75 25-30	0.40-1.0 15-40	(9, 11)

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Phosphorus	mmol mg	0.30-0.40 10-12	0.15-0.65 5-20	0.40-0.55 13-17	0.30-0.80 10-25	0.40-0.50 12-16	0.30-0.65 10-20	(9, 11)
Magnesium	mmol mg	0.12-0.14 2.8-3.4	0.11-0.16 2.7-3.8	0.12-0.13 2.8-3.2	0.09-0.18 2.1-4.3	0.13 3.1	0.09-0.16 2.1-4.0	(11, 13)
Iron	µg	-	-	-	-	20-40	-	(14, 15)
Zinc	µg	250-500	200-800	200-470	200-900	150-400	100-800	(11, 13, 15)
Copper	µg	50	25-80	40-45	25-75	22-40	20-60	(11, 13-15)
Selenium	µg	-	-	-	-	1.2-1.8	0.3-8.4	(14, 15) term
Manganese	µg	-	-	-	-	0.4	0.3-3.0	(14) term
Iodine	µg	-	-	-	-	5-10	5-15	(14, 15) term
Fluoride	µg	-	-	-	-	-	0-10	(14) term
Chromium	µg	-	-	-	-	-	0.02-1.1	(14) term
Molybdenum	µg	-	-	-	-	0.25	0.07-0.4	(14) term
Vitamin A (RE)	µg	135	35-235	240	215-265	23-53	8-60	(13-15) term
Vitamin D	µg	-	-	-	-	0.025-0.20	-	(14) term
Vitamin E (TE)	mg	2.4	1.3-3.5	1.2	1.1-1.3	0.32-0.49	0.23-0.49	(13-15) term
Vitamin K	µg	-	-	-	-	0.20-0.25	0.085-1.0	(14, 15) term
Ascorbic acid (vitamin C)	mg	-	-	-	-	3.0-9.0	-	(14, 15) term
Thiamine (B1)	µg	-	-	25	2.5-50	7.0-21	3.0-30	(13-15) term
Riboflavin (B2)	µg	-	-	-	-	30-60	-	(14, 15) term
Pyridoxine (B6)	µg	-	-	-	-	13-26	-	(13-15)term
Niacin (B3) (NE)	mg	-	-	-	-	0.18-0.27	-	(14, 15) term
Pantothenic acid (B5)	mg	-	-	-	-	0.25	-	(14) term
Biotin (B8)	µg	-	-	-	-	0.5	-	(14) term

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Folate (B9/B11)	µg	-	-	-	-	3.2-8.5	-	(13-15) term
Cobalamin (B12)	µg	-	-	0.10	0.03-0.25	0.03-0.12	0.02-0.12	(14, 15) term
L-Carnitine	mg	-	-	-	-	0.6-1.1	-	(14) term
Choline (total)	mg	4.2-7.0	2.5-7.0	-	-	14-17	11-21	(13, 14) term

* reported energy contents are gross contents. Bioavailable energy content is approximately 5 to 10% less (see text) (8).

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Supplementary Digital Content no.3

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: (February 2022)

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Introduction

Water is the major constituent of the human body and a key component of enteral nutrition, as an essential carrier for nutrients and metabolites. In preterm infants fluid requirements are higher compared to term infants, due to a number of factors including immature renal function with lower maximum urine osmolality, immature skin leading to increased insensible water loss, higher surface area to body volume ratio, but also because fluid needs are proportional to growth rates. Fluid requirements show considerable inter- and intra-individual variation, especially in very preterm infants that is greater than for most other nutrients (1). Maintenance of water volume is critical for body homeostasis and thermoregulation, cardiovascular and renal function, and certain adequate fluid intakes are needed in order to provide sufficient nutritional intakes. Total body water, as well as its major components, i.e. extracellular and intracellular fluid volumes, may change depending on clinical condition, and optimal fluid and dietary intakes need to be adapted. In preterm infants fed fortified breastmilk or formula the relationship between water and solute amounts is important, as it changes milk osmolality (2, 3), renal solute and acid load (4, 5). This also influences feed tolerance, acid-base status (5) and consequently water balance and requirements respectively, especially in preterm infants whose renal concentration ability, excretory capacity and regulation of acid-base metabolism are limited (6, 7).

Main text

Establishing recommendations for enteral fluid intake in stable growing preterm infants requires consideration of the following issues: 1) published data on body water and fluid intake in preterm neonates; 2) human milk composition and need for nutrient fortification; 3) the need to balance the fluid intake required to support normal growth and to optimize long term outcomes, whilst avoiding short term metabolic disturbances.
A general limitation of studies assessing enteral fluid intakes in preterm babies is that they often investigated the primary endpoint of fluid volume needed to achieve nutritional intakes, without considering parameters of water metabolism or kidney function.

1. Measurements of enteral fluid intake have been extrapolated based on water turnover using stable isotopes in 6-week-old healthy full-term (n=9) (8) as well as in growing preterm infants (n=13) (9) and derived values were 140-180 and 130-190 mL/kg/d (10), respectively. However, it is important to note that earlier studies in preterm babies were performed at a time when enteral macronutrient and electrolyte intakes were lower than current recommendations. Similar ranges were reported in international surveys of feeding practices as the targeted enteral feeding volume in preterm (11, 12) or VLBW infants (13). These studies have several methodological limitations as they were small sample size cohorts in healthy preterm infants often

with higher birth weight, post-conceptional and postnatal age, compared to current populations of stable growing preterm infants.

A few RCTs have compared enteral fluid intakes in stable preterm infants and of these, only three (14-16) were found eligible for a recent systematic review (17). One study included in the review (15) had compared high (300 mL/kg/d) versus standard (200 mL/kg/d) enteral fluid intakes in VLBW infants exclusively fed with expressed breast milk through nasogastric tube. These infants were able to tolerate fluid volumes of up to 300 mL/kg/d, but the study was not powered to detect differences for necrotizing enterocolitis or cardiopulmonary outcomes. It is also important to note that this trial was conducted in a low or middle income country, and that infants with a symptomatic patent ductus arteriosus (PDA) were excluded, the average gestation was 32 weeks and included a high proportion of infants who were SGA.

2. Mother's own milk composition changes over time: the protein content of expressed preterm milk declines from 2.4 to around 1.0 g/100 mL from the first days to the fourth week of life (18), and whilst breast milk is the best source for preterm infants, fortification with macronutrients and micronutrients using multicomponent fortifiers is necessary for most preterm infants (19-21). This increases solute concentration, milk osmolality and renal solute load. Potential renal solute load (PRSL) refers to solutes of dietary origin that must be excreted in the urine because they are not diverted into new tissue or lost through extra-renal routes. PRSL can be calculated by the following equation PRSL = [N]/28 + Na + Cl + K + Pa, where the dietary intakes of solutes are expressed in millimoles (mmol, or milliosmoles, mOsm), [N] is milligrams of nitrogen, and the factor [N]/28 represents the excretion of nitrogenous substances as urea (urea contains two nitrogen atoms with atomic weight 14). Na is sodium, Cl is chloride, K is potassium, and Pa is available (nonphytate) phosphorus, which is the same as total phosphorus in milk-based formulas (22).

   PRSL has been estimated at 9-19 mmol/kg in preterm infants fed human milk and at 11-32 mmol/Kg in those fed artificial milk formula (4). An expert panel on assessment of nutrient requirements for infant formula (23) suggested that minimum and maximum PRSL for preterm infant formula should be 22 mOsm and 32 mOsm/100 kcal. To date, only a few studies have calculated and reported the PRSL of fortified milk (5), and no studies of varying enteral water intake in preterm infants have explored this important issue (24, 14, 16).

3. Current recommendations for nutrition of preterm infants are based on the goal of approximating the growth and body composition of the healthy fetus at the same postmenstrual age, along with optimal functional development. Energy-protein ratios are important in ensuring appropriate quality of growth. The fluid intake necessary to achieve nutrient intake recommendations with different diets may by far exceed 300mL/kg/day for unfortified preterm human milk, ~150-200 mL/kg for fortified human milk and ~150–165 mL/kg/day for currently available artificial 'preterm' cow's milk-based formula. Large fluid volumes may be challenging for some preterm infants, in particular those with bronchopulmonary dysplasia (BPD) or PDA (25), but fluid restriction in return may lead to insufficient nutrient intake (unless feeds are concentrated) which will negatively impact postnatal growth (26). However, there are no detailed studies comparing different levels of fluid intakes whilst keeping nutrient intakes identical.

   It is important that fluid intakes support "appropriate growth" rather than simply focusing on weight gain, especially because short-term fluctuations in body weight

may simply reflect changes in hydration status (17). Although some clinicians are cautious about increasing intake volumes, prospective studies demonstrated improved growth with increased feed volumes up to 200 mL/kg/d (16, 27). These volumes appear well tolerated, improved weight gain and head growth, without adverse outcomes for body composition at 36 weeks of postmenstrual age or discharge. Very few studies have explored outcomes in infancy, but one retrospective study reported growth measures and neurodevelopment at 2 and 4 years corrected age respectively, and showed that higher-volume enteral feeding regimens (average 193 mL/kg/d, range 170-220 mL/kg/d from day of life 15 to 42) led to higher nutrient intakes, were safe and associated with improved growth and language development (28).
Whilst there are few studies exploring longer term renal outcomes, there is growing evidence from animal and human studies that prolonged states of mild dehydration cause renal hyperfiltration, especially when associated with high protein intake (29) which may be relevant to early development of metabolic disease in adulthood.

Conclusions and key recommendations

We conclude that fluid volumes as low as 135 mL/kg/d may be considered safe to maintain body homeostasis and avoid renal compromise, but, to achieve appropriate nutrient intakes, feeding volumes of 150–180 mL/kg/d, using artificial milk formula or fortified breast milk, are likely to be required for the majority of stable growing preterm infants. In individual preterm infants, enteral intakes up to 200 mL/kg/d (or higher) may be appropriate and safe depending on current health status such as the presence of a significant PDA or BPD.

In fully enterally fed preterm infants water balance, hydration status and renal function should be regularly assessed, especially when nutrient intakes or the clinical situation change, and fluid volume should be adjusted accordingly. Based on final osmolality, renal solute load and in accordance with previous recommendations, a reasonable range of fluid volume is 135-200 mL/kg/d. In infants receiving fluid intakes at the lower or upper margins recommended, growth and fluid tolerance must be considered regularly. Finally, the long-term renal effects of enteral feeding with high solute concentration deserve further investigation.
There is insufficient evidence to conclude whether routine enteral volumes higher than 200 mL/kg/d are beneficial, although this may be particularly relevant in specific contexts especially low-middle income countries where access to HM fortifiers is limited, or in infants that do not tolerate full strength fortification.
Finally, some subgroups of preterm infants, such as extremely low birth weight babies or severely growth-restricted infants may present substantial specificities in terms of fluid needs and body water composition. However, for these subgroups, there is insufficient evidence to recommend water intakes different from other preterm infants.

Conclusions, Recommendations

C1: Water requirements show considerable inter- and intra-individual variation, especially in preterm infants (LOE 2++)
C2: Water volume needed to maintain body homeostasis, cardiovascular and kidney function may be different from the volume needed to provide adequate nutrient intakes (LOE 3)

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C3: In fully enterally fed preterm infants water balance, hydration status and renal function should be regularly assessed and taken into account for the administration of fluid intake (LOE 2++)
R1: Most stable growing infants will require fluid intake of 150-180 mL/kg/d to achieve appropriate nutrient intakes. RGB (strong recommendation)
R2: If nutrition needs can be met, fluid intake as low as 135 mL/kg/d may be considered safe to maintain body homeostasis and avoid renal compromise. RG0 (conditional recommendation)
R3: In individual preterm infants, enteral fluid intakes up to 200 mL/kg/d may be appropriate and safe depending on current clinical status. RG0 (conditional recommendation).

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Energy

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ENERGY

Background

Energy is required by all cells of the body and supply needs to meet this 'basal metabolic rate' (BMR), plus the requirements of any physical activity (PA), diet induced thermogenesis (DIT), and importantly for preterm infants, growth. Energy intakes which exceed the amount required to carry out these functions can result in deposition of excess energy as fat stores with potential impacts on long-term metabolic programming(1). Conversely, inadequate energy supply can lead to impaired growth, catabolism of body tissues, impaired immunity and sub-optimal motor, cognitive and behavioural development(2).

Literature Search

A literature search was carried out to identify papers relevant to the energy needs of preterm infants using the search strategy outline in Table 1. Medline and EMBASE were searched using the OVID interface from 2000 until October 2020.

1. exp Infant, Premature/
2. exp Infant, Extremely Premature/
3. exp Infant, Low Birth Weight/
4. exp Infant, Small for Gestational Age/
5. exp Infant, Very Low Birth Weight/
6. exp Infant, Extremely Low Birth Weight/
7. prematurity.ti,kf.
8. prematures.ti,kf.
9. micropremie.ti,kf.
10. low birth weight.ti,kf.
11. low birthweight.ti,kf.
12. ((infant* or neonat* or newborn* or toddler*) and (preterm* or pre-term* or prematur* or pre-matur* or low birth weight or low birthweight)).ti.
13. ((infant* or neonat* or newborn* or toddler*) and (preterm* or pre-term* or prematur* or pre-matur* or low birth weight or low birthweight)).kf.
14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13
15. exp Energy Intake/ or exp Protein-Energy Malnutrition/ or energy.mp. or exp Energy Metabolism/
16. 14 and 15
17. limit 16 to yr="2000 - 2020"

Authors: MJ Johnson, SJ Moltu

Table 1: Search strategy used for OVID Medline and Embase

Estimating Requirements

To determine energy intake recommendations for preterm infants, there is a need to understand both the energy needs to meet BMR, DIT and PA, together with the energy requirements for growth(3). Direct measurement of BMR in preterm infants is not possible but basal needs can be estimated by measuring resting energy expenditure (REE) which is considered to be about 10% greater than BMR and includes the energy needed for maintaining the vital processes of the body (including breathing) and the energy cost of tissue accretion (but not including the growth related deposition of energy as fat, glycogen and protein in tissue). REE may change during conditions such as inflammation or chronic disease states. DIT is the energy expended when food is digested and absorbed and accounts for about 10% of all energy needs(4). The contribution of PA to REE in preterm infants is small as they mainly sleep or lie quietly, though does increase with postnatal age as infants become more active(3). Recommendations for energy intake depend on growth targets, and for preterm infants our position is to base recommendations on the aim of supporting growth, body composition and nutrient retention similar to the in-utero fetus(5), whilst acknowledging that nutritional needs are different in the ex-utero environment. It is noteworthy that the composition of weight gain ex-utero is often different to that seen in-utero, with relatively more fat deposition, and this is likely to be related to both the type and amount of nutrition delivered and the extrauterine environment. Adjustments need to be made for the ex-utero environment because of differences in nutrient supply and metabolism (e.g. the fetus receives only a small proportion of energy as fat) and energy lost through gastrointestinal malabsorption(5). Energy needs are higher per kg body weight at lower gestations and vary with growth velocity which decreases as postconceptional age increases(6). Of note, the Atwater factors used to estimate the metabolisable energy content of feeds are based on adult data and relatively unadapted for ELBW infants. In particular, fat absorption is reduced in VLBW infants compared to adults, so its Atwater factor is likely to need some adjustment. Such a difference could explain why a feed with a higher fat content could induce a lower growth rate than a feed that is predominantly carbohydrate, despite an apparently equivalent energy content.

Resting Energy Expenditure

Tissue synthesis is energy intense and influenced by intakes of protein and other nutrients, meaning that an adequate supply of protein and optimal protein:energy ratio (PER), alongside adequate

provision of other essential nutrients is important(7). Generally, REE increases with nutrient intakes, and also with postnatal age, possibly reflecting a shift from an early postnatal phase to a growth phase. Studies determining REE that were reviewed as part of previous ESPGHAN recommendations for enteral energy intake can now be considered along with more recent data. Bauer et al measured REE using indirect calorimetry in 75 preterm infants born between 26- and 28-weeks' gestation over a 7 week period and found that REE increased from 39kcal/kg/day in week 2 when infants were fully enterally fed on formula milk, to 80kcal/kg/day in week 7(6). However, these data suggest that REE increases by 3.75 Kcal per g/kg/day of weight gain, much higher than values from previous studies which suggested the energy cost of protein and fat deposition to be around 1 kcal per g/kg/day of weight gain(7, 8). An earlier study by the same group measured REE of another cohort of 17 appropriate for gestational age (AGA) preterm infants born below 28 weeks and weighing less than 1kg(9). Infants were fully fed from day 12 of the study through to completion on day 36, with REE increasing from 32kcal/kg/day to 74 kcal/kg/day during that time. During this study total energy intake at the end of the study was 119kcal/kg/day and was associated with weight gain of 19g/kg/day.

Romera et al studied REE using indirect calorimetry in 22 preterm infants born less than 27 weeks gestation after full enteral feeds were established. The infants were randomised to one of 3 groups to receive a formula milk containing 3.3g/kg/day protein and 147kcal/kg/day but with varying proportions of additional medium-chain triglycerides and carbohydrates, and compared these to a control group receiving a standard milk containing 3.3g/kg/day protein with 123kcal/kg/day(10). REE was measured at study commencement prior to randomisation of the different feeding regimens and ranged from 48-55kcal/kg/day with no significant difference between the groups in weeks 3-4.

A more recent study by Abranches et al, measured REE in 61 preterm infants using indirect calorimetry and found that REE increased from 44-47kcal/kg/day at the end of the first week of life up to 56-57kcal/kg/day by the end of the fourth week(11), when most infants were on full enteral feeds. Of note there was no difference in REE between small for gestational age (SGA) and AGA infants in this study.

The previous ESPGHAN recommendations assumed that REE in stable preterm infants was approximately 45 kcal/kg/day but did not include the energy cost of tissue deposition for growth(12). More recent studies suggest REE in healthy growing very preterm infants (Table 2) suggest that an REE in the range of 49 to 65 kcal/kg/day(6, 9-11, 13, 14). Of note, in all the studies, mean growth rate was lower than that based on in-utero growth (15-18)(17-20 g/kg/day). However,

these estimates include the energy cost of tissue deposition (1-1.2kcal/g tissue deposited(19)), and in fact once these studies are adjusted for the rate of growth seen, meta-analysis of these data (Figure 1) that shows the mean REE in stable enterally fed preterm infants is 43.9kcal/kg/day (95% confidence interval 43.0 to 44.8kcal/kg/day), plus the energy cost of tissue accretion of approximately 1-1.2kcal/g weight gain. **Assuming a growth rate of 17-20g/kcal/day, this means a total REE including the cost of tissue deposition of approximately 60-70kcal/kg/day** (table 3).

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Study	Group	Mean Birthweight	Mean Gestational Age at Birth	Number of infants	Timing of Measurement	Resting Energy Expenditure (kcal/kg/day)	95% Confidence interval	Weight Gain during study period (g/kg/day)	REE excluding energy cost of growth (kcal/kg/day)
Abranches 2018	AGA	1415	29	43	Day 28	55.7	52.5 to 58.9	8*	47.7
Abranches 2018	SGA	1260	33	18	Day 29	57	48 to 66	12.2*	44.8
Bauer K 2001	All	980	27	26	Day 30	53	51.7 to 54.3	10.1*	42.9
Bauer J 2003	Controls	1310	32	8	week 4	58	51.1 to 64.9	3*	55
Bauer 2009	26-28wks	Not available	Not available	75	week 4	57	54.7 to 59.3	12.2	44.8
Bauer 2009	29-32wks	Not available	Not available	49	week 4	65	61.9 to 68.1	13.9	51.1
Bell 2017	All	1320	29.3	15	Day 24	67.6	62.5 to 72.7	7.26*	60.34
Romera 2004	A	1130	29.3	8	Day 27	55.1	49.2 to 61	16	39.1
Romera 2004	B	1163	29.4	7	Day 27	51.6	46.3 to 56.9	14.9	36.7
Romera 2004	C	1166	29.7	7	Day 24	51.9	46.9 to 56.9	16.6	35.3
Romera 2004	D	1150	29.2	8	Day 23	52.4	49.9 to 54.9	13.8	38.6

Table 2: Studies of Resting Energy Expenditure in enterally fed preterm infants over the past 2 decades AGA – Appropriate for Gestational Age; SGA – Small for Gestational Age. *weight gain estimated from study population weight data

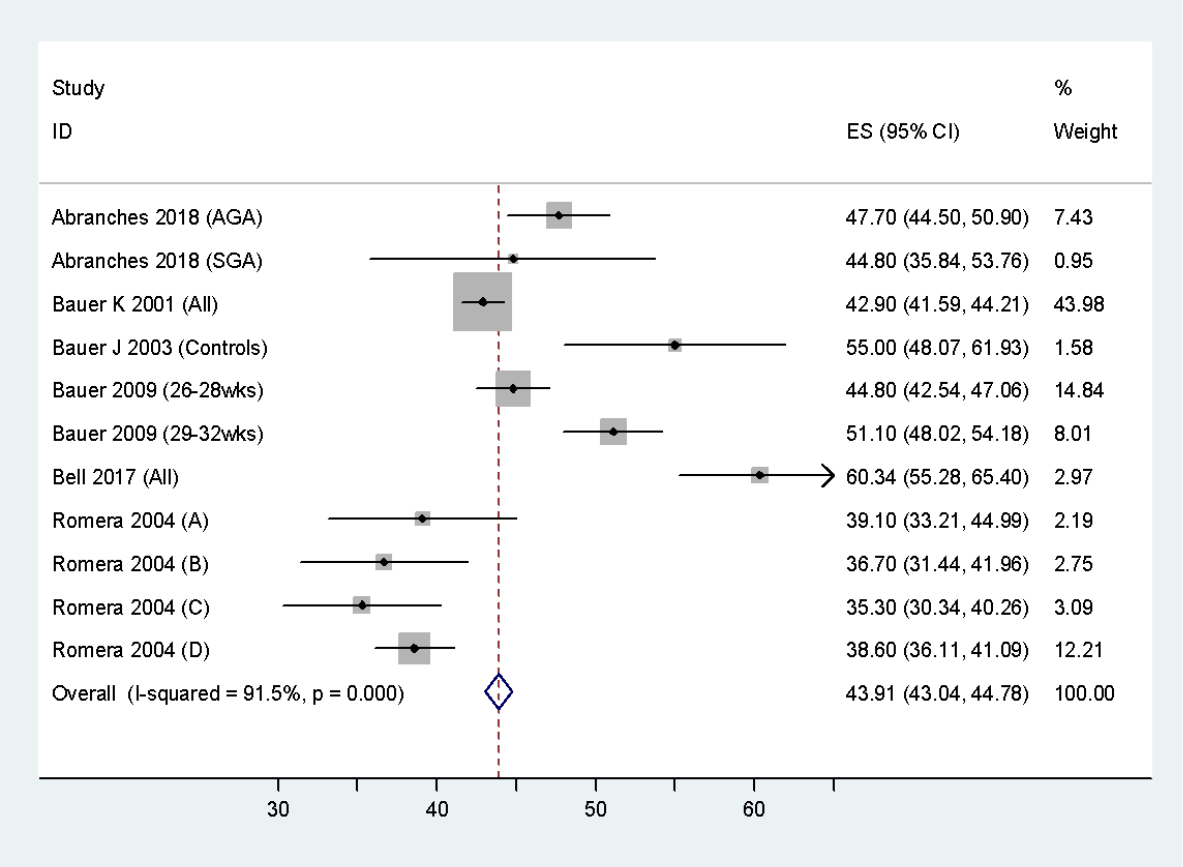

Figure 1: Meta-analysis of studies of Resting Energy Expenditure in enterally fed preterm infants over the past 2 decades

Energy intakes and growth

The overall energy intake needs to match metabolic and growth requirements (also called 'metabolizable energy'), plus an additional amount to account for energy lost in the stool. This can be represented by the equation:

Overall Energy Requirement = Metabolizable Energy + Energy lost in stool

Energy for growth represents energy deposition, and this will vary according to the composition of weight gain, with protein and fat deposition representing 5.4 and 9.3kcal respectively per gram of tissue. Therefore, the estimated average energy requirements for depositing new tissue with a composition of 13% protein and 20-30% fat are ~3.6-4.7 kcal/g(20, 21), plus the energy needed for synthesis of 1-1.2kcal/g tissue deposited(19). Taking these data together and assuming 17-20g/kg/day weight gain the metabolizable energy needed for growth based on a REE of 43.9kcal/kg/day would be between 106kcal/kg/day and 138kcal/kg/day (see table 3).

According to various studies, the energy lost in stool is around 6-12% with preterm formula (PTF) milk and 14-16% with human milk (HM)(8, 10, 21, 22). The percentage of energy lost in stool for fortified human milk is unclear, though likely to be in a similar range, and there may also be differences between raw maternal breast milk and donor milk(23). Therefore, adjusting the above metabolizable energy needs for energy lost in stool would equate to a total energy intake of approximately 115-160kcal/kg/day, regardless of feed type (see table 3). However, it is of note that the highest of this range of values (146-160kcal/kg day) is related to the upper end of weight gain and fat deposition, at 20g/kg/d and 30% respectively, and has been derived though a degree of extrapolation and theoretical assumptions. Energy intakes at the upper end of this range may potentially promote excess fat deposition, and are significantly higher than the previous ESPGHAN recommended upper limit of 135kcal/kg/day. Taking all this together, it is likely that an upper limit which is a little lower at 140kcal/kg/d is likely to be sufficient to allow adequate growth even after accounting for differences in energy lost in stool depending on feed type. **Overall then, an enteral energy intake of 115-140kcal/kg/day is recommended.**

Growth rate (g/kg/ day)	Composition of growth (%)		Energy stored (A)			Energy required for tissue deposition (kcal/kg/day) (B)			REE exc cost of tissue depos-ition (kcal/kg/ day) (C)	Total REE (=B+C) kcal/kg /day (D)	Total metaboliz -able energy required (kcal/kg/d ay) (=A+D)	Total Energy Intake required (allowing for loss in stool)
	Protein	Fat	Protein	Fat	Total (protein +fat)	Protein	Fat	Total (protein +fat)				
17	13	20	12.5	31.5	44.0	12.2	5.5	17.7	43.9	61.6	105.6	111.9. to 121.9
17	13	30	12.5	47.2	59.7	12.2	8.3	20.5	43.9	64.4	124.1	131.5 to 144.0
20	13	20	14.7	37.0	51.7	14.3	6.5	20.8	43.9	64.7	116.4	123.3 to 135.0
20	13	30	14.7	55.5	70.2	14.3	9.7	24	43.9	67.9	138.1	146.3 to 160.2

Table 3: Energy required for growth (storage and tissue deposition), REE and total energy intakes.

Several RCTs of formula milk or breast milk fortifiers have been carried out over the past decade, and are summarised in table 4, showing mean enteral energy intakes ranging from 110 to 138kcal/kg/day and mean enteral protein intakes of 3 to 4.5g/kg/day(15-17, 24-27). In study groups where a protein intake of >3.5g/kg/day was achieved, all but one study group achieved mean growth

rates of >16g/kg/day, even with energy intakes in the lower range, suggesting that an intake of 110-140kcal/kg/day may be sufficient to achieve desired growth rates, assuming adequate protein provision. Of note, study groups with growth in excess of 20g/kg/day had energy intakes over 130kcal/kg/day.

Following the previous ESPGHAN enteral nutrition recommendations in 2010, numerous studies have published data on their implementation in clinical practice (Table 5)(2, 18, 23, 28-35). In these cohorts, mean energy intakes ranged between 110 and 143kcal/kg/day, with mean protein intakes of 2.6 to 4.3g/kg/day although the majority achieved protein intakes of 3-4g/kg/day. Although there are important methodological limitations of pooling these data (figure 2), after excluding studies where protein intake was low (<3.5g/kg/day), there is a linear relationship between energy intake and weight gain ($p<0.01$). Figure 2 suggests that weight gain of 17-20g/kg/day will require an energy intake of approximately 130-150kcal/kg/day, albeit with wide confidence intervals. It is of note however, that in most of these studies the energy and protein intakes were not measured but estimated on the base of a standard composition of human milk. In the study by de Halleux et al (23), milk composition was measured and fortification adjusted to provide mean intakes of around 142kcal/kg/day, with a mean weight gain of 19g/kg/day. All these data therefore support a recommended energy intake of 140 kcal/kg/day.

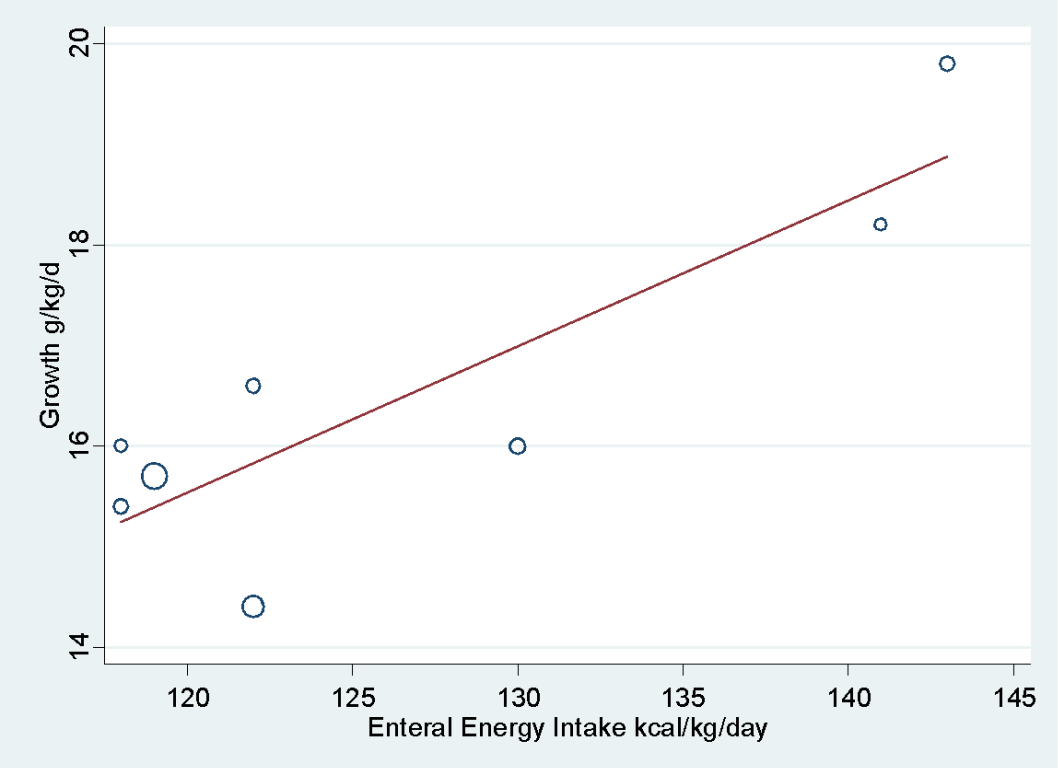

Figure 2: Meta regression bubble plot of the effect of enteral energy intake (kcal/kg/day) on

growth rates (g/kg/day) based on cohort studies of increased enteral nutritional intake in preterm infants. The size of the circles vary according to the weight given to the parameter estimate and is inversely proportional to the variance of the estimated treatment effect

Authors: MJ Johnson, SJ Moltu

Paper	Group	Number of infants	Mean Birth Weight	Mean Gestational Age at Birth	Mean Enteral Energy intake (kcal/kg/day)	95% Confidence interval	Mean Enteral Protein intake (g/kg/day)	95% Confidence interval	Mean Growth (g/kg/day)	95% Confidence interval
Arslanoglu 2006	STD	16	1407	31.3	120.5	116.4 to 124.7	2.8	2.7 to 2.9	14.4	13.1 to 15.8
Arslanoglu 2006	ADJ	16	1386	31.8	128	123.7 to 132.3	3.4	3.1 to 3.7	17.5	15.8 to 19.2
Bulut 2020	TF	16	1084	29.3	145	142.3 to 147.7	3.2	3.2 to 3.3	23.1	21.2 to 25.1
Bulut 2020	AF	16	1143	29.3	144.3	139.2 to 149.5	2.8	2.7 to 2.9	22.2	19 to 25.4
Brumberg 2010	MCT	12	862	27	124	118.9 to 129.1	3	2.7 to 3.3	11.5	8.8 to 14.2
Brumberg 2010	P/E	11	879	27	128	121.5 to 134.5	3.5	3.3 to 3.7	17	15.6 to 18.4
Kadioglu 2019*	SF	20	1090	29	128	Not Available	3.6	Not Available	12	9 to 17
Kadioglu 2019*	AF	20	1080	29	131	Not Available	4.3	Not Available	24	22 to 26
Kadioglu 2019*	TF	20	980	30	133	Not Available	4.5	Not Available	25.5	21 to 28
Maas 2017	Low Protein	30	1215	30	137	133.7 to 140.3	3.8	3.7 to 3.9	16.3	15.4 to 17.2
Maas 2017	High Protein	30	1193	29.7	138	135.7 to 140.3	4.3	4.2 to 4.4	16	15.1 to 16.9
Mcleod 2016	Intervention	20	1015	27	125.2	120.1 to 129.9	3.3	3.1 to 3.5	13.4	12.6 to 14.2
Mcleod 2016	Control	20	1009	27.1	128.6	123.6 to 133.6	3.4	3.2 to 3.6	14.3	13.6 to 15
Rigo 2017	nHMF	77	1147	28.8	125	Not Available	4.48	Not Available	18.3	17.5 to 19.1
Rigo 2017	cHMF	76	1156	28.7	125	Not Available	3.8	Not Available	16.8	16 to 17.6
Rochow 2020	Control	89	960	27	121	118.9 to 123.1	3.6	3.5 to 3.7	19.3	18.8 to 19.8
Rochow 2020	Intervention	90	980	27.1	140	137.9 to 142.1	4.5	4.4 to 4.6	21.2	20.7 to 21.7
Shah 2016	EF	49	990	27.5	110.7	106.1 to 115.3	3.71	3.6 to 3.8	17.97	16.8 to 19.2
Shah 2016	DF	50	990	28	111.3	106.5 to 116.1	3.55	3.4 to 3.7	17.53	15.3 to 19.8

Table 4: Randomised controlled trials of enhanced enteral nutrition in preterm infants. MCT – Medium Chain Triglyceride supplementation (increased energy) group; P/E – Increased Protein and Energy group; nHMF – new powdered Human Milk Fortifier group; cHMF – control Human Milk Fortifier group; EF – Early Fortification; DF – Delayed Fortification; *data given as medians and interquartile range

ESPGHAN SDC 4 Energy
July 2022

Authors: MJ Johnson, SJ Moltu

Paper	Group	Number of infants	Mean Birth Weight	Mean Gestational Age at Birth	Mean Enteral Energy intake (kcal/kg/day)	95% Confidence interval	Mean Enteral Protein intake (g/kg/day)	95% Confidence interval	Mean Growth (g/kg/day)	95% Confidence interval
Christmann 2013	1AGA	57	1200	29.1	117	110.8 to 123.2	3.29	3.2 to 3.4	8.9	8.2 to 9.6
Christmann 2013	1SGA	11	875	31.3	117	102.8 to 131.2	3.29	3.2 to 3.4	12.2	10.4 to 14
Christmann 2013	2AGA	65	1271	29.5	123	117.2 to 128.8	3.43	3.3 to 3.6	10.7	10 to 11.4
Christmann 2013	2SGA	14	881	30.9	123	110.4 to 135.6	3.43	3.3 to 3.6	12.2	11.1 to 13.3
Rochow 2012	Intervention	123	1060	28.7	121	119.2 to 122.8	3.2	3.1 to 3.3	18.5	18.1 to 18.9
Rochow 2012	Control	115	1080	29	108	106.4 to 109.6	2.9	2.8 to 3	17.7	17.2 to 18.2
Senterre 2011	SGA	82	959	28	122	119.8 to 124.2	3.7	3.7 to 3.7	14.4	13.9 to 14.9
Senterre 2011	AGA	20	1016	30.5	122	117.6 to 126.4	3.7	3.6 to 3.8	16.6	15.9 to 17.3
Senterre 2012	<28/40	39	895	26.8	118	112.6 to 123.4	3.9	3.8 to 4	15.4	14.7 to 16.1
Senterre 2012	28-30/40	45	1051	28.8	130	126.3 to 133.7	3.9	3.8 to 4	16	15.4 to 16.6
De Halleux 2019	>75%OMM	37	983	27.7	143	140.4 to 145.6	4.17	4.1 to 4.2	19.8	19.2 to 20.4
De Halleux 2019	>75%DM	33	901	27.5	141	139 to 143	4.15	4.1 to 4.2	18.2	17.4 to 19
Olsen 2014	All	56	1127	30.3	118	115.9 to 120.1	4.3	4.2 to 4.4	16	15.2 to 16.8
Collins 2010	Adelaide	138	1333	29.3	119	117.3 to 120.7	3.5	Not Available	15.7	15.3 to 16.1
Collins 2010	Singapore	148	1247	29.1	113	111.4 to 114.6	2.9	Not Available	13.8	13.1 to 14.5
Coviello 2018	All	131	934	27	110	107.6 to 112.4	2.6	2.6 to 2.6	14	13.1 to 14.9
Hu 2019	All	128	1310	30.8	113.2	113.2 to 113.2	3.42	Not Available	14.1	13.5 to 14.7
Hu 2019	EUGR	87	1296	31.5	110	Not Available	3.4	Not Available	13.5	12.8 to 14.2
Hu 2019	Non-EUGR	41	1310.9	29.1	120	Not Available	3.45	Not Available	15.6	14.8 to 16.4
Mcleod 2015	All	27	139.5	29	120	116.1 to 123.9	3.4	3.3 to 3.5	11.5	10.6 to 12.4
Peiler 2014	6-8 weeks	50	Not Available	28.5	133	133 to 133	3.3	Not Available	20.6	19.6 to 21.6

Table 5: Cohort studies of increased enteral nutritional intake in preterm infants. Includes intervention and comparator groups wherever applicable. AGA – Appropriate for Gestational Age; EUGR – Extrauterine Growth Restriction; DM – Donor Milk; OMM – Own Mother's Milk; SGA –

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Protein:Energy Ratio (PER)

Consideration of the enteral PER that optimises the quantity and quality of growth(36), with appropriate accretion of fat free mass (FFM) and fat mass (FM) is important. There is good evidence that many preterm infants fail to accrete adequate FFM as well as to achieve adequate linear growth, which may have important implications for long-term health and disease risk(37). Providing excessive energy without sufficient protein may lead to adiposity; conversely providing protein without sufficient energy for tissue accretion and protein turnover may increase the metabolic burden or result in poor growth.

Since the previous 2010 ESPGHAN guideline, studies of different nutritional products including formula milk and breast milk fortifier, have explored the effect of different PERs on growth. Costa-Orvay et al compared three enteral feeding regimens in preterm infants with different PERs to a control group. The study demonstrated that intakes of 150 kcal/kg/day leading to higher gains in weight (17g/kg/day) and greater FFM accretion compared to 130kcal/kg/day, regardless of PER (2.8 vs 3.1g/100kcal(38). Rigo et al found that an enhanced breast milk fortifier providing a higher PER (3.6g/100kcal) improved weight gain compared to standard fortifier containing a similar amount of energy (PER 3.1g/100kcal)(16).

Considering this evidence together with previous recommendations, optimal PER for enteral intake in preterm infants is likely to be around 2.8 to 3.6g/100kcal(5, 39), with PERs at the higher end of this range associated with improved weight gain and FFM accretion. However, one of the challenges of using PER to guide intakes is that low intake of both energy and protein will also lead to low growth rates despite a seemingly normal PER. Similarly, giving excessive amounts of energy and protein in parallel will keep an ‘optimal’ PER whilst leading to excessive fat deposition or a rise in blood urea nitrogen as excess protein is excreted (Figure 3). The isolated use of PER can be potentially unhelpful and maintaining both energy and protein intakes within recommended ranges is preferable.

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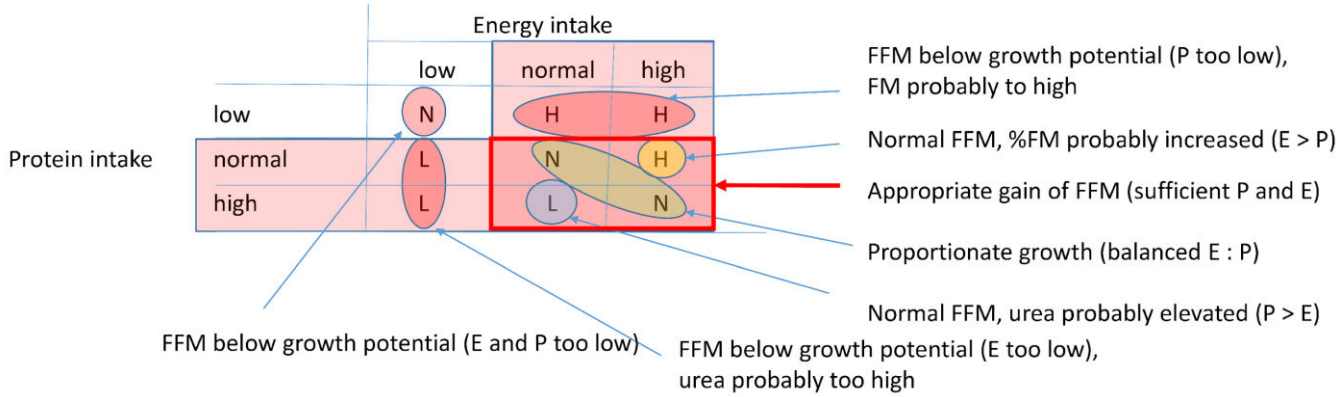

Figure 3: Relationship of Protein (P):Energy(E) Ratio (H - High; L - Low; N - Normal;) and quality of growth (FM – Fat Mass; FFM – Fat Free Mass)

Energy intakes and outcomes

Several studies over the past decade have suggested an association between energy intake and long-term outcomes, particularly neurodevelopment. A prospective observational study of 63 preterm infants, demonstrated an association between energy deprivation (mean intake <85kcal/kg/day vs 109 kcal/kg/d) during the first 2 weeks of age and increased risk of NDI(40). Another recent study also showed associations between increased energy intake and enteral feeds in the first 2 weeks of life and increased brain volume assessed by magnetic resonance imaging during the neonatal period(41). Furthermore, higher energy intakes from week 3 of life in preterm infants have been associated with higher IQ in adulthood(42). A recent cohort study has shown that increased energy intake between days 7 and 27 of life was associated with decreased risk of both retinopathy of prematurity (ROP) and bronchopulmonary dysplasia(BPD)(43). Two previous studies have also suggested that low energy intake during the first 4 weeks of life is associated with an increased risk of BPD(44) and an increased risk of severe ROP(45), though these were observational studies which also include some parenteral intake and need to be interpreted with caution. There are currently no prospective RCTs which show a benefit of an isolated increase in enteral energy intake.

Summary

One of the key challenges in determining energy requirements is the interdependence of the energy fractions provided by the respective macronutrients. Interpreting the results of feeding studies is thus difficult and is further compounded by a lack of recent RCTs of energy intake and methodological limitations of observational studies. Nevertheless, we consider that data from experimental studies on REE in preterm infants and observational feeding studies are helpful in determining our position on energy intake.

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Conclusions

- C1: The REE for healthy, growing very preterm infants is approximately 60-70 kcal/kg/day (LOE 1+)
- C2: Metabolizable energy intake needs to meet REE plus the energy needed for growth, adjusted for energy lost in stool. (LOE 1-)
- C3: To promote optimal quality of growth and longer term outcomes, energy intake recommendations also require consideration of the energy fractions provided by the respective macronutrients (LOE 1)

Recommendations

- R1: A reasonable range of total energy intake for most healthy growing preterm infants is 115-140kcal/kg/day. (GOR A)
- R2: Energy intakes >140kcal/kg/day may be needed where growth is below the recommended range, but should not be provided until protein and other nutrient sufficiency has been ensured, and should need exceed 160kcal/kg/day. (GOR B)
- R3: Provided that energy and protein intakes are within the recommended ranges, a protein to energy ratio of 2.8-3.6g/100kcal is recommended (GOR B)

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SDC 5: protein

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The literature has been reviewed to address studies published after the previous 2010 guidelines. A PubMed (http://www.ncbi.nlm.nih.gov/pubmed) search was conducted with the use of the following key words: ((((((((((((Infant, Premature) OR Infant, Extremely premature) OR Infant, Low birth weight) OR Infant, Small for gestational age) OR Infant, extremely Low birth weight) OR Infant, extremely Low birth weight) OR prematurity[Title]) OR prematures[Title]) OR micropremie[Title]) OR low birth weight[Title]) OR ((((infant*[Title] OR neonat*[Title] OR newborn*[Title] OR toddler*) AND Title AND (preterm*[Title] OR pre-term*[Title] OR prematur*[Title] OR pre-matur*[Title] OR low birth weight[Title] OR low birthweight)) AND Title)))) AND ((protein/) OR protein[Title]) AND (Clinical Trial[ptyp] AND ("2010/01/01"[PDat] : "2019/08/05"[PDat]))) AND (Randomized Controlled Trial[ptyp]).

Proteins form the key structural components of all human cells and are also involved in physiologic processes through their roles as enzymes, hormones and transport proteins. Amino acids are the building blocks for proteins and their polymers may vary in length from two (dipeptide) to thousands of amino acids. In addition, most amino acids have individual (e.g. signalling) functions as well or act as precursors for other metabolites (1, 2). Amino acids which are in excess and not used for protein synthesis, are irreversibly oxidised to CO_2 and ammonia (which is detoxified into urea) yielding approximately 4 kcal/g.

Protein intake is thought to be the main driver of lean body mass growth. Simultaneously, however, clinicians must make sure to provide sufficient energy, essential fatty acids, and all micronutrients to support the formation of new cells and thus tissue growth, as described in other sections. As well as determining total protein requirements, consideration of protein quality is also important, i.e. the optimal distribution of (conditionally) essential and non-essential amino acids (3).

The total (or crude) protein content of human milk is frequently subdivided into true protein (~75%) and non-protein nitrogen (~25%) which mainly consists of urea (~15%) but also individual amino acids. This must be considered when interpreting data as the biological role of intestinal urea is not well characterised. Stable isotope measurements indicate that around 15-50% of milk urea is metabolised into amino acids by intestinal microbes or salvaged after hydrolisation (4-6). Several meta-analyses with different inclusion criteria have shown that both the total as well as true protein content is initially relatively high in colostrum but declines rapidly in the first few days postpartum and then more slowly over the next few weeks (table 1) (7-9). Differences in macronutrients between milk expressed from women that delivered either term or preterm are relatively small, except for colostrum where protein content is higher in women delivering preterm. However, a recent large cohort study of almost 2000 milk samples after preterm birth showed slightly lower true protein content declining

from 1.7 g/100 mL in colostrum to 1.0 g/100 mL during postnatal week 4 (10). True protein content of donor milk is usually even lower at approximately 0.9 g/100 mL on average (11, 12). Recent data with more advanced techniques (proteomics and peptidomics) show differences in the immune proteome of milk from mothers that delivered preterm compared to term (13), although these differences seemed to disappear after one month of lactation.

Table 1: Protein content (g/100 mL) in human milk expressed from women that delivered either preterm or term, subdivided by lactational stage, and whether total, true, or biologically available protein was measured. Data are from 3 different meta-analyses (7-9). Biologically available protein was defined as true protein plus 27% of the non-protein nitrogen content (9).

	Preterm				Term	
	Gidrewicz (5)		Mimouni (6)	Boyce (7)	Gidrewicz (5)	
	Total (mean (SD))	True (mean (SD))	True (mean (SD))	Biologically available (mean (range))	Total (mean (SD))	True (mean (SD))
D 1-3	2.8 (1.1)	2.7 (1.5)	2.6 (1.4)	2.0 (1.7-2.6)	2.0 (0.6)	2.0 (0.9)
D 4-7	2.1 (0.5)	1.7 (0.5)	2.1 (0.4)		2.0 (0.5)	1.6 (0.3)
Week 2	1.9 (0.4)	1.5 (0.4)	2.0 (0.7)	1.7 (1.5-2.4)	1.8 (0.4)	1.3 (0.2)
Week 3-4	1.6 (0.4)	1.4 (0.4)	1.6 (0.5)	1.4 (0.9-2.1)	1.5 (0.3)	1.1 (0.2)
Week 5-6	1.4 (0.3)	1.1 (0.2)	1.4 (0.3)	1.4 (1.1-1.9)	1.1 (0.2)	1.0 (0.1)
Week 7-9	1.1 (0.2)	1.1 (0.2)	1.3 (0.2)	1.3 (1.0-2.0)	1.1 (0.2)	0.9 (0.1)
Week 10-12	1.3 (0.3)	1.0 (0.2)	1.3 (0.2)		1.1 (0.2)	1.0 (0.1)

Protein requirements based on the factorial approach

Ziegler et al., in a classic article, described the body composition of the reference fetus using carefully selected literature reports of whole-body chemical analyses of deceased fetuses or neonates immediately after birth at a range of gestations (14). From those analyses, daily protein and other nutrient accretion rates were calculated. Protein accretion per day was around 2.5 g/kg in fetuses weighing 500 g declining slightly to 2.2 g/kg at a body weight of 1800 g. However, obligatory nitrogen losses must also be considered (around 1.0 g/kg/d), along with intestinal utilisation of amino acids during the digestion and absorption process and suboptimal dietary protein absorption (in total around 0.5 g/kg/d). In theory, an extremely preterm neonate would require around 4.0 g/kg/d of enteral protein of optimal quality to grow at a rate comparable to the in-utero fetus (15), which will not be met with an intake of 150 to 180 mL/kg/d of unfortified human milk.

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Protein requirements based on experimental evidence

Both human milk without fortification or standard term infant formula provide far less protein than theoretically required to match in-utero lean mass accretion rates. The optimal amount of protein and its effect on weight and length gain, and metabolic and neurological outcome has been a key focus of research in recent years. Unfortunately, there are only few randomised controlled trials (RCTs) or other studies that include long-term functional outcomes. There are data, mainly from cohort studies, showing that relatively higher amounts of enteral protein are positively associated with growth as well as with reduced neonatal morbidities and improved neurodevelopmental outcome during infancy (16-22). Whilst all of these cohort studies remain important in evidence, they are at risk of residual confounding and reverse causation. Therefore, synthesis and development of intake recommendations is mainly based on available experimental data from RCTs.

Formula:

A recently updated Cochrane review assessed studies on nutrient-enriched preterm formula versus standard term formula given to preterm infants (23). The systematic review included 7 trials involving 590 preterm infants in total; all trials were performed between 1984 and 1992, were industry sponsored and had several methodological weaknesses. The interventions increased not only protein concentrations (typically from 1.4 to 2.1 g/100 mL), but also energy (typically from 67 to 80 kcal/100 mL) and several micronutrients. These formulas resulted in higher rates of weight gain and head growth, though not length gain, during neonatal hospitalization. Only limited data were available for growth and developmental outcomes assessed beyond infancy, and these do not show consistent effects. Another Cochrane review, last updated in 2014, compared different levels of protein intake in preterm formulas. Six small RCTs, with 143 included infants, compared low (<3.0 g/kg/d) versus medium high (3.0 – 4.0 g/kg/d) protein intake and demonstrated higher weight, length, and head circumference gains in the higher intake group. Two RCTs with 95 infants compared medium high versus very high (>4.0 g/kg/d) protein intake, and a higher weight gain was described in the latter group. Clinical morbidities during neonatal hospitalization were only sparsely described but showed no differences between any of the groups. Long-term follow-up at 6 years of age was only described in the studies by Goldman et al. in 1969 and 1974. They administered 6.0 to 7.2 g/kg/d (or half of that amount in the control group) of protein of casein source which is nowadays seen as protein of inferior quality (24, 25) and found a higher proportion of infants with IQ scores < 90 in the 21 infants who received the very high protein diet. Another systematic review from 2014 assessed growth in 12 RCTs (419 preterm infants) comparing formulas with differing protein content, ranging from 1.6 to 4.7 g/kg/d (26). In most studies, growth was greater in the high protein group. Functional outcomes were not described. Current typical preterm formulas nowadays contain approximately 2.6 g/100 mL and 80 kcal/100 mL (27) and therefore provide 3.9 to 4.7 g of protein/kg/d at intakes of 150 to 180 mL/kg/d.

Human milk:

To increase the nutrient content of human milk, a sole protein product, a multi-nutrient fortifier containing protein, or a combination of both may be added. The strategy of

supplementing human milk with solely protein was summarised in a recent Cochrane review where 6 small RCTs, predominantly conducted in the 1980s, were included describing 204 preterm infants in total (28). The intervention consisted of a protein supplement of 0.4 to 1.0 g/100 mL, sometimes accompanied by small amounts of minerals. Low-quality evidence showed that this strategy increased short-term growth, but the very low number of included infants precluded further conclusions. Moreover, the use of sole supplementation with protein alone is typically not practised nowadays because of the higher requirements of many other nutrients, and most practitioners use a multi-nutrient fortifier (see separate section). The use of multi-nutrient fortifiers has also been summarised in a Cochrane review with data from 14 trials published between 1987 and 2012 and included a total of 1071 preterm infants (29). The intervention typically consisted of protein supplementation at 0.4 to 1.0 g/100 mL amongst other nutrients. Similarly, increased in-hospital growth rates were seen, whereas neurodevelopmental outcome was only assessed in one trial which did not show significant differences. Further discussion on the use of multi-nutrient fortifiers is described in a separate section, as we will here focus on specific protein requirements.

Many of the studies that were included in the previous 4 Cochrane reviews (23, 28-30), were also included together with additional RCTs, in the 2014 systematic review by Tonkin et al. (26). This review featured a graph of protein intake and weight gain based on included studies. As can be seen in figure 1, there appears to be a positive linear relationship in protein intakes ranging from 1.9 to 4.6 g/kg/d, albeit with wide variation. Most of the studies also showed greater linear growth and head circumferences in the higher protein groups. A 2015 meta-analysis of 5 studies comparing higher versus standard protein levels in human milk fortifiers (31), showed similar beneficial effects on various anthropometric indices, but neither this study nor the systematic review from Tonkin et al. (26) assessed functional outcomes.

Figure 1: Relationship between protein intake and growth, figure from a systematic review by Tonkin et al. (26). Formula studies are indicated with black, unfortified versus fortified human milk studies with white, and studies comparing different human milk fortifiers with grey markers.

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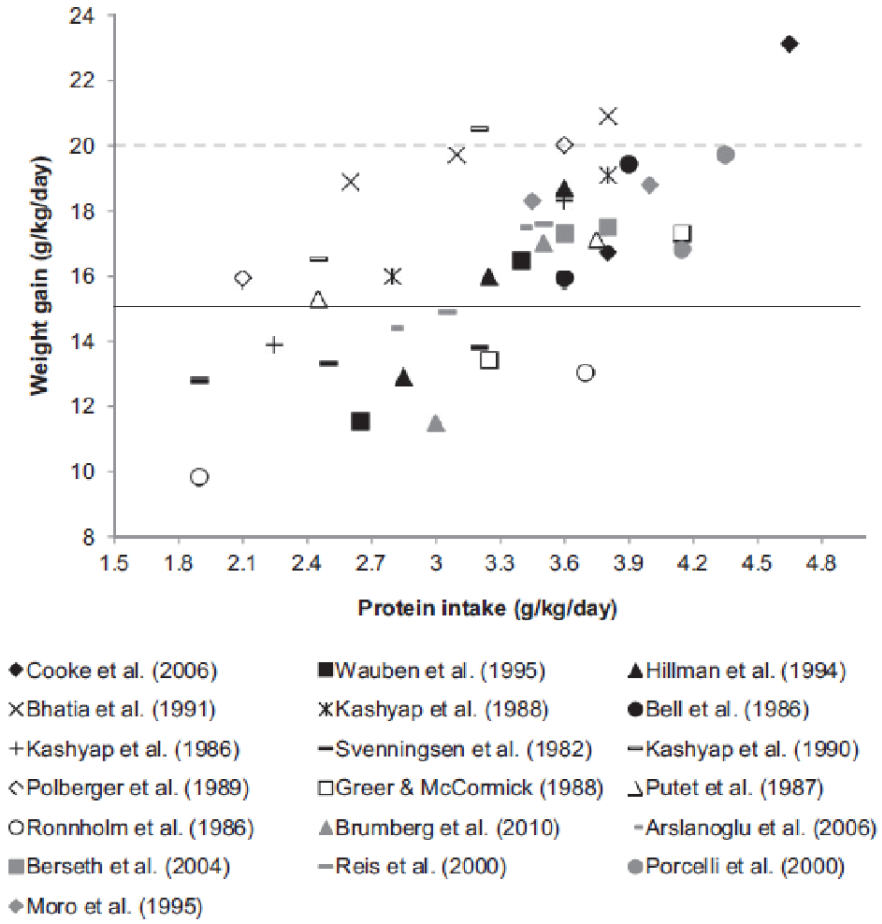

In table 2 we depict an overview of our systematic search on RCTs that have been published since 2010 on higher versus lower protein intakes in preterm infants and their anthropometry and clinical or functional outcomes. None of these studies were included in the aforementioned Cochrane reviews (23, 28-30). Frequently, the difference between groups was not only in amount of protein, but also amounts of energy and micronutrients where breast milk fortifiers were compared. Similarly, some other studies compared a liquid versus a powdered fortifier with either hydrolysed or intact protein. This may hamper a pure comparison of macronutrient intake and complicates interpretation of pure protein requirements. Furthermore, depicted protein intakes were frequently largely based on gross estimates of ingested milk volume and estimated protein content of supplemented milk.

Most studies in the table showed improved growth rates during hospitalisation in the higher protein group, similar to the studies outlined in figure 1; this frequently also included increased head circumference or length gain. When both protein and caloric intake were increased, all the studies (Rochow, Moltu, Gupta, Costa-Orvay) showed greater weight gain. When only protein intake was increased, six studies showed improvements in anthropometric parameters (Biasini, Bulut, Dogra, Rigo, Brumberg, Kadioglu Simsek) and nine showed no differences in weight gain (Atchley, Kim, Miller, Moya, Reid, Ditzenberger, Maas, Quan, Bellagamba). Protein intake in the control group ranged from 3.0 to 4.2 g/kg/d. The range of

the higher protein intake group was between 3.5 and 5.1 g/kg/d so that sometimes the higher group was equivalent to the lower group in other studies, although we would like to stress again that often, total protein intakes were not actually measured, but rather estimated.

Growth after hospitalisation was only occasionally reported and did not differ between groups. Functional outcomes such as mortality, neonatal morbidities (e.g. sepsis, necrotising enterocolitis, or bronchopulmonary dysplasia), or longer-term outcomes were often not different between groups in the individual, usually small, highly underpowered studies. Meta-analysis was not possible due to high heterogeneity of study design and study outcomes which hampers a more general conclusion as to whether higher amounts of protein also benefit functional outcomes besides growth alone.

Table 2: Overview of RCTs that have been published since 2010 on higher versus lower protein intake in preterm infants and their outcomes (anthropometry and clinical or functional outcomes). Studies are divided in subcategories.

First author, year, country (ref)	Population. Type of feeding	Intervention and duration	n	Daily weight gain (g/kg/d)	Main outcomes
RCTs with extra protein supplement:					
Atchley, 2019, USA (32)	GA <32 wks. Born AGA and current weight ≥1000 g OMM, DM, or PF	- CON: HM with BMF (or PF); aPI 3.5 (0.3) g/kg/d - INT: HM with BMF (or PF) + protein supplement; aPI 4.7 (0.3) g/kg/d Duration: from fortification initiation for 28 days or discharge	- 17 - 16	- 19.1(1.1) - 21.6 (1.1)	Similar rates of weight and length gain. Lower HC gain in high protein group.
Biasini, 2018, Italy (33)	BW <1250 g and GA <32 wks. OMM/DM	- CON: HM with BMF (1.3 g protein/dL), tPI 3.5 g/kg/d - INT: HM with BMF (1.3 g protein/dL) + protein supplement (0.9 g/dL), tPI 4.8 g/kg/d Duration: start fortification until 50% direct breastfeeding or discharge	- 27 - 34	- 18.8 (2.1) - 19.0 (2.2)	Improved weight, length, and HC gain in subgroup of infants <1000 g only. No differences in anthropometry z-scores until corrected age 2y, except for length z-score which was better at 9 months only. Higher hearing and language scores at neurodevelopmental testing at 1 and 1.5y CA.
Brumberg, 2010, USA (34)	BW ≤1250 g and growth <15 g/kg/d. OMM or PF	- CON: HM with standard BMF or PF plus MCT supplement; aPI 3.0 g/kg/d - INT: HM with standard BMF or PF plus protein (0.3 g/dL) and energy supplement; aPI 3.5 g/kg/d Human milk nutritional content is based on estimated content of mature human milk. Duration: start after ~3 weeks after birth, for ~3 wks	- 12 - 11	- 11.5 (4.8) - 17.0 (2.4)	Improved gain of weight and HC, but similar length gain. Incidence of clinical morbidities similar.
Costa-Orvay, 2011, Spain (35)	GA ≤32 wks, BW <1500 g, and AGA. PF	- CON: PF; tPI 3.7 g/kg/d - INT1: PF + protein & energy suppl; tPI 4.2 g/kg/d - INT2: PF + protein & energy suppl; tPI 4.7 g/kg/d Duration: start 2-3 wks after birth, until 4 wks after randomisation	- 8 - 12 - 12	- 12.8 - 18.0 - 17.5	Weight gain and fat-free mass higher in both intervention groups than in control group. No differences between both intervention groups. Incidence of clinical morbidities similar.
Ditzenber	BW <1500	- CON: HM with BMF, tPI 2.8 g/kg/d	- 34	NA	No differences in any growth parameters

ger, 2013, USA (36)	g, AGA. OMM or PF	- INT: HM with BMF + protein supplement; tPI <1000g 4.0 g/kg/d; tPI 1000-1500g 3.5 g/kg/d; tPI 1500-2000g 3.0 g/kg/d Duration: start at fortification until 2000 g	- 30		during hospital stay
Reid, 2018, Australia (37)	GA 28-32 wks. Intention OMM (or PF)	- CON: HM with BMF (1.0 g protein/dL), aPI 3.5 (0.93) g/kg/d - INT: HM with BMF (1.0 g protein/dL) + protein supplement (0.8 g/dL), aPI 4.2 (1.3) g/kg/d. Duration: from start fortification, until removal of nasogastric tube or CA 40 wks	- 29 - 31	- 17.3 (2.0) - 17.3 (3.0)	No differences in in-hospital anthropometry measurements or incidence of clinical morbidities.
RCTs with combined enhanced PN and EN:					
Bellagamba, 2016, Italy (38)	BW 500-1250 g. OMM/PF	- CON: tPI during PN 2.5 g/kg/d, aPI during EN 3.5 g/kg/d with HM and BMF - INT: tPI during PN 3.5 g/kg/d, aPI during EN 4.2 g/kg/d with HM, BMF, and a protein supplement Duration: until 1800 g.	- 82 - 82	- 16.8 (2.3) - 17.5 (2.4)	No differences in any growth parameters during hospital stay or at 2 years corrected age. No differences in clinical morbidity rates or Bayley III scores at 24 months CA.
Moltu 2013 and 2014, Strømmen 2015, Blakstad 2015, Norway, (39-41)	BW <1500 g	- CON: start tPI during PN 2.0 g/kg/d; during EN HM with BMF (1.1 g/dL); aPI 3.5 g/kg/d - INT: start tPI during PN 3.5 g/kg/d; during EN HM with BMF (1.1 g/dL) and a protein supplement (0.6 g/dL) (once breastfed daily protein shot); aPI 4.2 g/kg/d Duration: from birth until 3 m CA	- 21 - 23	- 13.8 (13.2-15.2) - 17.4 (16.3-19.0)	Improved weight and HC gain during hospitalization. More late onset sepsis in high protein group, mainly during first 2 postnatal weeks, attributed to hypophosphatemia and neonatal refeeding-like syndrome. Other clinical morbidities not different. Subset of infants MRI at term CA: decreased regional white matter mean diffusivity, suggesting improved maturation of cerebral connective tracts. Subset of infants MRI at 5m term CA: stronger visual event-related potential responses. Adiponectin concentration at 5 months CA was higher in the intervention group. Early nutrition and growth may affect metabolic markers in infancy.
RCTs with new BMF with higher protein content:					
Dogra, 2017, India (43)	BW <1500 g or GA <32 wks. OMM or PF	- CON: HM with standard BMF (0.4 g protein/dL), aPI 3.6 g/kg/d - INT: HM with study BMF (1.0 g protein/dL), aPI 4.2 g/kg/d Duration: start fortification until direct breast-fed or discharge	- 60 - 60	- 12.0 (3.3) - 13.7 (3.8)	Improved weight and HC gain, but not length. Incidence of clinical morbidities similar. No differences in anthropometry or neurodevelopmental outcome at 12 to 18 months CA.
Kim, 2015, USA (44)	GA ≤33 wks and BW 700-1500 g. AGA Mainly OMM (or DM)	- CON: powdered intact protein BMF; aPI 3.3 g/kg/d - INT: concentrated extensively hydrolysed protein liquid BMF; aPI 3.9 g/kg/d Duration: start fortification, for 28 d	- 63 - 66	- 17.5 (4.8) - 18.2 (2.4)	Similar growth in both groups in ITT analysis; improved weight and length gain in PP analysis. Incidence of clinical morbidities similar.
Miller, 2012, Australia (45)	GA <31 wks Intention OMM (or PF)	- CON: HM with standard BMF (adding 1.0 g protein/dL); aPI 3.6 g/kg/d - INT: HM with study BMF (adding 1.4 g protein/dL); aPI 4.2 g/kg/d Duration: start fortification until discharge or term CA (~10 wks). An aliquot of unfortified BM was collected once per week from the infant's daily volume and used to represent the weekly composition of BM. Protein and fat content	- 49 - 43	- 15.7 (2.0) - 15.9 (2.0)	No differences in in-hospital anthropometry measurements or incidence of clinical morbidities.

		was determined by infrared spectroscopy with the use of a MilkoScan Minor (Foss)			
Moya, 2012, USA (46)	BW ≤1250 g, GA ≤30 3/7 wks OMM/DM	- CON: HM with powdered BMF (total 2.6 g protein/dL), tPI 4.2 g/kg/d - INT: HM with hydrolysed protein liquid BMF (total 3.2 g protein/dL), tPI 5.1 g/kg/d Duration: 4 wks	- 55 - 51	- 15.8 (3.7) - 16.6 (3.6)	Improved linear growth, but gain of weight and HC were similar. Incidence of clinical morbidities similar.
Rigo, 2017, France, Belgium, Germany, Switzerland, Italy (47)	GA ≤32 wks or BW ≤1500 g. OMM or DM.	- CON: HM with standard BMF (adding 1.0 g protein/dL); aPI 3.8 g/kg/d - INT: HM with study BMF (adding 1.4 g protein/dL); aPI 4.5 g/kg/d Duration: start fortification, for 21 days	- 67 - 64	- 16.8 (3.7) - 18.3 (3.7)	Higher weight gain during first 3 study weeks. Higher HC at discharge. Similar length gain. Incidence of clinical morbidities similar.
RCTs with targeted or individualised fortification:					
Bulut, 2019, Turkey (48)	GA ≤32 wks. OMM	- CON: HM with BMF (1.1 g protein/dL) + 0.8 g/dL extra protein if low urea; aPI 4.0 g/kg/d. - INT: HM with BMF (1.1 g protein/dL) + extra protein based on BM analysis to tPI 4.5 g/kg/d; aPI 4.5 g/kg/d. Duration: From full enteral feeding for 4 weeks	- 16 - 16	- 18.7 (4.3) - 23.1 (4.3)	Improved weight gain and HC growth, but not length gain. Incidence of clinical morbidities similar.
Kadıoğlu Şimşek, 2019, Turkey (49)	GA <32 wks, BW <1500 g	- CON: HM with standard BMF (0.8 g protein/dL); aPI 3.6 g/kg/d - INT1: HM with standard BMF (0.8 g protein/dL) + protein supplement (0.8 g/dL), adjusted on urea; aPI 4.3 g/kg/d - INT2: HM with standard BMF (0.8 g protein/dL) + protein supplement based on BM analysis, tPI 3.5-4.5 g/kg/d; aPI 4.5 g/kg/d Duration: start when FEF, study nutrition for 4 weeks	- 20 - 20 - 20	- 12 (9–17) - 23.5 (22–26) - 25.5 (21–28)	Improved weight, HC, and length gain in both intervention groups. Incidence of clinical morbidities similar
Maas, 2017, Germany (50)	BW <1500 g and GA<32 wks. Intention of OMM (no DM available)	- CON: HM with standard BMF (1.0 g protein/dL), tPI 3.5 g/kg/d - INT1: HM with study BMF (1.8 g protein/dL), tPI 4.5 g/kg/d - INT2: HM with standard BMF (1.0 g protein/dL) plus individually adjusted fortification with added protein and fat, tPI <1500g 4.5; >1500g 4.0 g/kg/d Duration: start fortification until near discharge	- 30 - 15 - 15	- 18.3 (2.7) - 18.3 (2.3)	No differences in in-hospital anthropometry measurements or incidence of clinical morbidities.
McLeod, 2016, Australia (66)	GA <30 wks, OMM	- CON: HM with standard BMF (and if fluid restricted extra protein and lipids); aPI 3.4 g/kg/d - Int: HM with standard BMF + extra protein and fat based on BM analysis to tPI 3.8-4.4; aPI 3.3 g/kg/d Duration: start fortification until near discharge	- 20 - 20	- 14.3 (1.6) - 13.4 (1.9)	No difference in achieved protein intakes. No differences in in-hospital anthropometry measurements or incidence of clinical morbidities.

Parat, 2020, USA, (67)	BW <1500 g; OMM	- CON: HM with standard BMF; aPI 3.1 g/kg/d - INT: HM with standard BMF + extra protein based on BM analysis, tPI 4.0 g/kg/d; aPI 4.1 g/kg/d Duration: start fortification, for ~30 d on average	- 19 - 17	NA	No differences in in-hospital anthropometry measurements
Quan, 2019, China (51)	GA <34 wks and BW 800-1800g. ≥80% OMM; rest PF	- CON: HM with standard BMF (1.1 g protein/dL); aPI 4.0 g/kg/d. - INT: HM with BMF (0.8-1.4 g protein/dL) + extra protein (0-0.6 g/dL), both based on BM analysis and urea concentration (total range 0.8-2.0 g/dL); aPI 4.2 g/kg/d. Duration: From start fortification, during approx. 3 weeks	- 27 - 24	- 15.4 (14.5-18.9) - 16.7 (15.3-18.6)	No differences in in-hospital anthropometry measurements
Rochow, 2020, Canada (68)	GA < 30 wkn OMM or DM	- CON: HM with standard BMF (1.1 g protein/dL; +0.4 g/dL if DM); aPI 3.6 g/kg/d. - INT: HM with standard BMF (1.1 g protein/dL; +0.4 g/dL if DM) + targeted protein, fat, and CHO supplements; aPI 4.5 g/kg/d. Duration: start fortification, for >21 days	- 51 - 52	- 19.3 (2.4) - 21.2 (2.5)	Higher weight, but similar head circumference and length gain. Incidence of clinical morbidities similar.
RCTs with unfortified versus fortified milk:					
Gupta, 2019, India (52)	BW <1500 g and GA <34 wks	- CON: unfortified HM (~200 mL/kg/d) except some micronutrients (tPI ~3.0 g/kg/d) - INT: HM (~200 mL/kg/d) fortified with formula powder (tPI ~4.0 g/kg/d) Duration: until 1800 g	- 73 - 75	- 16.1 (2.9) - 18.0 (2.9)	Improved weight gain and linear growth, but not HC gain. Incidence of clinical morbidities similar.

Abbreviations: AGA appropriate for gestational age; aPI achieved protein intake (mean or range); BMF breast milk fortifier; BW birth weight; CA corrected age; CON control group; EN enteral nutrition; GA gestational age; HC head circumference; HM human milk; INT intervention group; PN parenteral nutrition; tPI targeted protein intake.

Daily weight gain is reported as mean (SD) or as median (IQR).

Urea concentration measurements

Plasma urea is frequently considered a marker of protein tolerance but in fact is the end-product of successful amino acid oxidation after ammonia detoxification and not a marker of accumulation of amino acids or protein, and urea is also influenced by fluid status and renal function. More direct indications of protein intolerance would be measurement of plasma amino acid or ammonia concentrations. Whilst interpretation of urea concentrations is complex, elevated concentrations in the absence of fluid or renal derangements indicate that proteins are not being fully utilised for protein synthesis but are oxidised instead. This might imply suboptimal protein quality, absence of sufficient concomitant energy or other nutrient administration, presence of an inflammatory state and/or catabolic hormones hampering anabolism, or a true surplus of protein.

During enteral nutrition, several papers have shown a correlation between protein intake and plasma urea concentrations (54, 55) or the urea-creatinine ratio (55). Increased protein administration based on presence of a low urea plasma concentration resulted in greater head and somatic growth (53). The upper limit of urea concentrations indicating optimal or maximal protein utilisation are, however, unknown. Several small studies based the level of human milk

fortification on the height of plasma urea concentrations (49, 53). The threshold range of plasma urea to adjust protein intakes was selected arbitrarily between 3.2 and 5.0 mmol/L (19-30 mg/dL; or 9-14 mg N/dL) while the maximal protein intakes in these studies were relatively low (~3.4 g/kg/d). The European Milk Bank Association recently published fortification guidelines and suggested to maintain urea concentrations between 3.6 and 5.7 mmol/L (21-34 mg/dL; or 10-16 mg N/dL) (56), although the underlying evidence base remains limited. The upper threshold ref value of 5.0 or 5.7 mmol/L could not be appropriate during the early weeks of life when the creatinine level remains over 50-60 µmol/L . Whether tolerating higher concentrations of plasma urea during enteral feeding in preterm infants is beneficial for growth and other outcomes is unknown. There is even less data on the role of urinary urea-creatinine ratios (55).

Individual amino acid requirements

In addition to total protein requirements, it is also important to consider protein quality in terms of the individual amino acid composition of administered proteins. From a factorial approach, fetal individual amino acid accretion rates can be assessed (57), though this does not necessarily equate to postnatal requirements after preterm birth. Individual amino acid requirements for normal growth can be assessed using stable isotopes with the indicator amino acid oxidation method (58), but to date, enteral requirements have only been assessed for phenylalanine and cysteine in moderately preterm infants (59, 60). The estimated requirements of phenylalanine in these studies amounted 80 (95% CI: 40–119) mg/kg/d and of cyst(e)ine <18 mg/kg/d provided that methionine intake is adequate.

Additional individual amino acid supplementation

In addition to protein needs to support growth, extra separate specific amino acid supplementation might be considered to reduce morbidity in preterm infants due to individual theoretical beneficial roles of several amino acids (1) such as glutamine, arginine, and taurine. Extra supplementation of glutamine could be beneficial as it serves as a fuel source for enterocytes and is also considered a key amino acid involved in growth and immune function. Data from 12 RCTs were summarised in a recent Cochrane review. Six studies randomised 1095 preterm infants to receive enteral glutamine or not; whereas the other 6 studies investigated the role of intravenous supplementation (61). There was no consistent evidence of benefit for additional enteral (or parenteral) glutamine supplementation when outcomes including mortality, sepsis, necrotising enterocolitis (NEC) and time to reach full enteral feeding were considered.

Arginine has also been proposed as an amino acid that should be supplied in higher amounts because it is a precursor for nitric oxide and may play a key role in intestinal epithelial blood supply and health. Three small RCTs on 285 infants in total, summarised in a Cochrane review, do indeed suggest that arginine may decrease the risk of NEC and the risk of mortality due to this disease (62). A different meta-analysis confirmed these findings for glutamine and arginine supplementation (63).

Taurine is the most abundant free amino acid in breast milk. Evidence exists that taurine has important roles in intestinal fat absorption, hepatic function, and auditory and visual

development in preterm infants. Observational data suggest that relative taurine deficiency during the neonatal period is associated with adverse long-term neurodevelopmental outcomes in preterm infants. Two meta-analyses, summarising nine small RCTs (n= 189 in total), all conducted in the 1980s or early 1990s, on the effects of taurine supplementation, showed no clear benefits, although studies were highly underpowered and included mostly stable moderately preterm infants (64, 65). Yet, since the early 1990s, it has been common to add taurine not only to parenteral nutrition solutions, but also to formula milk.

Conclusions:

C.1. True protein content of human milk decreases rapidly over time, from around 1.5-2.0 g/dL before two weeks of age to around 1.0-1.5 g/dL during the weeks thereafter. Donor milk contains around 0.9-1.0 g/dL of true protein. (LOE 1++)

C.2. Based on a factorial approach, an extremely preterm neonate would require around 4.0 g/kg/d of enteral protein of optimal quality to grow at a comparable rate as intrauterine. (LOE 2+).

C.3. Several recent RCTs have been performed comparing higher versus more moderate protein intakes, sometimes accompanied by higher other macro- and micronutrients as well. Study designs vary considerably, most are highly underpowered especially for functional outcomes, and actual protein intakes are often estimated or imprecisely measured, meaning it is difficult to draw firm conclusions. It seems that enteral protein intakes ranging from 3.5 up to 4.5 g/kg/d are justified to support somatic growth (including head growth), although data on functional outcomes are extremely limited. (LOE 1-)

C.4. Plasma urea concentrations may form a tool of assessing protein utilisation adequacy, although one must note that other parameters of protein metabolism such as ammonia and individual amino acid concentrations are not routinely measured. The evidence base for cut-off values of plasma urea concentrations to adapt protein intake is very limited. Yet, elevated urea concentrations in the absence of fluid or renal derangements indicate that proteins are not fully used for protein synthesis but are oxidised instead. This thus hints at either optimising concomitant nutritional intakes or decreasing protein intake. (LOE 1-)

C.5. Separate supplementation of certain extra amino acids (e.g. glutamine, arginine, or taurine) may reduce several neonatal morbidities but data are limited. Whilst arginine appears promising in reducing NEC rates, the number of infants studied remains limited. (LOE 1-)

Recommendations:

R.1. We strongly recommend very preterm infants are given at least 3.5 to 4.0 g protein/kg/d (together with sufficient other macro- and micronutrients). Protein intake may be further increased up to 4.5 g/kg/d where growth is considered to be

slow, provided protein quality is good, concomitant energy and other micronutrient intakes are optimal, and there are no other causes for suboptimal growth. (A)

R.2. We conditionally recommend monitoring plasma urea concentrations at regular intervals in case standard fortification is insufficient to support desired growth and more protein is added. There are no data that allow conclusive determination of optimal levels, and every baby is unique and clinicians have to individualise and exercise judgment. Low urea concentrations may indicate enteral protein intakes can be increased up to 4.5 g/kg/d. If urea concentrations are above 5.7 mmol/L (34 mg/dL; or 16 mg N/dL) during the enteral nutrition phase in the absence of fluid or renal derangements, while providing sufficient concomitant energy, lowering of protein intake should be considered. (C)

R.3. We conclude that no recommendation can be made in either direction regarding the use of separate supplementation of glutamine, arginine, or taurine next to enteral nutrition to decrease neonatal morbidity incidence. (B)

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Supplementary Digital Content no. 6

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Fat Supply (February 2022)

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Background

Dietary fats provide a preterm infant with a major portion of its energy needs, as well as numerous bioactive compounds such as essential polyunsaturated fatty acids, lipid soluble vitamins, and complex lipids. The natural source of fats is the mother's milk which contains lipids mainly in the form of triacylglycerols, but also in complex mixture of other lipid classes that are concentrated in the milk fat globule membranes (MFGM)[1, 2].

Numerous biofunctional roles of fat substrates provided with human milk have been proposed and care should be taken to provide optimal fat intake to ensure adequate metabolic response, growth, tissue composition and function. With no significant fat stores of their own, preterm infants are entirely reliant on external sources of nutrition to supplement the necessary essential fatty acids (FAs) and long-chain PUFAs (LC-PUFAs). Therefore, the supply of an adequate amount and composition of lipids from soon after birth is important for preterm and particularly for very and extremely preterm infants.

Literature search

A literature search was carried out to identify papers relevant to the fat needs of preterm infants using the following search strategy: (lipid OR fat OR omega 3 fatty acid OR docosahexaenoic acid OR omega 6

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fatty acid OR arachidonic acid OR medium chain fatty acid OR linoleic acid OR linolenic acid OR cholesterol OR eicosapentaenoic acid) AND (Prematurity OR premature infant OR low birth weight OR very low birth weight OR preterm infant)) AND (enteral OR supplement OR breastmilk OR fortifier OR milk OR formula) AND Clinical Trial[ptyp]. Medline was searched using the PubMed interface from 2005 until July 2020; the search was limited to clinical trials. Manuscripts were also retrieved from references and previous ESPGHAN recommendations[3].

Total fat intake

Very preterm infants have small amount of body fat which is almost entirely contained in structural lipids such as cell membranes and which does not represent an exchangeable energy storage that could compensate for a low or absent postnatal supply. The average daily body lipid deposition in the fetus increases from about 0.5 g/d at 24 weeks of gestation to about 5.5 g/d at 36-37 weeks of gestation. Assuming a mean daily intrauterine fat deposition of 3 g/kg, losses due to fat malabsorption in the range of 10-40%, and a further 15% accounting for losses for unavoidable oxidation and for conversion of absorbed triglyceride to deposited triglyceride in tissue, the estimated minimum fat intake to meet needs would be 3.8-4.8 g/kg/d[3]. With a caloric contribution from fat at 45-55%, a minimum supply of 4.8 g/kg/d is needed to assure 96 kcal/kg/d of non-protein calories. On this basis, a minimal dietary enteral fat intake of 4.8 g/kg/d is recommended.

Mature breast milk contains approximately 3.2 to 4 g fat/100 mL and a daily volume of 160–180 mL of breast milk/kg leads to a mean oral fat intake of up to 7 g/kg/d [4, 5]. Considering the upper interquartile range observed in human milk samples the upper interquartile range of observed lipid intake is 8.1 g/kg/d. Such intakes appeared to be safe even in extremely low-birth weight infants who exhibited appropriate weight gain and normal serum lipid concentrations [6].

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Complex lipids and cholesterol

Mammary alveolar cells produce milk fat globules containing a core predominantly consisting of triglycerides (comprising 98–99% of milk lipids) and small amounts of monoglycerides, diglycerides and non-esterified fatty acids, surrounded by a milk fat membrane with different phospholipids, esterified cholesterol, glycosylated polypeptides, filaments, mucin, lactadherin and other components [(1)]. Whether or not the formula fed preterm infant would benefit from a dietary supply of MFGM similar to that provided by the human milk remains not known.

Considerable amounts of cholesterol are deposited in tissues, including the brain, during growth. The higher cholesterol concentration of human milk is most likely the reason for the higher blood levels of cholesterol and low-density lipoprotein cholesterol levels in breast-fed infants compared with formula-fed infants. The major proportion of deposited cholesterol appears however to be derived from endogenous synthesis and there is yet no evidence that the dietary supply of cholesterol affects nervous system development [(7)]. Fat intake from human milk of up to 8.1 g/kg/d leads to normal serum total cholesterol concentration but low HDL cholesterol concentration compared to adult values [(6)]. Whether or not the formula fed preterm infant would benefit from a dietary supply of cholesterol similar to that provided by the human milk (i.e., 10-20 mg/dL) remains not known.

Metabolically dispensable fatty acids

Saturated FAs provide energy and have structural and metabolic functions. Saturated FAs can be synthesized from nonfat sources or by β-oxidation from unsaturated FAs. Typically about 40-45% of human milk fatty acids are saturated [(1, 2)]. Most of them have a chain length of 12 to 18 carbon atoms and the most predominant saturated FA is palmitic acid (C16:0) which represents approximately 25% of all FAs. In human milk approximately 70% of palmitic acid is esterified in the central position (*sn*-2 position, i.e. β-palmitate) of the triglycerides which facilitates absorption. In comparison, palmitic acid in

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bovine milk is only found in 45% in the *sn*-2 position of triglycerides and in most plant oils it is even less than 20%. The use of synthetic β-palmitate increases fat and mineral absorption and may offer other health benefits, although this is not yet definitely proven [8].

The content of medium chain triglycerides (MCT, C8–C10) in human milk is low (2-10%). Medium chain fatty acids can be absorbed to a large degree by preterm infants even at low intraluminal bile salts and pancreatic lipase levels. Absorbed medium chain fatty acids (MCFAs) enter directly into the portal vein and are thus transported to the liver, where they can be oxidized for energy [7]. The rapid and high oxidation of MCFAs can reduce the oxidation of other provided polyunsaturated fatty acids (PUFAs) and thereby enhance plasma concentrations of PUFAs and their derivatives LC-PUFAs. In preterm infants with intestinal immaturity, facilitation of fat absorption through the inclusion of MCTs in the diet may be useful, but there is no demonstrated benefit for energy balance or growth [1, 9]. This may be due to the 16% lower chemical energy content of the shorter chain length MCFA compared to long-chain saturated FA, the benefit in percentage absorption of MCT being not compensated for this difference in energy content. The use of MCT instead of long chain saturated FAs 16:0 and 18:0 in preterm infant formulas can increase calcium and magnesium absorption but similar benefits on mineral bioavailability can be achieved by other formula modifications. Overall, formulas for stable growing preterm infants do not necessarily need addition of MCT oils.

Monounsaturated fatty acids usually contribute to a similar proportion of human milk FA as saturated FA, and in many populations they represent the largest fraction, where oleic acid (C18:1n-9) is the dominant component [1]. Inclusion of a considerable fraction of monounsaturated FAs into formula fats appears advisable since they are better absorbed than saturated FAs.

The available evidence does not allow to set quantitative recommendations for the provision of saturated and monounsaturated fatty acids. The provision of MCT may furnish some benefits but high amounts exceeding 40 % of total fat are not recommended [3].

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Essential fatty acids

About 15-20% of FAs in human milk are polyunsaturated fatty acids (PUFAs) with considerable compositional variation induced by differences in maternal dietary intakes [10, 11]. Both the omega-6 FA linoleic acid (LA; C18:2n-6) and the omega-3 FA alpha linolenic acid (ALA; C18:3n-3) are the two essential FAs. The relative provision of LA and ALA is of importance for the endogenous synthesis of the respective LC-PUFAs because these 2 precursor FAs compete for desaturases and elongases in the PUFA conversion pathways. Current guidelines for the levels of LA and ALA in infant formulae aim at avoidance of an extremely high LA:ALA ratio, which may reduce ALA conversion to n-3 LC-PUFA [1]. High neonatal LA has been associated with impaired development up to 18 months in preterm infants [12] and with impaired neurodevelopment up to 2–3 yrs of age in term infants [13]. Overall, there is not enough relevant additional data that would support a significant modification of previous recommendations [3].

Long-chain polyunsaturated fatty acids (LC-PUFAs)

Arachidonic acid (ARA) and docosahexaenoic acid (DHA) are actively transferred through the placenta during the 3rd trimester of pregnancy and accumulate in the fetal body, especially the brain [9, 14]. Postnatally, ARA and DHA synthesis from the essential fatty acids LA and ALA, occurs to some degree in premature infants but is insufficient to meet requirements [15–17]. Breast milk naturally contain LC-PUFAs that are absorbed at 70-80% by very preterm infants and then stored in tissues in the form of triglycerides or phospholipids, but they can also undergo total or partial β-oxidation [7]. LC-PUFA content in breastmilk is highly variable and, particularly for DHA, dependent on the mother's diet [11].

Possible effects of enteral LC-PUFA supplementation include improved neurological and visual development and also growth and modulation of immune functions, and are extensively reviewed elsewhere [9, 18–21]. Benefits on neurodevelopment of supplementing preterm formulas with LC-PUFA is not fully supported by meta-analysis, possibly because of the extreme variability in study design of

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studies, the selection of relatively mature and healthy preterm infants which are likely less DHA deficient than VLBW infants, and the doses provided in most studies which were well below the *in utero* accretion rate [9].

Strategies to increase DHA intake of preterm infants by supplementing lactating mothers with fish oil is very efficient to induce changes in milk DHA content but its effect is highly variable [22]. Adding LC-PUFAs directly into the infant feeding is a reliable method for delivering known amounts of LC-PUFAs to preterm infants [23, 24]. It should be considered that the matrix (e.g., human milk or formula), the mode of administration (e.g., enteral or buccal) and the presence or absence of an emulsifier may all significantly affect the efficacy of LC-PUFA supplementation, thus suggesting that LC-PUFA supplementation should only be done by using a mode of administration that has been previously tested [25–27].

Overall current data demonstrate that exogenous supply of LC-PUFAs is critical in preterm infants, that both DHA and ARA are conditionally essential nutrients in preterm infants.

DHA

One approach for defining adequate DHA intake for preterm infants is to target the concentrations of DHA in the fetal blood *in utero*. Preterm infants fed breast milk or current preterm formulas containing 0.2 to 0.37% of total FAs as DHA (which corresponds to 13 to 24 mg/kg/d based on milk fat content of 4g/100mL and a daily milk consumption of 160 mL/kg), does not produce the expected rise in their DHA in plasma and/or red blood cell phospholipids between birth and term age (or hospital discharge) [13] while those receiving ≥50 mg/kg/d of DHA do [7, 23, 24, 27] **(Table I)**.

Another approach is to assess the functional effects of the DHA supplementation. There is possibly a dose effect relationship for DHA supplementation **(Figure 1)**. Three studies report neurodevelopment short and/or long term outcome data in preterm infants fed either human milk or formula, with a DHA content between 32 to 64 mg/kg/d (i.e. 0.5% to 1% of total FA) [23, 28–31] **(Table II)**. In the largest trial

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published to date providing a DHA intake equivalent to about 64 vs. 22 mg DHA/kg/d (i.e., 1% vs. 0.35% of total FAs) until expected term at a constant ARA intake (32 mg DHA/kg/d; 0.5% total FAs), with ARA:DHA-ratios of 0.5 and 1.5, respectively, improved early visual acuity at 4 months corrected age, reduced the risk of a very low mental development index (MDI) score at the Bayley score, improved MDI scores in girls at 18 months.

Numerous other development scores or health parameters tested in these large randomized trials did not show any significant differences or only subtle differences between the supplemented and non-supplemented groups [20, 31–34]. A recent randomized trial showed that enteral supplementation with 50 mg/kg/d of DHA and 100 mg/kg/d of ARA from soon after birth to expected term lowered the risk of severe ROP by 50% [24].

The data available to date do not support a further increase of DHA supply to maximize effects on respiratory function. A DHA intake of ~90mg/kg/d with no concomitant ARA supplementation (ARA:DHA ratio 0.24) resulted in an increased risk of bronchopulmonary dysplasia (BPD) or death before 36 weeks postmenstrual age [35] with no beneficial effects on development at 18 months [36].

Overall, the effects described above, despite being modest and transient, support a DHA intake of 30 to 65 mg/kg/d assuming sufficient intake of ARA.

ARA

Enteral supplementation strategies of FAs in preterm infants have relied on an n-3–dominant approach but the lack of supplemental ARA in these strategies may be of critical importance [37]. Determining appropriate ARA intake is difficult because requirements are partially covered by endogenous synthesis of ARA which is more efficient than that of DHA and because most of the studies have not assessed the requirement of this FA. Fetal transfer of ARA is about 3 times as high as that of DHA, and many studies

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have shown a decrease in ARA levels suggesting insufficient supply and insufficient endogenous synthesis from LA [13, 38].

Reduced serum levels of ARA in the postnatal period are associated with increased risk of retinopathy of prematurity [39] nosocomial sepsis [40] and severe BPD [35]. In formula-fed very preterm infants receiving 0.30% of FAs as DHA, adding ARA to achieve an ω-6/ω-3 ratio of 2/1 leads to higher blood levels of essential FAs during the first year of life, and better psychomotor development at 2 years compared with very preterm receiving a similar DHA enriched formula with an ω-6/ω-3 ratio of 1/1 [41]. A recent randomized trial showed that enteral supplementation with 100 mg/kg/d of ARA combined with 50 mg/kg/d of DHA from soon after birth to expected term did not prevent a decline in serum ARA content but significantly lowered the risk of severe ROP by 50% [24].

With the range of DHA intake defined above (i.e., 30 to 65 mg/kg/d), a ARA/DHA ratio ranging from 0.5 to 2 (i.e., 15 to 100 mg/kg/d) appears to be safe. However, to limit the decline in circulating ARA the minimum ARA intake was set based on the average value observed in human milk (i.e., 0.5% of FAs, which translates into 30 mg/kg/d).

EPA

Fish oil also contains eicosapentaenoic acid (EPA), but has not yet been proven to be safe in preterm infants when provided at a high amount [9]. Limited data are available to define if there is any benefit for including EPA in the diet of preterm infants. Therefore, we recommend not exceeding 20 mg/kg/d of EPA, which is the mean amount + 1 SD of EPA provided daily by human milk when fed at 180 ml/kg/d.

Conclusions

- C1: There is not enough new data to support a significant modification of previous recommendations for linoleic and linolenic acids nor medium chain triglycerides (LO2+)

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- C2: DHA supplementation has modest and transient effects on neurodevelopment outcomes but may help achieve intakes close to intrauterine accretion rate (LO1+)
- C4: With the range of DHA intake of 30 to 65 mg/kg/d, a ARA/DHA ratio ranging from 0.5 to 2 (i.e., 15 to 100 mg/kg/d) appears to be safe (LO1-). The minimum ARA intake was set based on the average value observed in human milk (LO2++).
- C5: Limited data are available to define if there is any benefit for including EPA in the diet of preterm infants (LO3)

Recommendations

- R1: A total fat intake of 4.8 to 8.1 g/kg/d is recommended although higher intakes may be safe (LO2, RGB).
- R2: Amounts of medium chain triglycerides exceeding 40 % of total fat are not recommended (RGB).
- R3: Linoleic acid intake of 385 to 1540 mg/kg/d, a minimum linolenic acid intake of 55 mg/kg/d, and a linoleic acid/linolenic acid ratio of 5-15:1 (wt/wt) are considered acceptable (RGB).
- R4: A DHA intake of 30 to 65 mg/kg/d is recommended assuming sufficient intake of ARA (RGA).
- R5: An ARA intake of 30 to 100 mg/kg/d is recommended (RGB).
- R6 : EPA intake should be < 20 mg/kg/d (RG0).

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Supplementary Digital Content no. 6

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Fat Supply (July 2022)

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Figures and tables

Figure 1: Relationship between DHA intake (expressed as % of total fatty acid in milk during hospitalization) and Bayley MDI score at 18 to 20 months corrected age of preterm infants (adapted from [23, 29, 42])

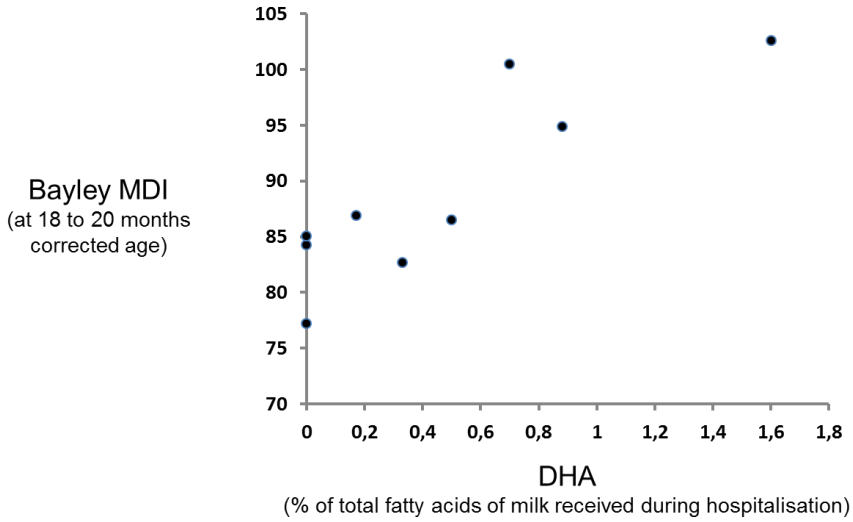

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Table I. Changes of DHA status of preterm infants. DHA status of preterm infants fed current preterm formulas containing 0.2 to 0.37% fatty acids as DHA or breast milk exhibit a decline in their DHA status between birth and expected term (or hospital discharge). Those fed a DHA dose > 50 mg/kg/d exhibit a DHA status that either increases during hospitalization or reach a level comparable to that of term infants.

Reference	DHA (mg/kg/d)	Effects on DHA status
Current DHA intake	13-24	Decline in DHA status
Henriksen 2008	32*	Decline in DHA status (PPL)
Smithers 2008	45	RBC DHA at expected term < term values
Baack 2016	38-57**	Raise of total blood DHA during hospitalization but < term values
Hellström 2021	50**	Raise of serum phospholipid DHA from birth to expected term
Smithers 2008	54[£]	RBC DHA at expected term similar to term values §
Henriksen 2008	59[#]	Increase in DHA status by 12% (PPL)

PPL: plasma phospholipid; RBC: red blood cell

* human milk from Danish mothers likely consuming fish

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** supplementation given at a fixed dose per day

£ human milk supplemented with a DHA supplement

mother's milk of women receiving 3 g of tuna oil per day

§ values observed for RBC DHA in term infants at birth is ~8% [59]

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Table II. Summary of findings showing an improvement in neurological outcomes in preterm infants receiving a DHA intake between 0.50% and 1% of total fatty acids.

	Intervention	**Short term**	**Long term**
Fewtrell 2004	I (n= 122): 0.5% DHA; 0.9% γ-linoleic acid C (n=116): 0% DHA; 0% ARA	= Primary outcome (Bayley 18mo) ↑ mental development in boys at 18 mo	No effects at 10 y overall ; ↑ word reading and spelling scores in girls
Henriksen 2008	I (n= 68): 1.18% DHA; 0.94% ARA C (n=73): 0.64% DHA; 0.44% ARA	↑ Problem solving and recognition memory at 6 mo (primary outcome) ↑ Attention at 20 mo	No effects at 8 y
Makrides 2009	I (n= 322): 1% DHA; 0.43% ARA C (n=335): 0.26 % DHA; 0.45% ARA	= Primary outcome (Bayley 18mo) ↑Visual function at 4mo; fewer infants with MDI less than 70 at 18 mo; Less developmental delay in girls	No effects at 3, 5, 8 years

I: Intervention; C: Control; mo: months

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Supplementary Digital Content no. 7

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: CARBOHYDRATES

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Introduction

Carbohydrate along with fat is the major source of energy derived from enteral nutrition. Due to the lower caloric content of carbohydrates (4 kcal/g) compared to fat (9 kcal/g), carbohydrate comprises about 40% of the total caloric content of preterm own mothers' milk (OMM) (1-4). Digested carbohydrates are utilized by the cells in the form of glucose, which is effectively transported across blood-tissue barriers through the glucose transporter GLUT-1 that is present within most tissues during the early neonatal period and has a very high affinity for glucose (5). After birth, GLUT-1 is gradually replaced by other GLUTs, such as GLUT 2 (liver), -3 (brain) and -4 (muscle), that are specifically suited for the metabolic characteristics of the specific tissue (5). In addition to being the primary energy source for the brain and other glucose-dependent organs, glucose is an important carbon source for de novo synthesis of non-essential fatty acids and amino acids (6, 7).

Lactose, glucose and glucose polymers

The predominant digestible carbohydrate in human milk is the disaccharide lactose (4, 8), but 15-30% of total carbohydrates comprise free glucose, galactose and human milk oligosaccharides (HMOs) (4). The carbohydrate concentration is the most stable of the macronutrients in human milk (HM) and reported total carbohydrate concentrations increase from about 6.2 g/100mL in the first week to 7.1 g/100mL after the fourth week of life (2, 3), whereas reported lactose concentrations are about 15% lower (4). Prior to absorption, lactose is hydrolyzed to glucose and galactose at the intestinal brush border by β-galactosidase (also called lactase) (8). The majority of the galactose is subsequently converted to glucose or glycogen during the first pass through the liver (8).

Lactase activity gradually increases with advancing gestation until about 36 weeks gestational age (GA), when it reaches the level of term infants (9). Incomplete digestion of lactose in the small intestine of the preterm infant may limit the availability of energy from carbohydrate, but early feeding and early achievement of full feeds increase intestinal lactase activity (10). A double blind, randomized controlled trial in 130 infants at 26-34 weeks of gestation showed that adding lactase to the diet increased weight gain at 10 days of age, but the difference was no longer significant at study

exit (11). Undigested lactose may be metabolized after bacterial fermentation in the colon to short-chain fatty acids (i.e. acetate, propionate, and butyrate), lactate and organic gases.

Because glucose absorption is well developed in enterally fed preterm infants (12), current commercial preterm infant formulas regularly contain glucose polymers as a partial replacement for lactose. Glucose polymers, such as maltodextrin and corn syrup solids, are preferred to glucose due to the lower osmolarity. Glucose polymers consist of glucose chains, predominantly of medium length (6 to 10 glucose units), and the proportion of free glucose is usually less than 2% (6). In the newborn, glucose polymers are primarily digested via intestinal glucoamylase and isomaltase (8, 13). Similar to lactose, undigested maltose and glucose polymers are salvaged by colonic bacteria (8, 13, 14).

It has been suggested that carbohydrate malabsorption may increase the risk of feeding intolerance and necrotizing enterocolitis (NEC). Studies in a piglet model of NEC show that lactose-based formulas reduce the risk of NEC as compared to formulas based on glucose polymers (15-17). In these studies, lactose was significantly better absorbed than the glucose polymers. In contrast, a previous study in preterm infants found that absorption of lactose was significantly less than that of lactose-glucose polymers or glucose polymers (14). Reduction or elimination of lactose and replacement with more readily digestible glucose polymers have shown inconsistent results in regard to feeding tolerance, weight gain and calcium absorption (18-22). The optimal proportion of lactose to total carbohydrate is still unknown, but the content of lactose in preterm formulas is usually in the range of 3.7-6.2 g lactose/100 mL and the carbohydrates constitute 40-45% of total caloric content.

Requirements

Minimum glucose requirements were originally determined with the use of stable isotopes from measuring the basal or endogenous glucose production rates (GPR) during fasting that were sufficient to maintain normoglycemia. The exact endogenous GPR in preterm infants is estimated to be around 6-8 (10) mg/kg/min in extremely preterm infants compared to 4-7 mg/kg/min in term infants and 2-4 mg/kg/min in adults (5, 23-26). The low glycogen reserves and limited fat stores of preterm infants restrict their ability to mobilize energy from other sources (e.g. ketones and lactate) to meet brain glucose requirements and put preterm infants at risk of hypoglycemia (5). Infants less than 30 weeks are possibly more vulnerable due to insufficient suppression of peripheral glucose utilization (25). On the other side, if energy intakes are high and the maximum rate of glucose oxidation is reached, excessive glucose will be converted to fat (5, 27, 28). This imposes enhanced metabolic stress to the infant because endogenous lipogenesis is energy demanding and increases O_2 consumption and CO_2 production (28, 29). Moreover, preterm infants have a high risk of developing hyperglycemia (30).

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The risk of hyperglycemia increases with decreasing gestational age due to immature glucose regulatory mechanisms. These involve persistent hepatic gluconeogenesis ("hepatic unresponsiveness"), decreased pancreatic beta-cell activation and partial insulin resistance (5, 25). The risk of hyperglycemia persists for a long period after birth in extremely preterm infants, even if the infants predominantly receive enteral nutrition (30). Hyperglycemia is associated with increased mortality, higher rates of neonatal comorbidities such as white matter injury of the brain, necrotizing enterocolitis and retinopathy of prematurity, and with adverse neurodevelopmental outcomes (30, 31).

The relative contribution of carbohydrate to total non-protein energy intakes is also an important factor to consider. Carbohydrates constitute about 45% of non-protein calories in OMM and 45-50% of non-protein calories in standard preterm formulas. Earlier studies in stable formula fed preterm infants show that compositions with 65% of non-protein energy as carbohydrate increases nitrogen retention compared to compositions with 65% of non-protein energy as fat at equal protein and energy intakes, but also that high-energy, high-carbohydrate intakes increase fat deposition (27, 28). The long-term consequence of the increased metabolic stress imposed by such a strategy is unknown, but higher mean carbohydrate intakes during postnatal weeks 1-8 were associated with higher blood pressure assessed at 6.5 years of age in extremely preterm infants (32).

Recommendations

In the previous ESPGHAN position paper on enteral nutrient supply in stable growing infants, carbohydrate recommendations ranged from 11.6-13.2 g/kg/d (or 10.5-12 g/100 kcal), albeit these numbers do not align (6). The minimum recommended intake was derived from studies on endogenous rates of glucose production in preterm infants (5, 27, 28) and aimed at preventing muscle breakdown and lipolysis. The maximum recommended intake of 13.2 g/kg/d was based on the carbohydrate equivalents of the total energy requirements (total energy expenditure minus the calories from the minimum requirements for protein and fat), and did not include requirements to cover cumulative deficits (33). These upper recommendations are lower than the enteral recommendations given by Tsang et al (34) and the maximum recommendation for carbohydrates given in the 2018 European guidelines for parenteral nutrition (35).

The recommended food for premature infants is fortified OMM, or alternatively fortified donor HM or preterm formula, all of which are composite nutrients. Thus, providing truly evidence-based guidelines for a single nutrient is difficult. For the update of the Carbohydrate section, a literature search was conducted using the MEDLINE and Cochrane systematic database covering the period from 2005 until October 8th 2019. This resulted in 318 publications. After screening the titles

and abstracts, 13 publications were selected for evaluation. Finally, the reference list from relevant publications was used to identify additional relevant studies.

In 2018, an update of the 1999 Cochrane Review on carbohydrate supplementation of breast milk in preterm infants was published (36), but the authors did not identify any applicable RCTs for their review. Due to lack of relevant RCTs on the effect of exclusively enhancing carbohydrate intakes on short- and long term outcomes in preterm infants, studies on breast milk fortification and studies on higher volume intake (>180 ml/kg/d) were also included as part of the evidence base for the present carbohydrate recommendations.

Studies on breast milk fortification in preterm infants generally show improved in-hospital growth, but a recent large Cochrane review and meta-analysis found only very limited data for growth and developmental outcomes assessed beyond infancy, and these showed no effects of fortification (37). The high carbohydrate content of some fortifiers has led to concerns about providing an unfavorable protein to energy ratio by exceeding carbohydrate intakes recommendations (4). In contrast, other studies suggest that not only protein targets, but also carbohydrate targets may not be met with standard fortification at milk volumes around 150 ml/kg/d (38, 39). A few small studies on targeted fortification of HM (3.0 g protein, 8.8 g carbohydrates and 4.4 g fat/100 mL HM) suggest improved growth rates as compared to standard fortification alone (38). In a Canadian study of targeted fortification, the authors found a linear relationship between milk intake and weight gain (39), and the authors suggest that this could be explained by less variation in the HM composition as compared to standard fortified milk. Collins et al performed a retrospective study of 138 infants born before 32 weeks' gestation and found that higher energy intakes were associated with improved growth (40). In agreement with the findings of Kashyap et al (27, 28), only the contribution of carbohydrate to total energy was positively associated with weight, length, and head circumference gains at isocaloric intakes and with protein supply in line with current recommendations (40). In Collins' study the estimated median enteral energy and protein intakes from full feeds to discharge were 125 kcal/kg/d and 3.7 g/kg/d, respectively, whereas the carbohydrate intake was between 12.5-14 g/kg/d. In contrast, fortification with a human milk based fortifier with high protein and low carbohydrate content resulted in improved in-hospital growth compared to fortification with a standard breast milk fortifier (41).Also in the large EXPRESS cohort study, only protein intake was consistently associated with better growth after adjusting for energy intakes (42). .

A common strategy to enhance nutrient intakes is to increase target feeding volumes to more than 150-160 ml/kg/d (43-46). If the intake of fortified human milk is increased from 150 to 180 ml/kg/d, the total carbohydrate intake increases 2-3 g/kg/d (from about 10-15 g/kg/d to 12-18 g/kg/d)

depending of the carbohydrate content of the fortifier. Providing 181 ± 16 ml/kg/d of fortified human milk/formula has been shown to be safe and to improve in-hospital growth compared to 157 ± 14 ml/kg/d in a recent RCT in preterm infants (1001-2500g) (46). In a retrospective population-based study of infants < 30 weeks gestation, Klingenberg et al found that a median intake of 193 mL/kg/d of fortified HM (range 180-220 mL/kg/d) was associated with good head growth and low rates of postnatal growth restriction (44). The change in head circumference z-scores from birth to discharge was associated with improved language scores at 2 years of age. Standard fortifier in this study was (Nutriprem®, Nutricia, Schiphol, the Netherlands) and provided an additional 0.8 g of protein and 2.8 g of Carbohydrate/100 mL human milk, respectively.

Unfortified human milk given at volumes between 200-300 mL/kg/d has also been shown to improve nutritional outcomes as compared to volumes < 200 mL/kg/d (47). However, high quality studies to ensure that this nutritional strategy is feasible and safe in very preterm and extremely preterm infants are needed.

Conclusions and key recommendations

The balance of benefits and risks needs to be considered when determining carbohydrate intake recommendations. We did not identify any new data to alter the previous recommendations of a carbohydrate intake in the range of 10.5-12 g/100 kcal (6). There were no data from randomized controlled trials demonstrating benefits of simply providing higher carbohydrate intakes than currently used routinely on short- and long term outcomes in preterm infants. However, when infants are given human milk fortified with commercially available fortifiers at feeding volumes > 160 ml/kg/d or they are fed high volumes of unfortified human milk (> 200 mL/kg/d), the estimated carbohydrate intake will easily exceed the previous maximum recommendation of 13.2 g/kg/d. Observational studies suggest that fortified human milk given at intakes that provide carbohydrates well above 13.2 g/kg/d are safe and improves weight, length and HC growth.

Assuming energy needs of 115-140 kcal/kg/d, enteral protein intakes of 3.5-4.0 (4.5) g/kg/d, and a carbohydrate composition contributing to 40-50% of non-protein energy intakes, a carbohydrate intake in the range of 11-15 g/kg/d may seem reasonable.

Lactase activity is reduced in preterm infants. Current commercial breast milk fortifiers and infant formulas regularly contain glucose polymers as a replacement for lactose, but the optimal lactose to total carbohydrate ratio is unknown. Due to the higher absorption rates of glucose polymers compared to lactose, the carbohydrate requirements of preterm infants fed formula may be somewhat lower than in infants fed fortified OMM or DHM.

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Conclusions and Recommendations

C 1: There are no new data from randomized controlled trials (RCTs) on the effects of exclusively increasing carbohydrate intakes on short- and long term outcomes in preterm infants, therefore we do not know for certain the optimal intake range
C 2: Observational data suggest that supplemental carbohydrate as part of fortified human milk improves in-hospital weight, length and head circumference growth at upper intake ranges (LOE 2++)
C 3: Preterm infants fed formula may need lower carbohydrate intakes than infants fed fortified human milk, due to higher absorption rates of glucose polymers compared to lactose (LOE 2, GOR B)
C 4: The optimal lactose to total carbohydrate ratio in human milk fortifiers or in preterm formulas is unknown

R 1: In preterm infants, a carbohydrate intake of 11-15 g/kg/d is recommended (GOR B).
R 2: Intakes above the recommendations may be considered during a short period of time to cover cumulative deficits and facilitate catch-up growth if tolerated (euglycemia), but should also be tapered accordingly to avoid overnutrition (LOE 4, GPP)

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Sodium

Sodium (Na) is the principal cation in extracellular fluid and concentrations influence intravascular and interstitial volumes, and blood pressure. Na also has a role in bone mineralization, nerve conduction, nitrogen retention and growth. To achieve normal intrauterine growth, a net storage of 1.8 mmol/kg/d of Na is necessary in near-term human fetuses (1) and Na retention rate has been estimated at 1.6-2.1 mmol/kg/d for a fetus of 27-34 weeks of gestation (2).

After birth, Na absorption from the gastrointestinal tract is effective in preterm infants. Fecal Na excretion is usually less than 10% of intake but is higher at lower gestational ages and diminishes with increasing post-conceptional age (3). Over a broad range of intakes, Na balance is maintained by renal Na conservation and excretion, but newborn infants, and especially those born preterm, have limited capacity both to conserve Na when challenged by Na restriction, and in excreting Na when challenged by a Na load. Hyponatremia in extremely preterm infants is common after the first 4 days of life (4). Na requirements change depending on maturational changes in renal function and tubular transport. Tubular Na loss (as estimated by the fractional excretion of sodium - FE_{Na}) is inversely associated with gestational age (5) and can be increased during critical illness and by common medications (6). In infants born less than 30 week of gestation urine sodium losses as high as 7 mmol/kg/d have been reported during the second week of life (7), suggesting that Na intakes higher than 7 mmol/kg/d can be occasionally necessary.

Determining intakes in fully enterally fed preterm infants is difficult because Na concentrations in human milk vary. The Na concentration of breast milk of both term and preterm infants shows a rapid decline over the first days after birth which is probably under the control of prolactin and aldosterone (8), with reported values of 70.9 mmol/L in colostrum decreasing to 17.3 mmol/L at 7 days, and 13.1 mmol/L at 8–14 days for infants born 27-34 weeks of gestation (9). More recent studies have confirmed this and shown that Na content was higher in the first week of life compared with the 8th week in infants born 23 to 33 weeks of gestation (10). Moreover, the Na content of preterm human milk is influenced by methods of expression, and maternal serum concentration (11, 12).

Several studies reported that early and late Na supplementation significantly reduced late hyponatremia and improved somatic growth and subsequent neurodevelopment in preterm infants (13, 14). One randomized controlled trial (13) showed that 53 very preterm infants who received either placebo or 4 mmol/Kg/d of sodium as a supplement from day of life 7 to 35 had greater percentage weight change from birth to 6 weeks of life, and were more likely to remain on their birth percentile for weight compared to infants receiving placebo.

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It is important to recognize that even some fortified human milk may still be insufficient to meet Na needs in preterm infants (15-17) especially those born <1000 g (18). Besides, plasma Na concentration and weight may reflect infant hydration status more than total body Na. Thus, identifying truly Na deficient preterm infants and guiding Na supplementation on a case-by-case basis remains a challenge for clinicians. Recently, Segar et al (7) showed that a sodium intake of 4-6 mmol/kg/d at 4 to 8 weeks of life versus 3-4 mmol/kg/d was associated with improved growth in infants born at 26-29 weeks of gestation. In this study an algorithm based on urine Na concentration was used to guide Na supplementation. However, the use of urinary sodium concentration to assess total body sodium status in the immature kidney has not been validated. Further studies are needed on appropriate methods to assess total Na body status and needs in stable, total enterally fed and growing preterm infants.
Finally, when calculating Na supplementation, clinicians should be aware that the addition of concentrated sodium chloride or sodium phosphate to expressed fortified human milk may increase milk osmolality. Where possible, Na supplements should be added to a sufficient volume of milk in order to maintain an osmolality ≤ 450 mOsm/kg (19).

Chloride

As the most abundant anion in extracellular fluid, chloride (Cl) is involved along with Na, in maintaining osmotic pressure and hydration. The daily turnover of Cl is high, and renal tubular reabsorption rate is 60-70%. Even if Cl balance parallels that of Na, and correlates well with extracellular volume balance, Cl losses and excretion can also occur independently from Na, mainly in equilibrium with bicarbonate status. Chloride is involved in maintaining ionic neutrality, and the simple difference between Na and Cl plasma concentration represents one independent variable determining hydrogen and bicarbonate ion concentrations.
Chloride content in preterm human breast milk is similar across gestational ages and does not differ from term milk (20).
In the early 1980s, a case series of chronic metabolic alkalosis induced by low-chloride milk formula in newborn infants was described by Rodriguez-Soriano (21). Severe hypochloremia was associated with a significant elevation in the serum concentrations of calcium and phosphate and in the urinary excretion of calcium and magnesium, creating an important risk of nephrocalcinosis. Others reported that deficiency of dietary Cl induced failure to thrive, decreased growth in body length and head circumference, and delayed neurological development (22, 23).
In enterally fed preterm infants receiving oral salt supplementation, Cl intake parallels that of Na or K, so, where there are higher intakes of Na or K there will also be higher Cl intakes (24). This also can occur in case of inappropriate fortifier composition. The relationship between the metabolically strong cations (Na + K) and anions (Cl) has been studied in parenteral nutrition according to the Stewart approach (25), showing that Cl intake should be slightly lower than the sum of Na and K intakes to avoid severe metabolic acidosis (26). The strong ion difference (SID) calculated as [(Na + K) - Cl] should be ~1-2 mmol/kg in parenteral nutrition (27). Nevertheless, the strong ion difference is lower in liquid acidified human milk fortifiers compared to non-acidified fortifiers. Shanler et al. (28) have recently shown that preterm infants fed acidified human milk fortifier had higher rates of metabolic acidosis and poor feeding tolerance, compared with infants fed a non-acidified fortifier. A previous RCT (29) in breast milk fed preterm infants showed that the addition of a fortifier with an inappropriate composition increased the severity and frequency of metabolic acidosis, with possible effects on growth and bone mineral content. The authors concluded that

inappropriate fortifier composition can create a higher renal acid load (mEq of Cl + PO_4 + SO_4 - Na - K - Mg - Ca) because the low renal capacity for maximum acid excretion is exceeded leading to metabolic acidosis. Adaptation of the fortifier content of sodium citrate, potassium citrate and sodium chloride, by increasing alkali, could decrease the incidence of metabolic acidosis.

Potassium

Potassium (K) is the most abundant cation in the human body and the major intracellular ion. The K concentration gradient across cell membranes is crucial for maintaining contractility and neuronal function and is maintained by the tight balance of the influx or efflux of K from intra- to extracellular spaces. Potassium is needed for somatic growth and the K pool correlates well with lean body mass. A growth rate of 15 g/kg/d results in a net storage of about 1.0-1.5 K mmol/kg/d (3). The total body K content depends on the balance of K intake and excretion and is mainly dependent on renal regulation. After an enteral feed, 80% of the absorbed K enters the cells due to increased insulin levels stimulated by the contemporary absorption of glucose and amino acids (30). Several pathophysiologic factors can increase gastrointestinal K loss including vomiting and diarrhea, but also changes in aldosterone, epinephrine, and prostaglandins. Renal K excretion is increased by loop and thiazide diuretics (30).
No differences in K concentrations were found in human milk from mothers delivering preterm compared to that from mothers delivering full term (20).
Several studies in parenterally fed preterm infants have demonstrated an increased incidence of hypokalemia while optimizing protein and energy intakes according to current recommendations (31-33). In enterally and parenterally fed VLBW infants during the first week of life, Bonsante et al. (31) showed that there is a linear association between the K balance and amino acid intake, and that with amino acid intakes of 3 g/kg/d, the K balance remains positive with K intakes ≥ 2 mmol/kg/d, and it seems reasonable to assume similar amounts for stable fully enterally fed growing preterm infants. Based on the above data, and considering the increased range of protein and energy intakes in present recommendations for enteral nutrition, minimum and maximum K intakes should be slightly increased accordingly. K balance in growing preterm infants receiving protein intake in the upper ranges deserves further investigation.
Finally, it is important to note that K concentrations in extracellular fluid are tightly regulated. This implies that intracellular K deficiency resulting in disrupted anabolism and impaired growth, may still occur in the presence of a plasma K concentration within the normal range.

Conclusions, Recommendations

C1: Na requirements show considerable inter- and intra-individual variation, especially in VLBW infants. Cl intakes parallel that of Na in case of oral salt supplementation (LOE 2++)
C2: Breastmilk with added fortifiers may still provide insufficient Na to meet needs in preterm infants (LOE 2++)
C3: The administration of Na additives by the enteral route exposes the infant gut to higher osmolality (LOE 3)
C4: High Cl intakes by additives and human milk fortifiers with low SID may induce metabolic acidosis (LOE2++)
C5: In enterally fed preterm infants there is a linear association between K needs and amino acid intake (LOE3)

R1: Na intake are recommended from a minimum of 3 mmol to a maximum of 8 mmol/kg/d. The upper range of Na intake is slightly higher than in previous recommendations and should be considered in infants receiving the upper range of energy and protein intake or with important sodium loss. RG0 (conditional recommendation)
R2: Na additives should be added to milk and divided between different feeds over 24 hours (LOE 2++)
R3: Cl intake are recommended from a minimum of 3 mmol to a maximum of 8 mmol/Kg/d. RG0 (conditional recommendation)
R4: Cl intake from HM fortifiers and preterm formula should be slightly lower than the sum of Na + K intakes, in order to avoid metabolic acidosis. HM fortifiers should provide buffers in order to compensate for high renal acid load. RGB (strong recommendation)
R5: A minimum K intake of 2.3 mmol/kg/d and a maximum K intake of 4.6 mmol/kg/d is recommended. The upper range of K intake should be considered in growing infants receiving the upper range of energy and protein intake. RGB (strong recommendation).

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SDC no. 8. Electrolytes: Sodium, Chloride and Potassium.
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Supplementary Digital Content no. 9

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Minerals (February 2022)

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Introduction

Bone mineral metabolism in fetal and early neonatal life is complex (1–5) and most estimates of bone mineral accretion in preterm infants show lower accretion rates compared to the in-utero fetus. This means that it is difficult to determine the optimal rate of bone mineral accretion in otherwise healthy enterally fed preterm infants and it is common for bone mineral content (BMC) at term corrected age to be lower in preterm compared to a term-born infant. There are few high-quality longitudinal studies, and the only RCT with long-term outcomes which was performed more than 30 years ago showed no effect of early mineral intakes on adult BMC but persisting benefits of higher proportional intakes of breast milk(6). However, this study was conducted in an era when few extremely preterm infants survived and when other aspects of nutritional management also differed. Studies in infancy using dual energy X-ray absorptiometry (DEXA) and/or quantitative ultrasound (QUS) show varying degrees of catch-up in mineral accretion in preterm infants after term corrected age. Whilst some studies show that adjusted BMC is similar to term infants in early infancy,(3,7,8) not all data are consistent(9).

Whilst there has been frequent use of terminology such as bone mineral disease, rickets, osteoporosis or osteopenia of prematurity, there is a lack of consensus over definitions and precise terminology. Changes in bone metabolism ex-utero means many preterm infants may experience bone mineral loss after birth(5) and some have suggested it might be more appropriate to use the term bone mineral ‘deficiency’, rather than ‘disease’, of prematurity(10).

Studies conducted more than 20 years ago showed that at least 40-50% of preterm infants had detectable mineral deficiency and at this stage around 7-30% also had fractures. In more recent studies, the incidence of fractures in preterm infants is substantially less, with one recent survey suggesting rib fractures (the most common fracture noted in preterm infants) occurred in <1% of preterm infants, despite the presence of mineral deficiency visible on X-ray(11,12). Unfortunately, there is no consensus on threshold levels of biochemical or radiological parameters defining bone mineral deficiency(10). Whilst severe osteopenia can be seen radiologically in some preterm infants, there are no available techniques to estimate BMC that can be used to guide routine clinical practice.

The dramatic reduction in bone pathology in preterm infants over the last 10-20 years undoubtedly reflects many improvements in nutritional care including improved parenteral mineral delivery(13,14), more appropriate use of human milk fortifiers and changes in preterm milk formula composition(8,15). It may also reflect other changes in neonatal care such as better positioning of infants inside incubators (so called 'nesting') which encourages better muscle development and mineral deposition, and more appropriate (gentle) handling of babies during procedures. Fractures, however, could still occur but are most likely to be seen in the most complex infants with additional risk factors such as unbalanced mineral parenteral nutrition solutions, ventilation and/or immobilization, steroids, diuretics, and gastrointestinal pathology that may limit mineral uptake(16–18). Adequate mineral and vitamin D intakes are essential, but matching in-utero bone mineral accretion is difficult and may often not be achieved.

Nutritional mineral requirements of the preterm infant can be estimated using the fetal mineral accretion rate and the rate of mineral absorption by the preterm intestine(19–21)(22) . During the second half of gestation, fetal accretion of calcium (Ca), phosphorus (P) and magnesium (Mg) is about 20 g, 10 g and 760 mg, respectively, which represents accretion rates of approximately 2.5 to 3.0 mmol/kg/d for Ca, 1.6 to 2.1 mmol/kg/d for P, and 0.12 to 0.21 mmol/kg/d for Mg(23,24).

Please note: With the exception of the data from carcass analysis in mass (g), further data will be presented in mmol/kg/d. The millimolar (1 mmol) to mass equivalence is: 40 mg for calcium, 31 mg for phosphorus, and 24.3 mg for magnesium. Although phosphorus in nutrition or in the body is exclusively in the form of phosphate (PO_4), we will adhere to elemental phosphorus requirements. Obviously molar requirements are equal for P and PO_4, whereas these would be different when mass equivalents are used (1 mmol PO_4 weighs 95 mg).

Ca accretion is mainly as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) in the bone accounting for about 98% of total stores, with a Ca:P molar ratio of ~1.7:1. By contrast, P stored in bone only represents around 80% of total P accretion. The remainder is incorporated in nucleic acids (DNA and RNA) and cell membranes (phospholipids), utilized in intra-cellular energy metabolism (ATP), or plays an important role for lean mass accretion as the major intracellular anion. This means that P intakes must be greater than that needed simply for bone mineral accretion(2,3,14). Furthermore, physiological changes in bone metabolism occur at birth stimulating bone remodelling, reducing relative bone mineral density but improving bone strength as suggested by postnatal bone development (2,5). This remodelling process releases minerals and could therefore reduce-mineral requirements and explain why breastmilk has a relatively low content of Ca and P compared to fetal supply.

Calcium and Phosphorus requirements

Studies conducted in preterm infants show that using modern human milk fortifiers and preterm milk formula, typical Ca retention rates of 2.25-2.75 mmol/kg/d and P retention rates of 2.2-2.6 mmol/kg/d can be achieved(2,3,25–27). The amount of mineral that needs to be provided to achieve these retention rates may be quite variable, as Ca and P absorption rates range between 30-70% and 70-90%, respectively (27). Multiple factors affect mineral absorption and bone deposition, and bioavailability may also differ between fortified human

milk and preterm formula milk. The use of calcium glycerophosphate improves bioavailability, and there may be also differences between powder or liquid milk formula, mainly due to the Maillard reaction induced by heat treatment during sterilisation (28–31). Furthermore, the use of intact versus hydrolysed protein (32), or the fatty acid composition (such as beta-palmitate) (33) may also potentially influence Ca and P availability and absorption. It can be challenging to obtain the optimal balance of Ca and P provision, and supplemental mineral provision may increase the risk of nephrocalcinosis. Nevertheless, adequate mineral intake are essential in order to optimise skeletal development. (34,35) In summary, multiple factors will affect the optimal amount of mineral to provide in enteral milk and it is therefore challenging to provide a narrow intake range in part due to the lack of high-quality studies (35). An individualized approach may be needed in some infants (36,37). However, direct assessment of bone mineral accretion for example, using whole-body dual energy x-ray absorptiometry, is not practical in routine clinical practice, and nutritional practice is often guided by periodic measurement of blood concentrations and/or urinary excretion, notwithstanding the potential imprecision of plasma and spot urine samples (see infra).

It is possible to estimate adequate intakes using the following:

(1) Ca intestinal absorption is typically around 50% so to achieve a Ca retention of 2.25-2.75 mmol/kg/d a Ca intake up to 4.5-5.5 mmol/kg/d might be required.

(2) Intestinal absorption rates of Ca may be as low as 30%, for example when using sterilized liquid formula. In this situation, intakes of 5.5 mmol/kg/d or higher might be needed. However, where bioavailability is higher, for example absorption from fortified breastmilk, absorption may be as high as 60% and Ca intakes as low as 3.7-4.5 mmol/kg/d might be sufficient.

(3) 0.35 mmol of P is required for every gram of protein that is accreted into tissue as lean body mass. To achieve a protein accretion rate of 2.0-2.5 g/kg/d (similar to fetal accretion rate) a P intake of approximately 0.9 mmol/kg/d will be needed which must be supplied in addition to the needs for bone mineral accretion.

(4) P intestinal absorption can be as high as 90%, so to support P retention in the range 2.2-2.6 mmol/kg/d, an intake of 2.4-3.2 mmol/kg/d would be needed. However, where P absorption is lower, intakes of 3.7 mmol/kg/d or higher might be needed.

(5) Using the Ca and P retention rates in points 1 and 4 would give a molar Ca/P ratio of approximately 1.4. Achieving a Ca intake of 4.0-4.6 mmol/kg/d and a P intake of 2.9-3.4 mmol/kg/d using fortified human milk resulted in bone mineralisation rates similar to that in paired term infants at the end of the second year of life (8,38).

Magnesium requirements

Mg accretion during the last trimester of gestation is around 0.12-0.21 mmol/kg/day with around half being accreted in the bone, and the remainder in muscle and soft tissue. Mg absorption rates change depending on Mg intakes but are typically around 40-50% (27) and in preterm infants, the serum concentrations seem to be higher than in adults or older infants with a range of 0.6-1.25 mmol/L (39) . In parenteral nutrition, a Mg intake of 0.1-0.3 mmol/kg/d in preterm infants maintains serum concentrations in the normal range (39,40) . Studies in preterm infants on fortified human milk providing 0.2-0.3 mmol/kg/d showed absorption rates of around 45-50 % leading to a Mg retention of 0.1 mmol/kg/d. In preterm infants fed formula, Mg intakes are in the range of 0.4-0.5 mmol/kg/d. Despite similar absorption rates of 45-50% with formula, Mg retention was only slightly higher, around 0.12-0.15 mmol/kg/d, similar to that estimated from fetal accretion. We therefore consider that an intake of 0.4-0.5 mmol/kg/d in preterm infants fed fortified human milk or formula is likely to be adequate. No randomised trials determining effects on bone accretion have been conducted.

Assessment of bone mineral needs in clinical practice

Serum Ca concentrations are tightly regulated, and preterm infants often maintain normal levels despite significant bone loss (25,26) meaning serum concentrations are not an appropriate marker for adequacy of Ca intake or absorption rates. However, high serum Ca

concentrations may reflect phosphorus deficiency. In contrast, serum phosphate concentrations more accurately reflect phosphorus status, with adequate serum concentrations of around 1.6-3.0 mmol/L, being higher than in adults (15,26,41). Data show these concentrations could be valid until 1 year of age. Recent studies using higher protein intakes from fortified human milk or preterm formula have resulted in an increased incidence of hypophosphatemia similar to that seen recently with higher amino acid intakes in parenteral nutrition (14,15,42–45). The optimum concentration of phosphate for bone mineralisation is unknown, but high serum concentrations (> 3 mmol/L) may indicate relative Ca/PO_4 imbalance or renal compromise.

Urinary Ca and phosphate concentrations depend on a complex interaction of intake, absorption, retention, losses via faeces and sweat, as well as renal function and water balance (46). Although studies have suggested that adequate bone mineral accretion can be achieved by establishing and maintaining a simultaneous urinary excretion of Ca and PO_4 at low concentration (1-2 mmol/L in urine) (36,37) there are few data to show this improves functional outcomes, and the approach is not widely practiced (47). An alternative could be to use the U_{Ca}/U_{PO4} ratio, avoiding the variability in dilution concentration in a spot urine (3) . High percent tubular phosphate reabsorption may also be an indicator of hypophosphatemia when tubular phosphate reabsorption is >95%, but this is rarely used in routine clinical practice (48,49).

Total serum Alkaline Phosphatase (ALP) does not directly predict bone mineralisation (49) but serial elevation over 800-1000iU may be useful as a screening marker for phosphate deficiency(43,49,50). Parathyroid hormone concentrations are elevated in the presence of inadequate bone mineral accretion, but this is rarely measured in clinical practice (10,51,52). Radiographs, typically done as part of standard clinical care, frequently show osteopenia in preterm infants, but bone mineral loss of 20-40% can occur before this is apparent radiologically (12,51). There are no clear thresholds of these parameters that enable accurate definition or quantification of bone mineral disorders in preterm infants. Furthermore, there is

no evidence that direct assessment of bone mineral density improves functional outcomes in preterm infants.

Conclusions, Recommendations

C1 The lack of a strong evidence base for determining mineral intakes that will optimise functional bone or other outcomes means that recommended reference ranges are wide. **LOE 2+**

C2 Inadequate mineral intakes postnatally result in osteopenia which increases the risks for bone fractures in preterm infants. However, there is no consensus regarding how best to assess BMC in clinical practice and there are few well-designed RCTs to determine optimal mineral intakes. **LOE 3**

C3 Targeting a Ca *retention* of 2.2-2.8mmol (90-110mg)/kg/d is appropriate to minimise mineral bone deficiency and the risk of fractures in preterm infants. The target for P *retention* is 2.2-2.6 mmol (70-80 mg)/kg/d and includes both the functional P requirements as well as the P requirement for bone- and soft tissue accretion. **LOE 3**

C4 Adequate phosphorus intakes are essential to accrete lean tissue (each gram of protein requires approximately 0.35mmol of phosphorus). The provision of PN with low phosphate and unfortified HM increase the risk of both early and late hypophosphatemia. **LOE 2+**

R1: It is recommended to fortify HM early with phosphate followed by early introduction of multi-component breastmilk fortifiers to optimise bone mineral outcomes. **GOR C**

R2 A Ca intake of 3.0-5.0mmol (120-200mg)/kg/d and a P intake of 2.2-3.7mmol (70-115mg P)/kg/d of P are recommended. **GOR C**

R3 The recommended molar calcium to phosphate ratio to ensure adequate Ca retention is ≤1.4 (≤1.8 in mass) **GOR C**

R4 Preterm infants fed artificial milk formula may require higher mineral intakes than those fed human milk. **GPP**

R5 Regular monitoring of P and Ca status is recommended. We do not recommend the routine use of bone imaging or other direct assessments of BMC in clinical practice. **GOR C**

R6 In preterm infants fed fortified human milk or preterm milk formula, a Mg intake of 0.4-0.5 mmol (9 to 12.5 mg)/kg/d is recommended. **GOR C**

Table 1 Summary of the recommendations /kg/d

/kg/day	ESPGHAN (2010)	ESPGHAN (2022)
Ca mmol (mg)	3.0-3.5 (120-140)	3.0-5.0 (120-200
P mmol (mg)	1.9-2.9 (60-90)	2.2-3.7 (70-115)
Mg mmol (mg)	0.3-0.6 (8-15)	0.4-0.4 (9-12)

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Supplementary Digital Content 10 – Trace elements

ESPGHAN Committee of Nutrition position paper on Enteral Nutrition for Preterm Infants 2022

Date: February 2022

Author: Magnus Domellöf

Literature search

1. iron [ti] (preterm OR premature OR "low birth weight") (randomized OR randomised OR meta-analysis) NOT pregnancy
2. zinc [ti] (preterm OR premature OR "low birth weight") (randomized OR randomised OR meta-analysis) NOT pregnancy
3. copper [ti] (preterm OR premature OR "low birth weight") NOT pregnancy
4. selenium [ti] (preterm OR premature OR "low birth weight") NOT pregnancy
5. manganese [ti] (preterm OR premature OR "low birth weight") NOT pregnancy
6. (iodine [ti] OR iodide [ti]) (preterm OR premature OR "low birth weight") (randomized OR randomised OR meta-analysis) NOT pregnancy
7. chromium [ti] (preterm OR premature OR "low birth weight") NOT pregnancy
8. molybdenum [ti] (preterm OR premature OR “low birth weight”) NOT pregnancy

Trace elements

Trace elements, including iron, zinc, copper, selenium, manganese, iodine, chromium and molybdenum are essential for many functions in different organ systems, as well as for normal growth and development. It is important to provide adequate amounts of microminerals in the diet since preterm infants are at increased risk of micromineral deficiencies but also may have adverse effects of excessive intakes.

Iron

Iron is an essential nutrient which is required for heme synthesis, oxygen transport, and many enzyme functions, especially cellular energy metabolism. Preterm infants are at high risk of iron deficiency (ID) due to low iron stores at birth, higher iron requirements due to rapid growth, and in very preterm infants, iron losses due to frequent blood samplings during neonatal intensive care (1). ID should be avoided since it causes anemia and is associated with impaired brain development (2). However, in contrast to most other nutrients, there is no mechanism for iron excretion from the human body and iron is a highly reactive pro-oxidant as well as an important substrate for pathogens, so excessive iron supplementation of infants should be avoided since it may have adverse effects, including increased oxidative stress, risk of infections, poor growth and even poor neurodevelopment (1).

The main public health problem associated with ID in childhood is the risk of poor neurodevelopment and many studies have shown that infants with iron deficiency anemia have long-lasting poor cognitive and behavioural performance up to adolescence. Prevention of iron deficiency is thus of great importance. A Cochrane systematic review from 2012 investigated the effects of enteral iron supplementation in preterm and LBW infants and concluded that infants who receive iron supplementation, compared to un-supplemented infants, have a lower risk developing iron

deficiency anemia but that there is a complete lack of studies investigating long-term benefits in terms of neurodevelopmental outcomes. There was no discernible benefit in exceeding standard doses of iron (i.e. 2-3 mg/kg/day) (3).

Since the Cochrane meta-analysis, there has been only two new published placebo-controlled, randomized clinical trials investigating neurodevelopmental outcomes of iron supplementation of preterm infants. The first was a follow-up of a randomized controlled trial (4) where 385 marginally low birth weight infants (most of which were moderately or late preterm) were randomized to receive 0, 1, or 2 mg/kg/day of iron supplements from 6 weeks to 6 months of age. The infants from the original intervention as well as 95 normal birth weight reference children were assessed at 3½ years and 7 years. At 3½ years, there was no difference in cognitive scores between groups, but children in the placebo group had significantly more behavioral problems: 13% compared to 3% in the two iron supplemented groups ($p = 0.027$) and also compared to 3% in the reference group (5). These effects largely persisted at 7 years and the effects were seen mostly for externalizing behaviour, suggesting that iron supplementation confers long-lasting health benefits for preterm infants (6).

In the second, recently published trial, 66 healthy late preterm infants ($34^{0/7}$-$36^{6/7}$ wk) were randomized to iron supplements (2 mg/kg/day) or placebo from 3 weeks of life to 6 months postconceptial age (7). At 12 months of age, iron supplemented infants had significantly higher developmental quotient as assessed by the Griffiths scale.

A meta-analysis from 2015 including mostly very low birth weight infants, suggests that early start of iron supplementation (2-3 weeks in most studies), vs late (4-8 weeks in most studies) is associated with a lower need for blood transfusions (8). A more recent systematic review in 2019 concluded that iron supplementation with a duration of at least 8 weeks results in reduced risk of iron deficiency and anemia in preterm and low birth weight infants (9). Regarding long-term effects, the review reports a lack of RCTs and suggests further studies. For late or moderately preterm infants with birth weights > 2000 g, iron supplementation lasting until 6 months of life seems to protect from iron deficiency at least up to 12 months of life (10). Iron intakes from 6 months of life is highly dependent on infant diet and iron-rich complementary foods are recommended for all infants from this age (11).

In VLBW infants, the timing of umbilical cord clamping, blood losses and blood transfusions related to neonatal intensive care, as well as erythropoietin treatment, will greatly influence iron status and iron requirements. Delayed umbilical cord clamping increases neonatal iron stores and is associated with a lower mortality, lower risk of intraventricular hemorrhage and lower need for red cell transfusions in preterm infants (12). Phlebotomy losses commonly amount to 6 mg/kg of iron per week (13) and in some cases much more. Each red blood cell transfusion typically adds 8 mg/kg of iron and hepatic iron stores as well as serum ferritin concentrations in preterm infants are highly correlated to the number of blood transfusions received (14). Erythropoietin may reduce the need for red blood cell transfusions in VLBW infants and is used in some centers. However, this treatment greatly increases iron requirements and high doses of oral or parenteral iron are thus recommended as an adjunct to this therapy. Factorial calculations suggest that parenteral iron requirements of VLBW infants would be 0.2-0.37 mg/kg/day (15). However, parenteral iron has been given in much higher doses, up to 3 mg/kg/day, in erythropoietin trials. Some studies have showed that 6 mg/kg/day of enteral iron supplements is as effective as parenteral iron in this context (16). There is insufficient data on safety of high doses of iron in combination with erythropoietin, but a Cochrane meta-analysis has shown that early erythropoietin treatment, which includes supplemental iron, increases the risk of retinopathy of prematurity (17).

Ferritin is a useful biomarker of iron status also in preterm infants but reference intervals are different from older infants and children. Ferritin concentrations lower than 35-40 µg/L indicate iron deficiency while concentrations > 300-350 µg/L indicate iron overload (1, 14, 18). Ferritin is not useful as a biomarker of iron status in patients with ongoing inflammation or liver disease.

Recommendations:

- A daily iron intake of 2-3 mg/kg/day starting at 2 weeks of age is recommended for very low birth weight infants. LOE 1+, RGA
- Infants who receive erythropoietin treatment need a higher dose (up to 6 mg/kg/day) during the treatment period. LOE 1-, RGB
- Since individual iron status in VLBW infants is highly variable, depending on the number of received blood transfusions and blood losses from phlebotomy, it is recommended to follow these infants with repeated measurements of serum ferritin during the hospital stay. LOE 4, RG0
- If ferritin is <35-70 µg/L, the iron dose may be increased up to 3-4 (or maximum 6) mg/kg/day for a limited period. LOE 4, RG0
- Prolonged dietary iron intakes of >3 mg/kg/day should be avoided in most cases because of possible adverse effects. LOE 1-, RGB
- If ferritin is >300 µg/L, which in the absence of ongoing inflammation and liver disease usually is the result of multiple blood transfusions, iron supplementation and fortification should be discontinued until serum ferritin falls below this level. LOE 4, RG0
- Iron supplements or intake of iron-fortified formula in the recommended doses should be continued after discharge, until 6-12 months of age. LOE 4, RG0
- Like all infants, preterm infants should receive iron-rich complementary foods from 6 months of age. LOE 1+, RGA
- Delayed umbilical cord clamping, whenever feasible, is recommended for all preterm infants. LOE 1++, RGA

Zinc

Zinc is an essential trace element which plays an important role in growth and tissue differentiation. Zinc deficiency in children and preterm infants is associated with stunted growth, increased risk for infections, skin rash, and possibly poor neurodevelopment (19). In contrast to iron and copper, zinc does not have a pro-oxidant effect and adverse effects of excess zinc intakes are rarely reported, with the exception of a negative effect on copper absorption with high zinc intakes.

Using a factorial method based on fetal accretion, the requirement for retained zinc has been estimated to be approximately 400 µg/kg/d at 1500-2500 g (30-32 weeks of gestation) (20). Based on data from 14 metabolic balance studies in preterm infants, it has been calculated that an enteral zinc intake of at least 2.0-2.25 mg/kg/d is required to achieve this zinc retention (21). Theoretically, zinc requirements are higher (500-600 µg/kg/day) in extremely preterm infants, due to their faster growth rates (20),and intakes up to 3 mg/kg/d have been suggested (22).

Even though the concentration of zinc in colostrum is high (up to 2.8 mg/L(23)), human milk alone does not cover zinc requirements in infants with a birth weight of less than 1500-2000 g, since it would only correspond to an intake of 0.4 mg/kg/day from 150 ml/kg/day of breast milk.

There have been few clinical trials of different zinc intakes in preterm infants, suggesting that an intake of at least 1.4-2 mg/kg/d is needed in order to achieve optimal growth in preterm infants (24, 25).

In a double-blinded study from 2013, Terrin randomized 193 preterm infants (average 28 weeks) to receive multivitamin drops with or without zinc from 7 days until discharge or 42 weeks of postconceptional age (26). Total zinc intake was 10.3 mg/day in the zinc group and 1.3 mg/day in the placebo group, corresponding to an average of 6.6 and 0.9 mg/kg/day. Neonatal morbidities (composite of sepsis, BPD, PVL and ROP) were significantly lower in the zinc group (27% vs 42%, p=0.03). The occurrence of NEC was significantly higher in the placebo group (6.3% vs 0%, p=0.014), as was mortality (RR 2.37; CI 1.08-5.18, p=0.006) but no growth effects were observed. This study is potentially very interesting and suggests that higher enteral doses of zinc are safe and may confer health benefits. However, as pointed out in an editorial, (27) it is important to distinguish between supplementation aiming to cover nutritional requirements and supplementation using pharmacological doses. In this case, the dose of zinc supplement used was much higher than the estimated requirements. Further studies are thus needed to ensure that high dose zinc supplementation > 3 mg/kg/d is safe and effective.

Two recent meta-analyses suggests that zinc supplementation improves weight gain and linear growth in preterm infants and may decrease mortality (Staub E, Cochrane Database Syst Rev 2021, Alshaikh B, J Perinatol 2022; 42: 430).

Very preterm infants can develop symptomatic zinc deficiency with acrodermatitis enterohepatica and/or poor growth, especially those infants who have an enterostomy after NEC sugery (Wulf K et al. Klin Pädiatr 2013; 225: 13).

<u>Recommendations</u>

- We recommend an enteral zinc intake of 2-3 mg/kg/d, based on the most recent randomized, controlled trial as well as on factorial calculations. LOE 4, RG0
- Measurement of serum zinc should be considered in preterm infants with dermatitis or poor growth and low alkaline phosphatase level, especially if they have excessive GI fluid losses. LOE 4, RG0

Copper

Copper is an essential nutrient with multiple functions as part of enzymes, including antioxidant enzymes, e.g. copper/zinc superoxide dismutase (CuZn-SOD).

Severe copper deficiency is a rare condition associated with anemia, neutropenia, thrombocytopenia and osteoporosis (28, 29). However little is known about the prevalence and possible health impact of marginal copper deficiency.

Low birth weight is a risk factor for copper deficiency (20, 28). Copper deficiency was reported relatively frequently in preterm infants in the 1970s and 1980s (30). However, most of these case reports were of infants who received copper-free long-term parenteral nutrition and there are no similar recent reports.

The intrauterine accretion rate of copper is approximately 50 µg/kg/d (31). Fractional copper absorption is about 60% from breast milk and generally lower from formula. Copper homeostasis is maintained by regulation of both intestinal absorption as well as biliary excretion.

Using a factorial method based on fetal accretion, the required net retention of copper in preterm infants has been estimated to 30 µg/kg/d, corresponding to an enteral requirement of 100 µg/kg/d (20).

The copper content of human milk declines from 600 µg/L during the first week of lactation (800 µg/L in preterm milk) to 220 µg/L by 5 months (32).

High doses of copper can damage the liver, kidneys and central nervous system (33).

A calculation, based on nine published studies of copper balance in preterm infants, has suggested that enteral copper requirements are around 210-232 µg/kg/d if zinc intake is 2-2.25 mg/kg/d, in order to achieve a net copper retention of 30 µg/kg/d (21).

There are very few clinical trials of different copper intakes in preterm infants. Enteral feeding of 41-89 µg/kg/d of copper in preterm infants has been associated with copper deficiency (34). Tyrala showed no clear benefit of a copper intake of 294 µg/kg/d compared to 121 µg/kg/d in preterm infants as assessed by copper balance, serum copper and ceruloplasmin (35). However, no adverse effects were observed in the high copper group. Zinc intakes in those infants were 2.0-2.3 mg/kg/d.

The ESPGHAN 2010 recommendations suggest 100-130 mcg/kg/d (36). However, to ensure adequate intestinal absorption, the zinc to copper molar ratio should not exceed 20 (31).Thus, since the zinc intake has been increased (see above), a higher copper intake is needed.

<u>Recommendations</u>

We recommend an enteral copper intake of 120-230 µg/kg/d. The lower value is based on the Tyrala study and the higher is based on the calculation of copper retention noted above. LOE 4, RG0

The zinc to copper molar ratio in infant formulas should not exceed 20. LOE 4, RG0

Selenium

Selenium is an essential trace element which plays an important role as a component of selenoproteins, including glutathione peroxidases, antioxidant enzymes which prevent free radical formation and oxygen toxicity, as well as deiodinases, which are required for the metabolism of thyroid hormones.

Dietary selenium is highly bioavailable. A stable isotope study showed that 60-80% of selenium in formula was absorbed in preterm infants (37). A balance study has shown that net absorption of selenium from breast milk was 77% in extremely low birth weight infants (38).

Selenium status is usually assessed by measuring serum or plasma concentrations of selenium or the activity of glutathione peroxidase in plasma or red blood cells. In preterm infants, glutathione peroxidase activity is not a useful marker of selenium status since it is affected also by immaturity and oxygen exposure (38).

Selenium concentrations in breast milk are significantly associated with maternal selenium intake, and most often range between 6-28 µg/L in the USA and Europe, with an average of around 15-18 µg/L (20, 38). Based on 15 µg/L in breast milk and an intake of 150 ml/kg/d, this corresponds to an intake of 2.3 µg/kg/d.

Children receiving long-term parenteral nutrition without selenium supplementation have been reported to develop low plasma selenium, erythrocyte macrocytosis, loss of hair and skin pigmentation and muscle weakness, which responded to selenium supplementation (39).

Preterm infants are at high risk for oxidative stress related disorders, including bronchopulmonary dysplasia (BPD), retinopathy of prematurity and cerebral white matter injury. Selenium deficiency has been associated with increased susceptibility to oxidative lung injury in rats. Several studies have demonstrated that plasma selenium concentrations decrease during the first weeks of life in preterm infants, suggesting possible selenium insufficiency (38). Furthermore, several studies have shown an association between low plasma selenium levels and BPD in preterm infants (40).

Excessive selenium exposure in adults leads to selenosis, with symptoms including headache, memory difficulties, alopecia and GI symptoms (41). There have been no reports of adverse effects caused by excessive selenium intakes in infancy.

A few studies have shown that selenium intakes of 3-5 µg/kg/d result in improved selenium status in preterms (42).

Darlow et al performed a randomized, controlled, blinded trial of selenium supplementation in 534 VLBW infants in New Zealand, a country in which soil and food are low in selenium (43). The supplemental dose was 5 µg/kg/d enterally (resulting in 7 µg/day when considering selenium from breast milk) or 7 µg/kg/d parenterally. Supplements were given from 4 days of life and continued until 36 weeks postmenstrual age or discharge. A significant effect was observed on plasma selenium concentrations, which reached similar levels as healthy, term infants. However, no significant effect was observed on oxygen dependency at 28 days of age, which was the primary outcome. Among the secondary outcomes, no effects were observed except that, among infants who were exposed to antenatal steroids (n=403), selenium supplementation was associated with significantly fewer sepsis episodes: relative risk 0.66 (95% CI 0.46-0.86). No adverse effects were observed in this study.

Aggarwal et al (2016) performed a blinded RCT of selenium supplementation in 90 very low birth weight and very preterm infants (< 1500 g, < 32 wk, average 1464 g) in India (44). Se (10 µg/day) or placebo was given orally daily from birth to 28 days of life. A large proportion of these infants were Se deficient at birth with mean Se level of 31 µg/L. Se supplementation increased serum Se levels significantly on day 28 (64 vs 41 µg/L, p<.01). Furthermore, the incidence of late onset sepsis was significantly lower in the Se group (15.6% vs 48.9%, p=0.001).

<u>Recommendations:</u>

We recommend an enteral selenium intake of 7-10 µg/kg/d, which in the studies by Darlow and Aggarwal (see above) has been shown to result in Se status similar to term infants and possibly a reduced risk of sepsis. LOE 1+, RGA

Manganese

Manganese is an essential trace element which is a cofactor for many enzymes. Manganese dependent superoxide dismutase (SOD2 or Mn-SOD) is important for cellular defense against free oxygen radicals. Reduced activity of this enzyme has been shown in manganese deficient animals, and mice lacking SOD2 die after a few days due to massive oxidative stress. Manganese dependent enzymes are also important for bone formation and manganese deficiency in animals leads to abnormal skeletal development.

There are very few reports of manganese deficiency in humans. The most comprehensive description is from a paper in which experimental manganese deficiency was induced in seven adult subjects (45). In that study, a manganese deficient diet for 39 days resulted in negative manganese balance,

biochemical evidence of bone resorption and clinical scaly dermatitis. There has been only one clinical report of manganese deficiency, in a 4-year-old girl with short bowel syndrome who since the neonatal period had received total parenteral nutrition which was deficient in manganese. She had short stature and brittle bones as well as a low serum manganese concentration. Bone density and longitudinal growth improved after manganese supplementation (46).

In contrast, there have been many reports of possible adverse effects of high manganese intakes. The main adverse effect of manganese is a neurotoxic effect, called manganism, which can occur with excessive occupational exposure to airborne manganese. Several studies have shown an association between dietary manganese exposure, blood magnesium concentrations and poor cognitive development in children (47). Manganese deposition in the brain can be detected with magnetic resonance imaging and recent studies have suggested that commercially available manganese containing parenteral trace element supplements can result in pathological manganese deposits in basal ganglia. This has been described in adult patients on long term total parenteral nutrition (48).

The main regulation of manganese homeostasis occurs at the absorption step. Intestinal absorption of manganese is usually assumed to be low. In a study performed in adults, fractional absorption of manganese was about 8% from human milk, 2% from cow's milk, 0.7% from soy formula and 2-6% from cow's milk based infant formulas with different iron contents (49). However, studies in rodents have shown that manganese absorption is higher in the postnatal period than later in life and it has been suggested that this is true also for humans. In rat pups, manganese absorption is 85% from preterm infant formula (50). The actual fractional absorption of manganese in preterm infants is unknown and may be higher than usually assumed.

The main excretory route for manganese is via the bile and a small amount is lost in urine. In conditions with poor bile excretion, e.g. parenteral nutrition associated cholestasis, manganese retention is increased.

Average manganese concentrations in breast milk range from 0.8 µg/L to 30 µg/L and in European studies, reported median concentrations vary between 3.2-11.8 µg/L (51). There is no clear correlation between maternal dietary manganese intake and breast milk manganese concentration. Assuming a breast milk manganese content of 6.5 µg/L and a milk intake of 150 ml/kg/day, a breastfed infant would receive 1.0 µg/kg/day of manganese.

Manganese is sometimes added as a supplement but may also be present as a contaminant in preterm formulas. Current European preterm formulas contain 5-13 µg of manganese per 100 mL, corresponding to an intake of 7.5-20 µg/kg/day.

Similar to their enteral counterparts, parenteral nutrition products can also be contaminated with manganese. Manganese excretion via bile is low, especially in long term TPN with cholestasis, so retention approaches 100%.

Based on fetal tissue concentration data by Casey et al, (52) the intrauterine accretion rate of manganese in a 1 kg fetus would be about 7 µg/kg/day.

There are no published intervention studies or observational studies comparing different doses of enteral or parenteral manganese in preterm infants.

Previous recommendations for enteral manganese intakes in preterm infants range from 0.7-7.5 µg/kg/d (53) and 6.3-25 µg/kg/d (ESPGHAN 2010) (36).

Conclusions

It seems to be prudent to recommend a lower intake than the estimated fetal accretion rate since manganese deficiency has never been described in a preterm infant and there is legitimate concern for manganese toxicity. LOE 4

Recommendations

Based on the average breast milk manganese content and the lower range of manganese in current preterm formulas, an enteral manganese intake of 1-15 µg/kg/d can be recommended. LOE 4, RG0

Iodine

Iodine is a trace element which is an integral part of the thyroid hormones thyroxine (T4) and triiodothyronine (T3), which are essential for regulating and stimulating metabolism, temperature control and normal growth and development.

Iodine deficiency results in hypothyroidism, thyroid enlargement (goiter), mental retardation (cretinism), poor growth and increased neonatal and infant mortality.

In utero iodine deficiency causes irreversible damage to the developing fetal brain. Iodine deficiency in pregnant mothers, which occurs in areas where iodine deficiency is endemic, results in cretinism in the newborn. Cretinism is characterized by severe mental retardation, deafness, strabismus and motor spasticity. There is also some evidence that even moderate or mild iodine deficiency in pregnant women increases the risk of neurodevelopmental deficits in the offspring (54).

Salt iodisation programmes have meant that iodine deficiency is now rare in the US, Canada, Australia and also in many European countries. However, iodine deficiency is still relatively common in some European countries, e.g. France and Belgium, due to a low proportion of households using iodized salt (55).

Excessive iodine has a well-known inhibitory effect on thyroid hormone synthesis and release, also resulting in hypothyroidism. In hospitals where iodine is routinely used as a disinfectant, preterm infants can be exposed to high doses of topical iodine, which is absorbed through the skin and can result in mild or severe hypothyreosis (56). Hypothyroidism has also been described in a breast-fed preterm infant whose mother was exposed to topical iodine disenfectants (57). We therefore recommend to avoid the use of iodine containing antiseptics during care of preterm infants and their lactating mothers.

Iodine is effectively absorbed in the intestine; in healthy adults the absorption is >90 % and it is assumed to be high in term and preterm infants. Excretion of iodine occurs through the urine.

Urinary iodine in spot urine samples is often used to assess iodine status at a population level but it cannot be used to determine individual iodine status due to variation in urine production and hydration status.

In the newborn, thyroid stimulating hormone (TSH) in serum is a sensitive marker of iodine status. Serum T4 is often used, even though it is a less sensitive biomarker of iodine nutrition status. It is well documented that many preterm infants have low serum T4 concentrations, especially those with a very low gestational age at birth and severe illness. It is unclear whether this transient hypothyreosis is caused by developmental changes or related to iodine nutritional status (58).

Average iodine concentration in European mothers' breast milk is 70-90 µg/L, corresponding to an intake of 12 µg/kg/d, assuming an intake of 150 ml/kg/d. Breast milk iodine concentrations in earlier

US studies gave concentrations that were about twice as high but a recent, small study showed iodine content in US breast milks to be 33-117 µg/L (59).

Based on urinary iodine in healthy, iodine-sufficient newborns, it has been estimated that the mean daily iodine intake during the first week of life is 30-50 µg/day, suggesting that the iodine requirement in term newborns is 8-10 µg/kg/d (60).

A 3-day metabolic balance study of 29 preterm infants and 20 full term controls in Belgium showed that 40% of the preterm infants had a negative iodine balance even when iodine intake was 17-25 µg/100 kcal (61).

Roghan randomized 121 preterm infants with an average birth weight of 1.4 kg to receive preterm formula with standard (68 µg/L) or increased (272 µg/L) iodine concentrations, resulting in iodine intakes of 10-13 µg/kg/day vs 32-52 µg/kg/day (58). No significant difference was observed in TSH, T3, free T4 or total T4 up to term age, suggesting that the lower intake may be sufficient. Long-term effects on neurodevelopment were not assessed in this study, but were performed in the UK, where iodine deficiency is rare in the general population (36). Iodine-containing topical antiseptics were not used on the infants in this study.

Williams et al randomized 1273 preterm infants (< 31 wk) to iodine supplementation (30 µg/kg/day) or placebo from 1 day after birth to 34 postmenstrual weeks (62). The primary outcome was neurodevelopmental status at 2 years. There were no significant differences between intervention groups in Bayley score (cognitive, motor or language). Infants supplemented with iodine had higher TSH levels (but not T4 or TBG) than the placebo group. In a prespecified subgroup analysis of hypothyroxinemic infants (n=288), iodine supplemented infants had significantly higher scores in the Bayley Language Composite Score and its subtest score Receptive Communication. No adverse effects were observed. The iodine intake in the placebo group was not measured but was assumed to be low (about 1-3 µg/kg/day).

A Cochrane meta-analysis from 2019 has concluded that there is currently no convincing evidence of beneficial clinical effects of iodine supplementation of preterm infants (63).

The previous recommendations according to ESPGHAN 2010 was 11-55 µg/kg/d (36).

There have been reports of clinical cases of iodine deficiency in patients receiving long term parenteral nutrition. A preterm infant with short bowel syndrome, was diagnosed with hypothyroidism due to iodine deficiency at 11 months of age. The infant was being fed almost exclusively parenteral nutrition and was receiving only chlorhexidine-based skin antisepsis after 3 months of age (64).

<u>Conclusions</u>

There is not enough conclusive evidence to change the previous recommendation. LOE 4.

<u>Recommendations:</u>

An enteral iodine intake of 11-55 µg/kg/d is recommended. LOE 4, RG0

Chromium

Chromium is considered to be an essential nutrient, even though this has been challenged (65). Its proposed main role is to potentiate the action of insulin and thereby improve glucose tolerance through a mechanism which has not yet been elucidated.

There have been no clinical reports of chromium deficiency in term or preterm infants, or any reports of adverse effects of excessive chromium intakes from enteral or parenteral nutrition. There are

isolated case reports of adult patients with long-term parenteral nutrition who have developed chromium deficiency but also some reports of high serum chromium concentrations in this patient group since parenteral nutrition solutions can be contaminated with chromium (66).

There is large variation between different studies with regard to reported chromium concentrations in breast milk, with mean concentrations ranging between 180-1000 ng/L. Even higher concentrations have been reported, but these may be caused by contamination of samples. In previous reviews, a chromium concentration or 250-500 ng/L in breast milk has been assumed (20, 53).

Chromium concentrations in preterm formulas are generally much higher than in breast milk and have been reported to range between 7.5-22 µg/L (20).

Chromium is poorly bioavailable with intestinal absorption in adults being <2% (67). Urinary excretion is proportional to dietary intake (67).

There is some evidence from an observational study that early parenteral administration of chromium (0.2 µg/kg/day) improves glucose tolerance in newborns, especially very low birth weight infants (68).

Conclusions

There is insufficient scientific data upon which to base recommendations for chromium intake in preterm infants LOE 4.

There is no reason to change previous recommendations for enteral chromium intake in preterm infants of 0.1-2.25 µg/kg/d (53) or 0.03-1.23 µg /kg/d (36), which were based on the concentrations of chromium in breast milk and preterm formulas respectively. LOE 4

Recommendations

A chromium supply in the range of 0.03-2.25 µg/kg/d can be recommended as prudent. LOE 4, GPP.

Molybdenum

Molybdenum is an essential cofactor for several enzymes involved in oxidation and reduction, including xanthine oxidase, sulfite oxidase and aldehyde oxidase.

There is only a single report of molybdenum deficiency in humans; this was an adult patient receiving long-term parenteral nutrition without molybdenum who developed neurological symptoms and biochemical findings suggesting impaired metabolism of sulphur-containing amino acids, purines and pyrimidines. The biochemical abnormalities in this case were normalized after administration of molybdenum. There are no reports of molybdenum deficiency in children, including preterm infants.

Molybdenum has a very high bioavailability and > 90% of dietary molybdenum is absorbed in infants (69). Molybdenum homeostasis is regulated primarily by urinary excretion and a large proportion of absorbed molybdenum is excreted in the urine.

Balance studies in adults have suggested a minimum requirement of 25 µg/d, corresponding to 0.4 µg/kg/d (70).

Molybdenum concentrations in breast milk vary between mothers but the mean concentration is regarded to be approximately 2 µg/L (71), corresponding to an intake of 0.3 µg/kg/d, assuming an intake of 150 mL/kg/d. Molybdenum concentrations in preterm formulas are generally much higher than in breast milk, often between 20-30 µg/L (20), corresponding to an intake of 3-4.5 µg/kg/d.

There have been no randomized trials of different molybdenum intakes in preterm infants. Based on an observational balance study in 16 VLBW infants, Friel et al suggested that an enteral intake of 4-6 µg/kg/d or a parenteral intake of 1 µg/kg/d would be adequate (72). A stable isotope metabolic balance study in preterm infants by Sievers et al suggested that an intake of 3 µg/kg/d or lower may be sufficient (73).

Conclusions

There is no convincing evidence to change the previous recommendations. LOE 4.

Recommendations:

An enteral molybdenum intake of 0.3-5 µg/kg/d is recommended. RG0.

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Supplementary Digital Content no. 15

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Water soluble vitamins (February 2022)

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Water soluble vitamins are essential nutrients for whole body function and homeostasis. In newborn infants, the total daily vitamin requirements are often lower than those of older children and adults, but the requirement per kg body weight, or per calorific intake is usually significantly higher.

A literature search was carried out in March 2020 using Pubmed to identify relevant publications. Search terms included: [(preterm* OR premature*) AND (vitamin* OR thiamin* OR riboflavin* OR niacin* OR pantothen* OR pyridox* OR folic OR folate OR ascorb* OR biotin*) AND (infant* OR neonat* OR newborn* OR toddler*). Primary search was restricted to studies published in English, since 1990. From the 1,662 titles retrieved, 35 studies were selected following screening of study title and abstract content. Additional literature, including key research published prior to 1990, was identified from articles' reference lists and the previous ESPGHAN 2010 commentary[1].

In brief, there is major lack of nutrient balance or isotope studies to inform the development of water-soluble vitamin requirements for preterm infants. Except for few key vitamins, there is also limited evidence from supplementation studies aiming to improve clinical outcomes in preterm infants. Most of the available evidence reported here originates from research using cross-sectional design and/or retrospective analysis of patient medical charts. Such studies describe the vitamin intakes of preterm infants who have a nutrient biomarker in blood within certain reference intervals or who do not present any overt clinical symptoms of deficiency.

The approach we used for developing our current position on water soluble vitamin intakes considered the following principles:

1. Vitamin dosage from supplementation studies demonstrating improvement of clinical outcomes in preterm infants: while these studies are not indicative of minimum requirements, they are important in providing information on intakes which are likely to optimise disease outcomes.
2. Vitamin intakes from preterm infants who do not manifest deficiency symptoms, or intakes which prevent deterioration, or correct a nutrient biomarker in blood: an important consideration here is that these children are predominantly fed with infant milk formula

designed specifically for preterm infants which often contain augmented amounts of vitamins and other micronutrients.

3. The daily dietary recommendation of the European Food Safety Authority for term infants (< 6 month): these are based on the concentration of vitamins in mature human milk and assume a milk intake of 0.8L/day by healthy exclusively breastfed term infants. Considering the stringent methodology applied by the EFSA Panel to estimate vitamin milk content in breastmilk and generate dietary reference values, we cautiously propose their conditional use for nutrients for which we have evidence that requirements either do not differ to healthy term infants, or for vitamins for which we have no other available evidence. Theoretically, the EFSA daily recommendations may underestimate the increased requirements of the rapidly growing sick preterm infant. However, because preterm infants are much smaller than term infants, we considered that daily recommendations for term infants are likely to be adequate for preterm infants as the weight difference results in an approximate a 3-5 fold higher intake per kg body weight. In addition to the EFSA recommendations for daily intake we have also calculated weight (per kg) standardised recommendations. To do this, we expressed the EFSA daily recommendations per kg of body weight using a reference weight of 6.1 kg for a 3 month infant[2].
4. Using the EFSA best estimate of vitamin content in mature breastmilk, an average calorific value of 70 kcal/100 ml for mature breastmilk and a minimal energy intake in preterm infants of 115 kcal/kg/day[3]. Vitamin recommendations per kg of body weight was calculated using the formula: "*Vitamin recommendation [unit per kg/d]= (Nutrient concentration in breastmilk*1.15)/7"*
5. Vitamin composition of routinely available 'preterm formula' with the underlying hypothesis that use of these formulas in routine practice has not been associated with clinical deficiencies; hence these formulas are assumed to supply at least the minimum requirements. However, we are also mindful that these concentrations are likely to be inflated, particularly for preterm formula, as their design and composition adhered to the 'concept of prudence' i.e erring on the side of giving more rather than less. This is clearly displayed in Table 1 where the vitamin ranges in infant formula are compared against the weight standardised EFSA recommendations. In order to express the concentration of vitamins in preterm formulas from units per 100 kcal to units expression per kg, we assumed a minimal energy intake for preterm infants of 115 kcal/kg/day[3].

Thiamine (B1)

Thiamine (B1) acts as co-enzyme in reactions related to energy metabolism and intakes are therefore critically related to overall energy intakes. In human milk, thiamine is mainly present

as thiamine monophosphate and free thiamine[4]. Maternal thiamine intake does not influence thiamine concentration in breast milk, except for women who are deficient[5]. The concentration of thiamine is higher in term compared to preterm human milk and increases progressively with duration of lactation, and more so in full than preterm milk[6]. Based on the EFSA Panel report, the average concentration of thiamine in mature breast milk is estimated at 0.18 mg/L[2] based upon a recommendation of 42 µg/100 kcal for healthy term infants at 0-6 months has been made. There is a decline in whole blood thiamine disphosphate (TDP) with postnatal age, and low weight at birth was related to low vitamin TDP concentrations. No significant correlation between gestational age at birth with TDP in whole blood was observed although on average TDP levels are higher in late (32-37 weeks) compared to very (28-32 weeks) or extremely preterm infants (<28 weeks)[7]. In the same study, intake of thiamine did not correlate with blood levels and it is noteworthy that infants receiving only breast milk were not at risk for low TDP concentrations[7].

In previous research, mean thiamine intake of 510 µg/kg/d, from supplemental/total parenteral nutrition, and 254 µg/kg/d from enteral feeding did not influence erythrocyte transketolase activity, suggesting thiamine saturation[8]. In addition, concentrations remained similar to those at birth suggesting adequate provision of vitamin B1 during hospitalisation[8]. In an earlier study by the same group, the authors estimated B1 balance calculated from intake minus urinary output, in infants receiving enteral nutrition that provided 247 µg/100 kcal[9]. No infants had abnormal levels of transketolase activity, and the authors estimated requirements at 225 µg/100 kcal. Thiamine content in infant formulas varies between 120 to 250 µg/100 kcal providing between 140 to 290 µg/kg when minimum energy intake is 115 kcal/kg (Table 1).

Considering the available evidence we propose an intake of 140-290 µg/kg/d based on the content of infant formula milk [LOE 3, RG0] . Considering the findings by Kroner et al[7] it is also possible that B1 content in breastmilk or the EFSA daily recommendations for B1 (42 µg/100 kcal or 46 µg/kg) might also be adequate to maintain optimal levels of B1 in preterm infants[2] (Table 1).

Pantothenic acid

Pantothenic acid is a component of coenzyme A and acyl-carrier proteins, and serves in acyl-group activation and transfer, which is essential for fatty acid synthesis and oxidative degradation of fatty acids and amino acids[2]. There is significant variation in the content of pantothenic acid in breast milk from mothers delivering preterm or term infants[6]. The concentration of pantothenic acid in mature human breast milk correlates with maternal intake and urinary excretion of the vitamin[10]. According to the EFSA Panel report, the mean

concentration of pantothenic acid in mature human milk is approximately 2.5 mg/L. For healthy term infants 0-6 months, EFSA estimated pantothenic acid requirements at 2 mg/d. Assuming a reference weight of 6.1 kg for a 3-month healthy term child, the EFSA recommendation for pantothenic acid per kg of body weight is estimated at 0.33 mg/kg/d. Using the average concentration of pantothenic acid in breastmilk, a calorific value of breastmilk of 70 kcal/100 ml and a minimum energy intake of 115 kcal/kg, recommendations for pantothenic acid intakes in preterm infants are estimated at 0.41 mg/kg. The range of pantothenic acid content in preterm infant formula varies from 0.55 to 1.9 mg/100 kcal which will provide 0.63 to 2.2 mg/kg at a minimum energy intake of 115 kcal/kg (Table 1). There is currently no published evidence to determine precise requirements for preterm infants. **Considering the available evidence we propose an intake of 0.6 to 2.2 mg/kg based on the content of infant formula milk [LOE 3, RG0].**

Biotin

Biotin is co-factor for enzymes which play critical roles in the synthesis of fatty acids, the catabolism of branched-chain amino acids and gluconeogenesis, including acetyl-CoA carboxylase and propionyl-CoA carboxylase[2,4]. Like pantothenic acid, biotin concentration varies considerably in breast milk from mothers of term (0.21 to 5.33 ng/ml) or preterm infants (0.22 to 5.37 ng/ml)[6]. According to the EFSA Panel report, mean concentrations of biotin in mature human milk is approximately 5 µg/L; which is equivalent to estimating daily biotin intake recommendations for healthy term infants (< 6 months) at 4 µg/day. For a reference weight of 6.1 kg for a 3-month healthy term child, the EFSA recommendations for biotin per kg of body weight would be 0.66 µg/kg/d. Using the average concentration of biotin in breastmilk, a calorific value of breastmilk of 70 kcal/100 ml and a minimum energy intake of 115 kcal/kg, recommendations for biotin acid are estimated to be 0.82 µg/kg. The range of biotin concentration in preterm infant formulas varies considerably from 3 to 37 µg/100 kcal which will provide 3.5 to 43 µg/kg at a minimum energy intake of 115 kcal/kg, considerably higher than the amount provided from breast milk (Table 1). There is no published evidence to allow estimation of biotin requirements for preterm infants. **We recommend using the lowest concentration of biotin that would be provided using preterm formula and the upper level in the previous recommendations by ESPGHAN[1]. We propose an intake of 3.5 to 15 µg/kg based on the content of infant formula milk [LOE 3, RG0].**

Niacin

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Niacin is the precursor of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP+), which are involved in oxidation/reduction reactions and associated with both catabolic and anabolic processes[2,4]. Niacin concentration in human breast milk increases progressively with duration of lactation in mothers of both preterm and full term infants[6]. According to the EFSA Panel report, the mean concentrations of niacin in mature human milk is approximately 2100 µg/L and the daily recommendations for a healthy term infant (<6 months) are set at 1700 µg/d. Since the EFSA Panel used a reference weight of 6.1 kg for a 3-month reference child, the daily EFSA recommendation standardised per kg of body weight for infants is equivalent to 279 µg/kg/d. Using the average concentration of niacin in breastmilk, a calorific value of breastmilk of 70 kcal/100 ml and a minimum energy intake of 115 kcal/kg, recommendations for niacin are estimated to be 345 µg/kg/d. In preterm infant formula, niacin content varies from 1000 to 5000 µg/100 kcal providing 1150 to 5750 µg/kg at a minimum energy intake of 115 kcal/kg (Table 1). There is no published literature to allow precise estimation of requirements of niacin for preterm infants. EFSA daily recommendations overlap with the content of niacin in preterm infant formulas and the previous recommendations proposed by ESPGHAN[1] (Table 1). **Considering the available evidence we propose an intake of 1100 to 5700 µg/kg based on the content of infant formula milk [LOE 3, RG0].**

Ascorbic acid (vitamin C)

Vitamin C (L-ascorbic acid) is an enzyme cofactor in biochemical reactions catalysed by oxygenases. Vitamin C plays an important role in the biosynthesis of collagen, is essential for the synthesis of carnitine and catecholamines, and is also involved in the metabolism of cholesterol to bile acids[2,4]. Vitamin C concentration in human milk reflects maternal intake[11]. According to EFSA, the mean human milk vitamin C concentrations are in the range of 35-90 mg/L with a midpoint concentration of 50 mg/L. An average intake of 28 mg/kg/d was associated with adequate plasma vitamin C concentrations[8]. In a double-blinded RCT, low or high vitamin C supplementation and corresponding plasma levels showed no significant benefits or harmful effects on oxygen requirements and retinopathy of prematurity in preterm infants[12]. A higher dosage of parenteral nutrition administered vitamin C (100 mg/kg) prevented the immediate postnatal drop in vitamin C but no differences in haemolysis, renal function, rate of infection, bronchopulmonary dysplasia, or intraventricular haemorrhage were seen between this group and a control group receiving saline[13]. Likewise, oral supplementation of 50 mg/kg of vitamin C was not associated with evidence of increased erythrocyte destruction, hyperbilirubinemia, or other morbidity[14]. The EFSA recommended for term infants aged 0-6 months an intake of 40 mg/day or 6.6 mg/kg using a weight of 6.1 kg for a 3-month

reference child. Using the average concentration of vitamin C in breastmilk, a calorific value of breastmilk of 70 kcal/100 ml and minimum energy intakes of 115 kcal/kg, recommendations for vitamin C are estimated to be 8.2 mg/kg/d. The concentration range of vitamin C in preterm infant formula varies from 15 to 37 mg/100 kcal which will provide 17 to 43 mg/kg at minimum energy intakes of 115 kcal/kg (Table 1). **Considering the available evidence we propose an intake of 17 to 43 mg/kg based on the content of infant formula milk [LOE 3, RG0].**

Riboflavin (B2)

Riboflavin (B2) is the integral part of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) that act as the cofactors of a variety of flavoprotein enzymes such as glutathione reductase or pyridoxamine phosphate oxidase[2,4]. FMN and FAD act as proton carriers in redox reactions involved in energy metabolism, metabolic pathways and formation of some vitamins and coenzymes[2,4]. There are no differences in red cell measurements of glutathione peroxidase activity from umbilical cord blood of preterm, full-term and full-term small for gestational age infants potentially suggesting similar requirements[15]. Riboflavin (B2) concentrations in breast milk are related to maternal B2 intake and supplementation[16,17]. Mean B2 concentrations were similar in term and preterm breastmilk and showed no significant change with advancing lactation[6]. According to EFSA the average concentration of B2 in mature breast milk of unsupplemented women is about 364 µg/L and recommendations for healthy full term infants (<6 months) are 290 µg/d[2] or 47.5 µg/kg/d using a reference weight of 6.1 kg for a 3-month reference child. Using the average concentration of B2 in breastmilk, a calorific value of breastmilk of 70 kcal/100 ml and minimum energy requirements of 115 kcal/kg, recommendations for B2 are estimated 60 µg/kg/d. Riboflavin content in preterm infant formula varies between 150 to 620 µg/100 kcal providing 170 to 710 µg/kg at a minimum energy intake of 115 kcal/kg (Table 1). In the study by Levy, an enteral intake of 200 to 270 µg/kg was adequate to maintain the activity of glutathione peroxidase, a functional marker of B2, in preterm infants[18]. Infants with an intake of B2 of 370 or 620 µg/kg did not differ in erythrocyte glutathione reductase activity and none had functional results indicative of deficiency suggesting adequacy within this range. Similar results were presented in another study by the same group in which mean intakes of 430 µg/kg/ to 720 µg/kg exceeded mean daily urinary excretions, resulting in net positive balance[18]. **We recommend an intake of riboflavin of 200 to 430 µg/kg/d [LOE 3, RG0].** The daily recommendations made by the EFSA Panel may also be adequate considering the lack of evidence of differences in requirements between preterm and full-term infants (Table 1).

Pyridoxine

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Vitamin B6 is a generic descriptor for a group of derivatives including pyridoxine, pyridoxal and pyridoxamine, and their respective phosphorylated forms, pyridoxine 5'-phosphate, pyridoxal 5'-phosphate and pyridoxamine 5'-phosphate. Their phosphorylated forms act as cofactors of enzymes involved in amino acid metabolism, one-carbon reactions, glycogenolysis and gluconeogenesis, haem synthesis, niacin formation, and also in lipid metabolism, neurotransmitter synthesis and hormone action[2,4]. Vitamin B6 concentration of human milk is markedly influenced by maternal intake and use of supplements[19,20]. Breastmilk from mothers delivering preterm is considerably lower in total vitamin B6 concentration than that of term mothers, but in both cases this increases progressively during lactation[6]. Based on the ESFA report the mean concentrations of vitamin B6 in mature breast milk is 130 μg/L and daily recommendations for healthy term infants (<6 months) are set to 100 μg/day or 16.4 μg/kg using a reference weight of 6.1 kg for a 3-month reference child. Using the average concentration of B6 in breastmilk, a calorific value of breastmilk of 70 kcal/100 ml and a minimum energy intake of 115 kcal/kg, recommendations for vitamin B6 are 22 μg/kg/d. Vitamin B6 homeostasis in brain changes with gestation with preterm infants having a higher (inverse correlation with postmenstrual age) concentration of its active cofactor (pyridoxal phosphate) and direct precursor (pyridoxal); hence potentially suggesting higher requirements in preterm infants[21]. However, a study by Ramos found no difference in glutamic oxaloacetic transaminase activity, for which B6 acts as cofactor, in cord blood between low or normal weight term and preterm infants[15]. Porcelli et al showed that daily intakes of 300 μg/kg from preterm infant formula in preterm infants was associated with elevation of plasma B6 and urinary excretion of B6; hence suggesting that preterm milk formulas provide more than adequate amounts of B6 for this population[22]. Preterm formula content of pyridoxine varies from 60-250 μg/100 kcal providing 70 to 290 μg/kg at minimum energy intakes of 115 kcal/kg (Table 1). In the study by Friel et al, plasma concentration of B6 did not differ between preterm infants receiving 399 μg/100 kcal (439 μg/kg) from total or supplementary PN, and 194 μg/100 kcal (213 μg/kg) from full enteral feeding suggesting that the latter dosage is probably adequate to maintain normal blood levels of B6[8]. **We suggest a recommended intake in keeping with the concentration range that would be provided by commercially available preterm milk formula (70-290 μg/kg/d) [LOE 3, RG0].** This is close to the previous guidelines of ESPGHAN[1], the EFSA daily recommendations (Table 1) and the available evidence discussed above.

Folate

Folates function as cofactors for enzymes involved in one-carbon metabolism. Folate provides one-carbon units for the formation of nucleotides necessary for the synthesis of RNA and DNA. Folate is also fundamental for the normal functioning of the methionine cycle. Natural food

folates have a lower bioavailability than synthetic folic acid. In human milk, folate is bound to folate-binding proteins which are believed to facilitate folate uptake from the circulation, since human milk folate concentration is typically 5–10 times higher than that of maternal plasma[2,4]. Breast milk folate concentrations are maintained at the expense of maternal folate reserves and are not affected by low maternal folate intake, unless there is severe deficiency[23]. Folic acid supplementation in well-nourished lactating women does not affect breast milk folate concentration[23]. Mean concentrations of folic acid increased progressively with duration of lactation[6]. The EFSA Panel estimates average folate concentration of breast milk at 80 µg/L and daily recommendations for healthy term infants (<6 months) are 64 µg/day or 10.5 µg/kg using a reference weight of 6.1 kg for a 3-month reference child. Using the average concentration of folate in breastmilk, a calorific value of breastmilk of 70 kcal/100 ml and minimum energy intake of 115 kcal/kg, recommendations for folate are 13.1 µg/kg.

Folate intake has been associated positively with weight and length gain in extreme preterm infants in one study[24] whereas in another study low maternal folate intake and smoking were the strongest predictors of low folate intake in preterm infants in the first month of life[25]. Plasma folate is lower in children receiving only human breast milk rather than fortified human milk or preterm infant formula; however, at a mean intake of 23.4 µg/kg no infant developed folate deficiency as indicated by serum concentrations or hematological profile results; hence suggesting intake adequacy[25]. In a study by Jyothi, no child receiving preterm formula or breast milk fortifiers had raised levels of homocysteine, a biomarker of folate deficiency[26]. However, artificial milk formulas (Table 1) and breast milk fortifiers usually contain much higher amounts of folate than breast milk (Table 1). A relatively historical retrospective study by Fuller et al recommended supplementation in the range between 50 to 200 µg/day[27]. Haiden and colleagues showed that combination supplementation with erythropoietin, iron, vitamin B12 therapy and 100 µg/kg of folate stimulated erythropoiesis and improved haematological profile (haemoglobin, haematocrit) and reduced needs for blood transfusion, but the net effect of each of these components is unclear[28,29]. In an RCT daily supplementation of 100 µg folate in addition to other feeding modalities reduced the prevalence of anaemia in very preterm infants[30]. In the same study, plasma folate concentration declined compared with the supplemented group suggesting higher folate demands in preterm infants. In the study by Levy et al, a mean folate intake of 38 µg/kg/day was adequate to maintain serum and red blood cell folate within normal ranges in preterm infants[18]. In a follow on study by the same group an average intake of approximately 50 µg/100kcal or 55 µg/kg (assuming an energy intake of 110 kcal/kg) was adequate to maintain hematological profile, normal levels of folate in blood and folate urinary output, with the authors proposing a recommended folate intake of 37µg/100kcal (41 µg/kg) from infant milk formula[9]. **Considering also the range of folic acid content in preterm infant formula (20 to 45 µg/100 kcal, or 23 to 52 µg/kg) (Table 1) we**

propose an intake of 23-100 µg/kg/d [LOE 3, RG0]. Based on the evidence presented hereby, a folate intake at the maximum of the recommended range is likely to improve patients' outcomes too.

Cobalamin (B12)

Cobalamin (B12) acts as a coenzyme in two key reactions in humans. One is the rearrangement of methylmalonyl-coenzyme A to succinyl- CoA in propionate metabolism in mitochondria. The other is the cytosolic transmethylation of homocysteine by 5-methyl-tetrahydrofolate to methionine by methionine synthase[2,4]. The B12 concentration of breast milk reflects maternal concentration in blood[31], and mean vitamin B12 concentrations are highest in colostrum and higher in preterm than term human milk[6]. B12 content falls with duration of lactation and in mature human milk the mean concentration of B12 does not differ between preterm and term milk[6]. For term infants up to 6 months, the EFSA Panel estimated a B12 concentration of 0.5 µg/L in human breastmilk with a daily requirement of 0.4 µg/d [2] or 0.07 µg/kg using a reference weight of 6.1 kg for a 3-month reference child. Using the average concentration of B12 in breastmilk, a calorific value of breastmilk of 70 kcal/100 ml and minimum energy intakes for preterm infants of 115 kcal/kg, recommendations for folate are 0.08 µg/kg/d.

In an RCT, combined treatment with erythropoietin, intravenous iron, folate, and high vitamin B12 (3 µg/kg/day given subcutaneously or intravenously) stimulated erythropoiesis and reduced the need for transfusion in preterm infants[28,29] although it is impossible to discern the net benefit of B12 alone. In another study in preterm infants, a mean intake of 0.55-0.58 µg/kg was not associated with suboptimal blood levels within the first two weeks of life[18]; 0.6 µg of B12 via parenteral nutrition was associated with 41% of infants reaching levels above reference intervals suggesting potentially excessive intake. In an RCT of 184 preterm infants (<1,800 kg and <36 weeks gestation), 100 µg of intramuscular B12 monthly for 4 months reduced the severity of anaemia of prematurity and prevented a decline of B12 in plasma compared with the unsupplemented group[30]; hence potentially suggesting elevated requirements in preterm infants. Cobalamin content in preterm milk formulas varies from 0.11 to 0.55 µg/100 kcal providing 0.13 to 0.63 µg/kg at a minimum energy intake of 115 kcal/kg (Table 1). **We propose dietary recommendations of 0.10 to 0.60 µg/kg based on the cobalamin content in preterm infant formulas [LOE 3, RG0].** An intake >0.6 µg might be associated with excessively elevated B12 levels in blood.

For all water-soluble vitamins presented here, the weight standardised EFSA recommendations as well as those derived from breastmilk concentrations are substantially lower than the newly proposed recommendations which are based on the EFSA daily recommendations, current

evidence and the vitamin content of preterm infant formulas (Table 1). However, this does not mean that using the EFSA weight standardised recommendations or those estimates generated using breastmilk composition are necessarily inadequate. However, in the absence of evidence to address that the health practitioners are either discouraged to use these estimates or if they do so to monitor nutrient status with biomarkers.

Conclusions

- For most of the water soluble vitamins there are very little data upon which to produce evidence based dietary recommendations for preterm infants
- Previous and current dietary recommendations for water soluble vitamins predominantly reflect the amounts added from industry in preterm infant formulas
- Wide ranges in current dietary recommendations for preterm infants do not represent the distribution of intakes in a population
- In the absence of robust evidence dietary recommendations for water soluble vitamins for preterm infants are often inflated to ensure adequacy well in excess

Recommendations

- Intakes above dietary recommendations are unlikely to be of benefit in improving patients' outcomes
- Patients who do not meet minimal dietary recommendations should not be labelled as deficient until additional investigations are performed including monitoring of vitamin biomarker
- In preterm infants fed solely human breastmilk the health care professional is advised to monitor nutrient status with biomarkers or supplement water soluble vitamins.

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ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Vitamin A, D, E, K (February 2022)

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ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Vitamin A, D, E, K (July 2022)

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Vitamin A

Search strategy: Vitamin A – Preterm infants – Very low birth weight infants – Enteral nutrition

Background

Vitamin A is an essential micronutrient for growth and tissue differentiation [1, 2], and is especially important in lung maturation [3]. Whilst most term infants born in high-income countries receive adequate supplies of vitamin A *in ut*ero, clinical deficiency may still occur especially in very preterm infants. Preterm infants often have lower plasma concentrations of both retinol and retinol binding protein (RBP) at birth compared with term infants reflecting low hepatic stores [4]. Plasma concentrations of retinol remain low throughout the first year of life, especially in preterm infants of multiple births [4]. A plasma retinol concentration of ≥ 200 ng/mL is generally considered adequate [5], but due to the complexity of vitamin A metabolism and organ immaturity in preterm infants, vitamin A supplementation may still not result in adequate concentrations in blood. In addition to possibly impaired absorption or metabolism of retinyl-ester, low vitamin A concentrations may persist because vitamin A is consumed by vitamin A-deficient organs (liver, lung, eyes) [1, 6]. Animal studies suggest vitamin A deficiency during gestation might lead to abnormal conducting airways and impaired alveolar septation, resulting in suboptimal lung function [3].

The beneficial role of high dose intramuscular vitamin A for prevention of BPD in preterm infants has been demonstrated in a Cochrane systematic review [7]. However, because of the pain and discomfort associated with repeated intramuscular injections, this form of vitamin A supplementation is not common practice in most NICUs. Studies of higher enteral doses (5000 IU/day) produced inconsistent effects on BPD [8].

Interestingly, the United States experienced a nationwide shortage of intramuscular vitamin A in 2010, leading to cessation of therapy in all infants, but this transient enforced change in practice did not have an appreciable impact on the incidence of BPD [9]. When considering total vitamin A intake during NICU stay it is important to note that the majority of vitamin A

intake is enteral [11] and both oral and nasogastric tube administration is feasible [12]. Routine enteral vitamin A supplementation is common on NICUs once enteral feeding is established, but there are no good data on whether doses vary or are adjusted to the degree of prematurity or the intake of enteral feeds and fortifiers.

One RCT assessing high-dose fat-soluble oral vitamin A supplementation (5000 IU/day vs. placebo for 28 days) in 154 preterm infants failed to show a significant reduction of chronic lung disease (CLD) [13]. In contrast, another RCT including 196 preterm infants assessed higher dose enteral vitamin A supplementation (10,000 IU on alternate days for 28 days) and demonstrated a positive effect on the primary outcome parameter of all-cause mortality and oxygen requirement for 28 days (relative risk: 0.440 (95% CI: 0.229-0.844) [14] without any major adverse effects. A very recent RCT also assessed the effect of supplementation with 5000 IU/day of water-soluble vitamin A in 188 extremely preterm infants (< 28 weeks GA). In this trial, vitamin A supplementation improved plasma retinol levels, but did not affect the rate of BPD or other clinical outcomes as compared to placebo [15].

Despite these data the evidence base for vitamin A recommendations remains limited. Currently, one large RCT is still assessing the role of early high-dose oral vitamin A supplementation in ELBW infants and its effects on mortality and BPD [14]. Pending the results of this trial [14], we continue to recommend a daily vitamin A intake of 1333-3300 IU/kg body weight (400-1000 µg retinol ester/kg/d) in preterm infants [16]. Preterm infants with hepatic impairment or significant cholestasis may need higher intakes; conversely, renal impairment may require reduction of vitamin A dosage based on serum vitamin A levels.
In conclusion, very (VLBW) and extremely low birth weight (ELBW) infants are at increased risk of vitamin A deficiency, and therefore adequate vitamin A substitution is necessary. The currently recommended dosage of vitamin A supplementation for this cohort may undergo further adjustments, depending on the results of currently performed RCTs.

Recommendation:

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C1: There are insufficient data to change the previous recommendation of daily vitamin A intake in preterm infants (16), but infants with hepatic impairment may need higher intakes, and those with renal impairment may require lower doses (LOE 2++).

R1: Based on best current available data, we recommend a daily total intake of vitamin A of 1.333-3.300 IU/kg body weight (400-1000µg retinol ester/kg/d) (GOR B).

Vitamin D

Search strategy: Vitamin D – Preterm infants – Very low birth weight infants – Enteral nutrition

Background:

Vitamin D plays a critical role in multiple cellular processes especially bone metabolism and function of the innate immune system [17]. The intestinal receptor-dependent actions of calcitriol [1,25(OH)2D] are crucial for optimal calcium absorption. Evidence suggests that as early as the 20th week of gestation the human fetal intestine possesses functional calcitriol receptors that regulate the expression of calcium-binding protein and calcidiol-24-hydroxylase. Pathways of vitamin D absorption and metabolism are fully operative in babies <28 weeks GA [18-20]. Mineral bone deficiency in preterm infants is common and is primarily caused by suboptimal intakes of calcium and phosphate, but this can be compounded by Vitamin D deficiency [21]. Even though there is no consensus regarding the definition of vitamin D deficiency in infants, the ESPGHAN Committee on Nutrition has previously recommended the pragmatic use of a serum 25-hydroxy vitamin D concentration >50 nmol/L to indicate sufficiency and a serum concentration <25 nmol/L to indicate severe deficiency [22], whilst noting that excessive vitamin D intakes resulting in concentrations >120 nmol/L should also be avoided [23].

Several studies have assessed the relation between vitamin D3 intake and the mean circulating concentration of 25(OH)D. However, only a few studies have determined the effects of different vitamin D intakes in preterm infants on bone mineral density after the immediate neonatal period. In one study, 39 preterm infants randomized to either 200 IU/kg (up to a maximum of 400 IU/day) or 960 IU/day until 3 months of age showed no differences in bone

mineral density at 3- or 6-months corrected age [21]. Another RCT compared vitamin D supplementation of 800 IU with 400 IU/day in 96 preterm infants. The prevalence of vitamin D deficiency defined as a serum 25-hydroxy vitamin D level <50 nmol/L was significantly lower in the 800 IU group at 40 weeks and at 3 months corrected age, but bone mineral content and density were not different. [24].

In contrast, a study in 32 preterm infants compared 400 or 800 IU/day vitamin D3 supplementation and measured serum 25(OH)D3 levels demonstrated improved bone density with a daily dose of 800 IU [25]. A further study in very preterm infants of whom 72% were vitamin D deficient before supplementation, demonstrated that 1000 IU/d of vitamin D supplementation more effectively decreased the prevalence of vitamin D deficiency at 36 weeks postmenstrual age (PMA) when compared to supplementary intakes of 800 and 400 IU/d [26]. This is also in keeping with a study demonstrating a vitamin D deficiency rate of ~80% at 4 weeks of age despite vitamin D supplementation of 400 IU/day [27]. A small study in 40 preterm infants comparing 800 IU/d vs. 400 IU/d of vitamin D3 over a 4-week-period showed increased expression of T regulatory cells (at 1 week and 4 weeks) but was under-powered for other functional outcomes [28]. However, similar findings on immune parameters have been seen in other small studies included in a systematic review in preterm infants [29] Current data suggest that bone density is the only relevant clinical outcome to consider for the use of vitamin D supplementation in preterm infants [22].

Bronner et al. [30] showed that calcium absorption in VLBW and low birth weight infants was directly proportional to the daily calcium intake in the range from 40 to 142 mg/kg, and was independent of daily vitamin D supplementation of up to 2000 IU Conversely, *Senterre et al.* demonstrated that calcium net absorption increased from 50% to 71% by feeding appropriate-for-gestational age VLBW infants banked human milk alone or supplemented with 30 µg of cholecalciferol/day (1200 IU) without additional calcium fortification [31].

When calculating vitamin D intakes per kg per day, it is useful to analyze the above study results in relation to the body weight of the infants when receiving the supplements: The higher doses of vitamin D supplementation that have been associated with reduced risk of

vitamin D deficiency among infants in the weight interval 1500-2000 g are 800-1000 IU/day, corresponding to 400-670 IU/kg/day [24-26]. The corresponding doses in the weight interval 1000-1500 g are 500-1000 IU/kg/day [25]. However, other studies have suggested that lower doses (200-300 IU/kg/day) may be sufficient [24, 27]. In the weight interval 600-1000 g, no data is available from randomized, controlled trials, but an observational study has shown that a vitamin D dose of 400 IU/day, corresponding to 400-670 IU/kg/day, was safe and not associated with vitamin D deficiency in this weight interval [24, 28]. Conversely, another study suggested a daily vitamin D supplementation of 800 IU/d in extremely premature infants with a gestational age < 28 weeks. [32]. There is a lack of safety data on higher doses of vitamin D in preterm infants < 1000 g.

Based on this, we recommend a daily vitamin D intake of 400-700 IU/kg/d (10-17.5µg/kg/d) during the first months of life for preterm infants with a body weight < 1800 g. This corresponds to 300-525 IU/kg/d at 750 g body weight, 400-700 IU/kg/d at 1000 g body weight, and 600-1000 IU/kg/d at 1500 g body weight. The maximum recommended intake is 1000 IU/day. However, preterm infants who are vitamin D deficient due to maternal vitamin D deficiency or cholestasis may temporarily need higher doses. Adequate vitamin D supplementation could be monitored by measuring serum 25(OH)D at 3-4 weeks of life and then every month until discharge to adapt vitamin D supplementation to each individual's needs.

C1: Ensuring adequate vitamin D intakes in preterm infants is essential for bone health and may possibly have positive effects on immune function, even though this is not conclusively shown. **LOE 2+**

C2: There are few adequately powered controlled trials on which to base firm recommendations in preterm infants, and even fewer trials provide clinically relevant outcomes beyond vitamin D concentrations, e.g. markers of bone health. **LOE 4**

R1: Based on currently available data, we recommend a daily vitamin D intake of 400 to 700 IU/kg/d (10 µg-17.5 µg/kg/d) during the first months of life with a maximum dose of 1000 IU/day (25 µg/d) **(LOE 2++, GOR B).**

Vitamin E

Search strategy: Vitamin E – Preterm infants – Very low birth weight infants – Enteral nutrition

Background:

Vitamin E comprises a group of eight biologically active tocopherols, among which d-alpha-tocopherol has the highest antioxidant activity. It is one of several antioxidant agents that preterm neonates can use as scavengers of free radicals, thereby potentially limiting lipid peroxidation which can lead to chronic lung disease/bronchopulmonary dysplasia (CLD/BPD) and retinopathy of prematurity (ROP), and other long-term complications like hemolytic anemia [33, 34]. Alpha-tocopherol inhibits inflammation by modulating cellular signaling and by regulating transcription, and it stimulates immunity as well [35].

VLBW infants are at risk for vitamin E deficiency because of low storage due to low body fat, inadequate intakes, impaired absorption and higher requirements compared with term infants [36]. Low concentrations of vitamin E were found at birth and at discharge in VLBW infants [36]. However, the clinical assessment of vitamin E deficiency remains challenging in preterm infants, because serum tocopherol concentrations may not reflect tissue concentrations and are dependent on serum lipid concentrations [37]. Moreover, milk from mothers who delivered preterm contains greater concentrations of vitamin E (4.5 mg/L) than those delivered at term, and preterm infants fed their own mother´s milk may be able to maintain adequate concentrations of vitamin E [36, 37].

In a study in 93 extremely premature neonates with a gestational age < 27 weeks' gestation and a birth weight < 1000 g, a single enteral 50 IU/kg dose of vitamin E raised serum α-

tocopherol concentrations, but to consistently achieve α-tocopherol concentrations > 5 mg/L, a higher dose or repeat doses of vitamin E may be needed [38]. In another study, long-term supplementation of alpha-tocopherol (20 mg/kg/day of alpha-tocopherol each day after full feeding) improved mental development, in particular, performance IQ, in school-aged ELBW children [39]. Reports of toxicity related to enterally administered vitamin E are rare, however an increased risk of sepsis and necrotizing enterocolitis (NEC) has been reported after enteral vitamin E, primarily when plasma (or serum) vitamin E concentrations exceeded 3.5 mg/dL [40]. A Cochrane meta-analysis on parenteral vitamin E supplementation in neonates demonstrated that vitamin E supplementation in preterm infants reduced the risk of IVH as well as the risk of severe ROP and blindness in VLBW infants, but increased the risk of sepsis [41]. Thus, the current best available evidence does not support the routine parenteral use of vitamin E supplementation at high doses [16, 40, 42].

Although no clear clinical benefit has been shown for routine vitamin E supplementation in premature infants, it seems prudent to maintain plasma vitamin E concentrations of 10 - 35 mg/L, and a ratio of serum-α-tocopherol of at least 1 mg to 1 g total lipids , implying a minimum dose 3.8 mg/kg/d. The recommended daily adequate intake of vitamin E in preterm infants is 2.2 (3.8)-11 mg/kg/d [16, 40, 42]. Infants with prolonged cholestasis may require higher intakes.

Recommendation:

R1: Based on the current available data, we recommend a daily dose for vitamin E supplementation in preterm infants of 2.2-11 mg/kg/d **(LOE 2++, GOR B).**

Vitamin K

Search strategy: Vitamin K – Preterm infants – Very low birth weight infants – Enteral nutrition

Background:

Vitamin K is a group of lipophilic, hydrophobic vitamins that belong to the class of 2-methyl-1,4-naphthoquinone derivatives. Vitamin K is necessary for the synthesis of coagulation factors (factors II (prothrombin), VII, IX, and X, and the anticoagulation proteins C and S in the liver. Maternal transfer of vitamin K across the placenta is very low with cord blood concentrations of vitamin K often below the detection limit of 0.02 ng/mL in healthy newborns [43]. While breast milk is the preferred diet for all neonates, it has very low levels of vitamin K [44, 45], and late onset vitamin K deficiency bleeding (VKDB) is primarily seen in exclusively breast fed infants or in those with cholestatic disease [46].

Several studies have evaluated the levels of vitamin K dependent clotting factors in both term and preterm infants. Term infants at birth have approximately 50% of the serum concentrations of adults of factors II, VII, IX, and X [43]. Plasma concentrations of vitamin K dependent clotting factors are inversely correlated with gestational age [47]. The impact of prematurity may be further exaggerated by concurrent hepatic disease, delayed milk feeding and repeated exposure to antibiotics that impact on gut bacterial colonization and, consequently, endogenous synthesis of vitamin K2 [44, 45]. Bile salts are necessary for effective absorption of vitamin K.

VKDB can occur as one of three distinct presentations: early (first 24 h of life), classical (day one to seven of life), or late (later than one week). Late onset VKDB typically presents with gastrointestinal, cutaneous, or intracranial hemorrhage [48] and has a high mortality rate with 50% of affected infants presenting with intracranial hemorrhage [49].
Whilst preterm infants are of particular concern because of their relatively immature hepatic and hemostatic functions, most preterm infants receive prophylactic vitamin K at birth, and vitamin K as part of parenteral nutrition, thus VKDB in preterm infants is not widely reported. Vitamin K can be administered intramuscularly, intravenously, and orally with different recommended dosing regimen. [50].

Worldwide practices in the administration of vitamin K vary widely, with differing doses and routes of administration in different NICUs [, 51]. Supra-physiological concentrations of

vitamin K and elevated concentrations of vitamin K epoxide have been found in studies that assessed vitamin K concentrations after 0.5 mg IM or 1 mg IM administration. This may be indicative of a relative immaturity of the preterm liver [52]. Studies that have looked at blood markers such as prothrombin time (PT) as a surrogate for vitamin K status have not found a correlation between prolonged PT and vitamin K. Preterm infants receive large quantities of vitamin K from prophylaxis, total parenteral nutrition (TPN) solutions, infant formula and breast milk fortifiers. Thus, vitamin serum concentration in preterm infants is usually higher than those found in in term formula-fed infants [53].

The AAP recommends administering 0.5 mg to 1 mg vitamin K IM after delivery to prevent VKDB in term infants [54]. Thereafter, term infants who are exclusively breastfed receive about 2 µg/day from breast milk without evidence of VKDB, and this forms the basis of the current Recommended Daily Allowance (RDA) of vitamin K of 2 µg/day for healthy term infants [55]. There are no RCTs in preterm infants, and recommendations for daily vitamin K intake vary, ranging from 4.4-28 µg/kg/d, to up to 100 µg/kg/day [16, 56, 57]. Moreover, preterm infants continue to receive vitamin K via parenteral nutrition until full enteral feeds are established. In case of prolonged cholestasis higher intakes are likely to be required.

Recommendation:

R1 Based on the current based available data, we recommend a daily dose for vitamin K of 4.4-28 µg/kg **(LOE 2++, GOR B).**

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Supplementary digital content no. 13

ESPGHAN Committee of Nutrition (CoN) position paper on Enteral Nutrition for Preterm Infants Feeding mode: minimal enteral feeding, feed advancement, gastric residuals and timeline of parenteral and enteral nutrition

Date : February 2022

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Minimal enteral feedings and enteral fasting during the first days of life

In the literature, various different terms for “minimal enteral feeding” (MEF) are used such as gut priming, minimal enteral nutrition, trophic feeding or hypocaloric feeding but not buccal colostrum application which is discussed in chapter 15. Usually MEF and its synonyms are defined as nutritionally insignificant small volumes of milk (typically 12–24 ml/kg/day) without any advancement in feed volumes during the first three (1) to seven postnatal days (2). The primary aim of MEF is to stimulate gut motility (3-5), transit time (6), gut epithelial health and endocrine activity (5), enabling infants to transition to full enteral feeding more quickly. In most trials, infants received either expressed breast milk, formula milk or a mixture of both (7, 8). A Cochrane review (9) considered studies of enteral fasting versus MEF in VLBW-infants and the impact on feeding, morbidity and mortality. Most of these studies were performed in the 1990s. MEF was generally started within the first three days after birth and continued for 7 to 10 days and found to be safe in comparison to complete enteral fasting. A second Cochrane review focused on enteral fasting with delayed (4-7 days) versus early (up to 4 days) introduction of progressive enteral feeding to prevent NEC (8)**.** Meta-analysis did not detect an effect on the risk of NEC or all-cause mortality related to introduction of enteral feeding but infants who had delayed introduction of enteral feeds took 2-4 days longer to establish full enteral feeding (7, 8). Two recent RCTs investigated early progressive feeding without MEF or delayed progressive feeding after a 3 to 4-day course of MEF (delayed feeding group) and found conflicting results (10, 11). In the first trial 60 extremely premature infants were randomised to daily increases (25 mL/kg) in EN immediately after birth, or to similar increases but starting after a 4 d period of MEF. Time to full enteral feeding defined as 120 mL/kg/d was significantly reduced from 12 to 10 days on average in the group with the early increments, without increasing major morbidities (12). These findings confirmed results from an earlier performed observational study in 192 extreme low birth weight (ELBW)- infants with a gestational age between 23-28 weeks (1) investigating a period of short MEF of 3 days or less versus a period of MEF of more than 3 days. In contrast, the second RCT did not show that a 3 to 4 day period of

MEF delayed time to achieve full enteral feeding in 199 preterm infants with a birth weight <1250g (10). The authors suggested that prolonging MEF up to 4 days might have an advantage over early advancing enteral nutrition in terms of avoiding NEC and improving daily weight gain (10). In conclusion it remains unclear whether maintaining MEF for several days has any advantage compared to initial early progressive feeding.

C1: Minimal enteral feedings are defined as nutritional insignificant small volumes of milk (typically 12–24 ml/kg/day) without advancing the feed volumes for a period of 3-7 days. (LOE1+)

C2: There is no clear beneficial effect of enteral fasting or MEF of any duration compared to advancing feeds immediately after birth. (LOE1+)

R1: Start small volume enteral feeds as soon as possible after birth in most preterm infants and advance feeds as clinically tolerated. (GOR B)

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Advancement of enteral feeds

Historically, faster advancements of enteral feeds were thought to increase the risk of NEC, although this was not confirmed in a recent systematic review of 10 RCTs including a total of 3753 infants (13). In this meta-analyses, advancing feed volumes more slowly increased the time to full enteral feeds and it was suggested, that slower enteral increments increased the risk of invasive infections due to prolonged use of central venous catheters. Final data from the recently published Speed of Increasing milk Feeds Trial (SIFT)(14), which included 2804 infants did not provide evidence that advancing enteral feed volumes at daily increments of 18 mL/kg/day compared to faster rates of 30 mL/kg/day reduced the risk of late onset sepsis, NEC or mortality after 4 days of life. About one-third of all participants were extremely preterm or ELBW, and about one-fifth were small for gestational age (SGA), growth-restricted, or compromised in utero, as indicated by absent or reversed end-diastolic flow velocity in the fetal umbilical artery Doppler signal. However, careful interpretation of SIFT trial data (14) is important: infants were a median of 4 days old at time of randomisation and the trial therefore may not adequately inform the relative safety of these feeding volume increments during the first few days of life. In addition, the actual daily increment was slower than targeted in both groups and other factors such as the approach and definition of feeding tolerance or gastric residuals might have influenced practice and were not reported. Interestingly, in formula fed infants the rate of moderate or severe motor impairment at age 2y, obtained from parental reports, was slightly higher in the faster-increment group. However, in preterm infants between 1500-1999g

C1: After 4 days of life, faster feeding progression (30 mL/kg/day) of enteral feed volumes does not significantly increase the incidence of NEC or all-cause mortality compared to slower (15–20 mL/kg/day) feeding advancement (LOE1+).

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C2: Meta-analysis showed that faster increment of enteral feed volumes positively reduces the time to full enteral feeding and the length of hospital stay as well as possibly the incidence of invasive infections (LOE1+).

R1: In stable preterm infants where the clinician considers that feed volume can be increased, a routine daily increment of 18-30mL/kg/day is recommended, especially in breastmilk-fed infants. (GOR A)

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Gastric residuals

Gastric residuals (GR) are commonly used to define feeding tolerance although there are few data on the significance of its volume and colour. Preterm infants have immature gastric motility, resulting in delayed gastric emptying (15, 16). In addition, the average basal gastric secretions in preterm infants amount to 2.8 mL every 4 hours (17). Positioning after feeding influences gastric emptying and regurgitation, with crossover studies demonstrating that the left lateral position is associated with higher pre-feed gastric residuals and the right lateral position with increased regurgitation episodes (18, 19). The best compromise for such neonates may be the prone position for the first half an hour post-feeding and then changing the position according to the behavior cues of the infants (20). Gastric emptying is also influenced by the type of enteral feed, with breastmilk emptied almost twice as fast as formula (21, 22). However, the effects of breastmilk (23), pasteurization (23), and fortification with either casein or whey dominated protein (23) on gastric emptying are inconsistent (24).

Determination of “feeding intolerance” often includes emesis, colour and volume of gastric residuals, abdominal distension or tenderness, the presence/absence or quality of bowel sounds, absent or abnormal-appearing stools, heme-positive stools, or any combination thereof (25-27). There is therefore no consistent, agreed definition of feeding intolerance. There are no good data that GR are a predictive marker of feeding tolerance and definitions of what constitutes a “clinically significant” GR vary widely. RCTs refer to a range in volume between >2 mL (28) to 5 mL/kg (29) or from >33% (30) of the volume of previous feed up to >50% (31), respectively.

Few studies have examined the clinical importance of GR in the context of NEC. A case-control study in 34 infants reported that “large” GRs (mean 7.5 mL in NEC cases versus 4.0 mL in controls $p = 0.04$) were predictive for NEC, and that bilious colour of GR was irrelevant, although haemorrhagic residuals were predictive for NEC (32). These numbers reflected the mean gastric residuals from birth until the onset of higher stage NEC but did not provide information about what happened in the 2-3 days right before NEC onset. Another case-control

study found that infants who developed NEC had a median GR of 4.5 mL per feed (40% of previous feed) whereas the control group had 2 mL per feed (14% of previous feed). The total residuals as percentage of feeds and the average of maximum residuals increased in the NEC group during the 3 days prior to NEC-onset(33).

Three RCTs evaluated the predictive value of routine monitoring of pre-feed GR on time to full enteral feeding and NEC. One study compared the assessment of bloody GR without volume determination against routine assessment (34), and two other trials compared routine monitoring versus no routine monitoring of GR (35, 36). Evidence from studies suggest that routine monitoring of GR increases the risk of feed interruption episodes, the time taken to reach full enteral feeds and to regain birth weight, and PN days, but does not have an impact on NEC incidence. This is in line with a large retrospective study in 472 preterm infants (37) and a recent Cochrane review (38). However, all of these trials only included 843 infants in total and therefore have limited power to determine a true effect on NEC.

In summary, there is no data on the volume and/or colour of GRs that definitively indicate feeding intolerance, or are predictive of NEC (38). GRs alone are neither a sensitive nor a specific indicator for bowel injury in the premature gut. GRs may be present prior to NEC but are likely to be more helpful in combination with other classic signs of NEC. Similarly, the clinical importance of abdominal distension or 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 often results in intestinal gaseous distension (26, 39). So far, no consensus on whether to re-feed or discard the aspirated GR is available. Although re-feeding gastric residuals may replace partially digested milk, gastrointestinal enzymes, bile acids, hormones, and trophic substances that support digestion and promote gastrointestinal motility and maturation (40), re-feeding abnormal residuals may result in emesis, NEC, or sepsis (41). There are only data from one small unblinded trial on the efficacy and safety of re-feeding GR in 72 preterm infants (42). Re-feeding GR volumes in extremely preterm infants did not reduce time to achieve full enteral feeding but the available

evidence is insufficient to support or refute re-feeding of gastric residuals in preterm infants. (42).

C1: Positioning of the infant has an impact on gastric emptying with the prone position for the first half hour post feeding being quickest. (LOE 2+)
C2: The GR alone is neither a sensitive nor a specific indicator for bowel injury of the premature gut (LOE 2+)
C3: Routine monitoring of GR increases the time taken to reach full enteral feeds and to regain birth weight, and increases the number of PN days but does not have an impact on NEC incidence. (LOE 2+)
C4: There is no consensus on whether to re-feed or discard the aspirated GR (LOE 3)

R1: Routine monitoring of GR in the clinically stable infant is not recommended (GOR B)
R2: Assessment of GR should be performed only when other clinical signs associated with feeding intolerance or NEC are present such as extreme abdominal distension, tenderness, emesis, bloody stools, apnea, temperature instability (GOR B)

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Timeline of parenteral and enteral nutrition

The nutritional therapy timeline of very preterm and very low birth weight (VLBW) infants often involves use of parenteral nutrition (PN), enteral nutrition (EN), and a transitional period in between. This transitional period varies significantly from infant to infant but also between Neonatal Intensive Care Units (NICUs), and is influenced by local feeding practices, feeding intolerance and metabolic intolerance (43-45). The transition phase is a critical time period for poor growth, with growth rates often <10 g/kg/d (44) although early progressive PN and EN strategies may lead to reductions in the cumulative energy and protein deficits that occur during the first weeks of life (46, 47). Standardized feeding guidelines and protocols, designed to maintain targeted intake throughout the transition phase (48, 49) can help to achieve nutritional goals (50). NICU guidelines must consider the optimal duration of minimal enteral feedings, the daily advancement of milk feeds and the optimal breast milk fortification strategy. NICU guidelines should also consider the approach to gastric residuals, together with definitions of “feeding intolerance” e.g. stopping or withholding feeds (51) and “full enteral feedings” e.g. an enteral intake of 140-160 ml/kg/d (44, 52, 53) and/or the timing of the removal of central venous lines. Data from multiple observational studies suggest that the use of standardized feeding protocols allow preterm infants to achieve full enteral feeds faster, shorten the time on PN and hospital stay, decrease the rates of NEC, improve growth and promote improved neurodevelopment (54-62). One of the key challenges during the transition phase is to try and combine the different recommendations for parenteral and enteral intake of nutrients. In general, recommendations for enteral intake of protein and fat are higher than for the parenteral route. Therefore, it is recommended to use a process such as prescription software for parenteral and enteral nutrition that is able to adapt to changing reference values during the transition phase.

C1: Early progressive parenteral and enteral nutrition strategies may reduce cumulative energy and protein deficits. (LOE 2+)

Author: Nadja Haiden

C2: The transition phase between parenteral and exclusive enteral nutrition is a critical time period for cumulative nutrient deficits and for poor growth. (LOE 2+)

R1: To avoid nutrient deficits we recommend to establish a standardized feeding protocol in every NICU that defines the following parameters: duration of minimal enteral feedings, daily advancement of milk feeds, definition and management of gastric residuals, definition and approach to feeding intolerance, definition of full enteral feedings, and breast milk fortification strategy. (GOR B)

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Supplementary Digital Content no. 14

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Feeding mode (February 2022)

Authors J.B. van Goudoever, S. Meyer, N.D. Embleton

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Feeding Practice - Mode of Feeding

a. Nasogastric versus orogastric tube feeding
b. Bolus vs continuous
c. When to start oral (breast)feeding (and stop tube feeding)

a. Nasogastric versus orogastric feeding tubes

Orogastric (OG) and nasogastric (NG) feeding tubes are commonly used in neonatal intensive care units (NICUs) and are used to support enteral feeding in premature infants who are unable to feed orally.

NG tubes may increase nasal airway resistance, leading to increases in total airway resistance especially in the smallest infants (1). Increased airway resistance increases work of breathing and may cause pharyngeal airway collapse (2, 3). Although it is widely assumed that NG tubes increase the incidence of apneas because infants are obligatory nose-breathers, this has not been confirmed in studies. (4) Furthermore, the most recent Cochrane Database Systematic Review (2013) did not show any consistent effects on feed tolerance (time to achieve full enteral feeds) or the incidence or frequency of apneas, desaturation episodes or bradycardia (5). We did not identify any more recent studies.

OG tubes may be more prone to vagal stimulation which may provoke bradycardia (6, 7) due to tube movements in the hypopharynx because secure fixation in the oral cavity is challenging. It is therefore possible any advantage of OG tubes in avoiding the increases in airway resistance seen with NG tubes is counteracted by enhanced vagal stimulation. Adverse effects of both NG and OG tube placement have been described, including tube misplacement (8, 9), nasal damage (10) and oesophageal perforation.

No preferential method to use either nasogastric or orogastric feeding tubes for preterm neonates can be determined, so local preferences are allowed.

Supplementary Digital Content no. 14

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Authors J.B. van Goudoever, S. Meyer, N.D. Embleton

b. Bolus versus continuous feeding

When an infant is not capable of coordinating sucking, swallowing and breathing, feeding is provided through an oro- or naso-gastric tube. Feeding can be given as a continuous infusion or intermittently (bolus feeding). Bolus feeds are physiological postnatally, promoting the cyclical release of gastrointestinal tract hormones to stimulate gut maturation and motility, while the fetus has a continuous pattern of ingesting amniotic fluid (11). Marked variations in feeding interval protocols for infants <28 weeks' gestation exist (12). In 2012, 38% of neonatal units reported using continuous or hourly feeds, 35% of units reported using a 2-hourly interval, 7% reported a 3-hourly interval, and 20% reported otherwise. Low-quality evidence suggests feeding 3-hourly is comparable to 2-hourly feeding in VLBW infants from start of feed. However, extremely low-birth-weight infants reach full enteral feeds earlier when fed 2-hourly compared with 3-hourly (13). Bolus feeding increases splanchnic perfusion more than continuous feeding (14). Energy expenditure may increase upon bolus feeding as compared to continuous feeding, which may compromise growth on the other hand (15).

The Cochrane systematic review (published in 2011) included seven trials, involving 511 infants, found no differences in time to achieve full enteral feeds between feeding methods (16). A more recent meta-analysis, including eight studies involving 707 infants described a longer time to reach full enteral feeding in continuous feeding infants (weight mean difference 0.98 (95% CI 0.26-1.71, P = 0.008) days) compared with intermittent feeding infants (17).

Fat may adhere to the inner wall of the tube to a greater extent during continuous feeding when compared to bolus feeding. However, despite two studies showing a loss of energy and fat content (including an in vitro study indicating some loss of arachidonic and docosahexaenoic acid) after continuous feeding (18, 19), no significant effects were observed on growth (weight, length or head circumference) in the most recent available meta-analysis (20). Some reports demonstrate an association of a higher number of apneas and apnea related hypoxic episodes during continuous feeding (21, 22), others do not (23-25).

Bolus feeding (2-3 hourly) may be slightly more preferential than continuous feeding in preterm infants, but more well-designed studies are needed for more definitive advice.

c. When to start oral (breast)feeding and stop tube feeding

Infant oral feeding performance is the result of the skills to coordinate sucking, swallowing, breathing and esophageal transport plus the external factors imposed on the infant that may enhance or hamper the use of these inherent skills (e.g., milk availability, NICU environment, caregiver feeding approach) (26). In particular, infants with BPD often experience difficulties transitioning from gavage to oral feedings that can delay discharge (27). Introducing oral feeds to infants on CPAP or high flow nasal oxygen is practiced in some units, and whilst this is associated with shorter time to full oral feeds compared to those not fed in some studies(28, 29) there remain some concerns about the

possibility of micro-aspiration as a contributor to ongoing lung disease. However, the current evidence is limited. A recent feasibility study comparing infants on CPAP or on high flow nasal cannula was unable to draw definitive conclusions(30). Some hospitals discharge children home with feeding tubes in place (31), others wait till oral feeding is fully established. Consequently, hospital discharge can be delayed by the inability of preterm infants to feed by mouth safely and efficiently.

There is no strong or consistent evidence that early introduction and advancement of oral feeds based on the infant's individualized cues, state and behavior, rather than a predetermined feeding schedule, affects important outcomes for preterm infants or their families. Some evidence exists that preterm infants fed in response to feeding and satiation cues achieve full oral feeding earlier than infants fed prescribed volumes at scheduled intervals. However, this finding should be interpreted cautiously because of methodological weaknesses in the included trials (32).

Meta-analyses provided evidence of low to moderate quality that avoiding bottles increases the extent of breast feeding on discharge home (full breast feeding risk ratio (RR) 1.47, 95% CI 1.19 to 1.80; any breast-feeding RR 1.11, 95% CI 1.06 to 1.16) (33).

Non-nutritive sucking, where no milk/liquid ingestion is involved, is a good marker of the sucking process in itself, and as shown in a recent meta-analysis, demonstrates a significant reduction in time to full oral feeding (-5.5 d) and in reducing length of hospital stay (-4.5 d) (34). Non-nutritive sucking is usually twice as fast as nutritive sucking (1 cycle/s) (35). Nutritive sucking requires a functional dependence of the four processes involved (sucking, swallowing, respiration and esophageal function). Any delay of the nutrition during the oral, pharyngeal and/or esophageal phase will increase the risks of adverse events such as choking, respiratory distress or aspiration. A swallow event may last 0.35-0.7 seconds (36), whereas a preterm infant respiration rate may be between 40-60/minute leaving 1-1.5 seconds in between. Some infants may therefore not have sufficient time between swallows to breathe appropriately, while sensorimotor interventions may improve the sucking process (37, 38). Breastfeeding will require more effort from the infant than bottle feeding, where mature sucking with alternation of sucking and expression is not required. Nipple shields may help facilitate this process while breastfeeding, although recent reviews are contradictory on their effectiveness (39, 40).

In a small prospective descriptive design study (mothers of 15 infants, born at gestational ages between 26 and 31 weeks), semi-demand feeding with prescription of a total daily volume of milk was practiced during the transition from scheduled to demand feeding (41). Breastfeeding was initiated from a postmenstrual age (PMA) of 29 weeks. Full breastfeeding was attained at a median of 35 weeks, between 32 and 38 weeks. Initiating oral feeding attempts at 30 weeks' PMA does not result in earlier attainment of full oral feedings (42). Low birth weight, moderate to severe bronchopulmonary dysplasia and necrotizing enterocolitis were factors that negatively influenced progression to full oral feeding. In this study, which full oral feedings were attained at around 35 weeks PMA in a cohort of preterm infants < 32 wks at birth (mean birth weight 1325 g) although this varies between different units(43).

Some authors state that introducing oral feeding is indicated from 32-34 weeks at postmenstrual age, by trial and error (44), others recommend that the breastfeeding process is guided by the preterm infant's competence and stability, not a certain postmenstrual or postnatal age or weight(45).

Introducing oral feeding should be guided by the competence and stability of the preterm infant and may be started from 32 weeks postmenstrual age. Establishment of non-nutritive sucking prior to the introduction of oral feeding may reduce time to reach full oral feeding and length of hospital stay.

Conclusions, Recommendations

C1: No preferential method to use either nasogastric or orogastric feeding tubes for preterm neonates can be determined. **LOE 2**
C2: Bolus feeding (2-3 hourly) might be slightly more preferential than continuous feeding in preterm infants, but more well-designed studies are needed for definitive advice. **LOE 2+**
C3: Establishment of non-nutritive sucking prior to the introduction of oral feeding may reduce time to reach full oral feeding and length of hospital stay. **LOE 3**
R1: Introducing oral feeding should be guided by the competence and stability of the preterm infant and may be started from 32 weeks postmenstrual age. **GOR GPP**

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Supplementary Digital Content no. 15

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Growth (February 2022)

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Introduction

Growth is evaluated by measuring gain in weight, length/height and head circumference but also using measures of body composition. Plotting on a growth chart enables clinicians to compare individual size and growth velocity to a reference group throughout infancy, childhood and into adulthood. Growth charts are therefore essential for clinicians evaluating growth in infants and children.

In preterm infants, the optimal growth velocity for any individual is difficult to determine. Slow growth might be a sign of inadequate nutrition but might also be acceptable at certain periods, for example when direct breastfeeding is being established. Rapid growth may be acceptable during periods of 'catch-up' following periods of poor nutritional supply, but too rapid weight gain in particular, may also be harmful. Growth must be seen in the context of optimising nutritional status that improves short- and long-term functional outcomes rather than simply promoting an increase in anthropometric values.

The neonatal adaptation process and growth monitoring

The neonatal period involves major changes to growth and body composition, from the immediate processes involved in metabolic adaptation to ex-utero life, and further on to a period of stable growth. In preterm infants this has traditionally been divided into three phases: Phase 1. Initial weight loss, 2. Regain from nadir to birthweight and 3. Growth from regained birth weight and until discharge (1, 2).

We recommend only 2 phases:

- Phase 1. From immediately after birth until 3-4 days and often involves a loss of weight mainly due to a one-time irreversible contraction of extracellular water space* (3, 4)
- Phase 2. From weight nadir until discharge with growth for each infant following their current percentile or standard deviation score (SDS) in the chosen growth chart (ideally less than 1 SD below the birth percentile)

*Small for gestational age (SGA) infants often lose less or even demonstrate weight gain instead of loss (5, 6).

Monitoring growth requires local policies, standardized operating procedures (SOPs) and guidelines, accurate and precise measuring devices, training of health care professionals and the use of an appropriate growth reference. Weight should ideally be measured using the same device

each time (both stand-alone scales and those incorporated into an incubator are acceptable with adequate training); and head circumference (HC) should be measured using specifically designed measuring tape placed on the same reference points on the head each time, ideally taking three independent measurements and using the highest value of the three readings. Linear growth measures of crown heel length (CHL) is a composite of long bone and spinal growth and is best performed with two people using a hard surface length board for stable infants, an infant length stadiometer or specially designed measuring equipment suitable for use within an incubator.

Methodological issues discussing growth velocity and growth charts for preterm infants

A plethora of growth charts have been developed over recent decades and represent an impressive synthesis of auxology, statistical summary, smoothing, and chart design with some of them providing percentiles and others based on standard deviations.

Growth references and growth standards

Growth *references* describe how groups of individuals have grown at a particular time and place, and may represent historical cohorts from several decades earlier. Growth *standards*, on the other hand, are intended to reflect how a selected, typically healthy population grows when nutritional, environmental, and health constraints on growth are minimal (WHO-recommendation) (7). Based on these recommendations the WHO performed a Multicentre Growth study from 1997 to 2003, resulting in the publication of the 2006 WHO growth standard curves from term birth to 5 years of age, which provide a single international standard based on healthy breastfed term born infants (8). Longitudinal growth measurements from this cohort of 8.500 healthy children are now accepted as the normative model for postnatal growth of healthy term infants.

For preterm born infants, growth standards are more challenging to develop. Different references have been established typically based on national anthropometric data from measurements in-utero or at birth. Growth references have been based on continuous in-utero measurements by ultrasound, which only estimates weight from one- or two-dimensional lengths or circumferences measurements; available until term age (9), cross-sectional measurements of weight, length and HC at birth from gestational age 22 - 42 weeks (10), or cross-sectional measurements taken at birth (from 24 weeks to term) in combination with data from longitudinally followed term-born children up to the age of 24 months (11). Therefore, the majority of preterm growth charts are not true growth standards as they have been based on cross-sectional birth weight data rather than longitudinal population-based data. Another challenge, developing growth references, is when anthropometric data were collected as completed gestational weeks without further subdividing into days, further limiting the precision of the growth references for an individual infant.

One widely used chart is the Fenton preterm growth chart (GA 22-50 weeks), first published in 2003 and generated by combining growth data (foetal and postnatal) until term from four studies (12). To enable preterm infants to be tracked until the age of 50 weeks these charts were combined with year 2000 CDC postnatal growth data by linear extrapolation of all percentiles simply by drawing a straight line from 34 to 50 weeks (13). These growth curves were revised in 2013 by adding growth data from additional studies, which allowed gender specific growth references to be described. These charts have further been updated by merging the WHO 2006 postnatal growth data to enable preterm infants to be tracked until 50 weeks and thereby applying the same straight-line principle. Simple linear extrapolation gives a bold picture on how new-borns may transition into infancy. However, this principle ignores the impact of postnatal

extracellular water contraction physiology on an individual postnatal weight curve and the shape of a given percentile is therefore not optimally suited to precisely monitor growth during the first weeks after birth.

In 2017, the WHO published a multinational longitudinal study with standards on foetal growth estimated from in-utero ultrasound studies from 10 countries including 1319 foetuses. Data were collected between 2009 and 2014 and estimated foetal growth showed considerable inter-individual variation with significant differences between countries (14). In 2017, the INTERGROWTH-21 Project also published a foetal growth standard using longitudinal data on estimated weight derived from one- or two-dimensional in-utero ultrasound measurements performed in 8 countries including 1556 foetuses (15). The estimated foetal weights are slightly higher in the WHO foetal growth charts compared to INTERGROWTH-21. WHO growth charts for estimated weights are available regardless of gender but also gender-specific, while INTERGROWTH-21 has published a single foetal chart regardless of gender. Growth velocity based on ultrasound estimations also has limitations due to potential limited accuracy, but these intrauterine estimates can guide clinicians evaluating growth in a stable preterm infant. According to WHO in-utero data, estimated average foetal weight gain will be 20-23 g/kg/d (12-16 g/d) from 23-25 weeks postmenstrual age (PMA), 17-20 g/kg/d (17-23 g/d) from 26-29 weeks PMA, 13-17 g/kg/d (24-30 g/d) from 30-34 weeks PMA and 10-13 g/kg/d (30-32 g/d) from 35-37 weeks PMA.

Postnatal growth trajectory in preterm infants

Both term and preterm born infants lose weight during the first days of life, primarily due to weight loss from a once-only irreversible contraction of extracellular fluid space as part of a physiological transition from intra- to extra-uterine life. In term-born infants, normal metabolic adaptation occurs in the context of an abrupt cessation of placental macronutrient supply and temporary absence of tissue growth. The WHO term growth charts do not illustrate this weight loss on their charts, but a weight loss of 7-10% is probably acceptable for both term and preterm infants (2). Since SGA born infants lose less or sometimes even demonstrate weight gain, a weight loss of 4-7% will probably be acceptable. Recommending an acceptable loss in centiles or SDS is challenging since it depends on an infant's GA, BW and the growth reference / standard used. E.g., using WHO in-utero estimated weight data, an acceptable loss from BW will be (0.8 to) 1.0 SD (10%) in an appropriate for gestational age (AGA) infant and (0.4 to) 0.6 SD (7%) in an SGA infant, both receiving adequate nutrition.

Term born infant growth trajectory can be evaluated according to the actual centile or standard deviation (SD) once postnatal adaptation is completed and postnatal growth has been established. However, in preterm infants the optimal duration (and extent) of any catabolism, and/or the period until full macronutrient needs are met using parenteral or enteral nutrition, is unclear. In preterm infants, one "growth trajectory that fits all" will not reflect the individual physiology of postnatal adaptation.

Providing adequate nutrition, the weight nadir is usually reached at 3-4 days of age and after 4 days growth should normally follow the infant's current centile (16), with birth weight being regained around 7-10 days of age.

The WHO foetal chart describes considerable variations in foetal growth and birth weight even under optimal conditions, likely due to a range of genetic, epigenetic and maternal factors (14). INTERGROWTH-21 reports differences between in-utero estimated weight and respective

postnatal weight trajectories at corresponding gestational ages suggesting that preterm postnatal growth is a different biological process compared to in-utero growth (15).

There are a variety of approaches that can be used to determine the optimal growth trajectory or target percentile for preterm born infants. In 2015, an ESPGHAN expert group pragmatically recommended that the aim of postnatal growth is to avoid losing more than 1 SDS in weight and HC from birth to discharge (17). However, calculating changes in SDS from birth to discharge is confounded by skewed reference data (18), and even more for the more immature infants growing on lower centiles, which is due to an over representation of intrauterine pathologies resulting in restricted growth that can be associated with preterm termination of pregnancy. Other researchers have considered whether infants should regain their actual centile at birth, and then continue along this centile, or above, once growth has been established. Cole et al demonstrates a weight gain in very preterm infants during the first postnatal weeks, and the group is questioning using the infants target centile at birth, since this will require a rapid weight gain that could potentially be harmful to the infants (19). Two studies based on data obtained from a large population of preterm infants with a relatively straightforward postnatal transition (reflecting growth among selected healthy preterm infants) recommend that the infant's target centile should not be based on birthweight but based on weight at 1-3 weeks of age, once weight gain has stabilised (3, 4). The rationale for this is to individualise the approach and achieve appropriate growth following a period with acceptable weight loss (4).

Both INTERGROWTH-21, WHO foetal, and the above-mentioned references recommend preterm born infants after term PMA to be followed longitudinally using postnatal WHO-term age growth charts (4). For preterm infants, that did not deviate from their genetic growth potential or born very growth restricted, a gradual transition to the corresponding percentiles on WHO-term growth chart is recommended within the first weeks or months post term.

Intrauterine/fetal growth restriction (IUGR/FGR) and small for gestational age (SGA)

The terms SGA, intrauterine growth restriction (IUGR) and foetal growth restriction (FGR) is often used synonymously, but it is important to note that SGA only refers to a statistical definition based on gestation (and sometimes gender) specific birth weight distribution across a given percentile or SDS and may occur in the absence of IUGR. Conversely, IUGR may not result in an infant being SGA. An antenatal diagnosis of IUGR/FGR usually includes an abnormal umbilical artery doppler blood flow profile in addition to reduced growth velocity during foetal life (20). Since not all infants are antenatally detected as growth restricted prior to birth, a new consensus-based definition of growth restriction in new born infants has been developed that uses birthweight <3rd percentile or -2 SDS, or alternatively requires the presence of at least 3 of the following parameters: birth weight <10th percentile, HC<10th percentile, length <10th percentile, prenatal diagnosis of IUGR/FGR or maternal illness such as hypertension or pre-eclampsia (21).

Growth patterns in preterm SGA infants seem to be different from AGA infants: typically, they lose no or less weight in the first days of life, and frequently catch-up crossing above -2 SDS for weight before 2 months corrected age (CA), although full height catch-up is often not seen until 6-12 months CA (22). The optimal time frame and speed of catch-up growth of preterm SGA infants are not known. Concerns on the risk of obesity in childhood especially in SGA infants has been raised. Two recent studies on supplementation, growth, cognitive and metabolic outcome, based on individual participant data meta-analysis with data from more than 2000 preterm

infants, did not find an increased metabolic risk in childhood among preterm SGA born infants (23, 24).

Postnatal growth faltering and how to handle poor growth

Growth faltering (GF) describes an infant whose growth slows such that they do not follow a certain centile during the period of established growth (and is not defined as any anthropometric measurement below the 10th percentile). GF is associated with poor neurodevelopmental outcomes in preterm infants (25) but is also more common in sicker infants. It has become increasingly clear that optimising nutrient intakes, together with interventions that reduce illness severity in the first few weeks of life can reduce growth faltering. Growth faltering and poor neurodevelopmental outcomes are especially common in preterm infants with necrotising enterocolitis (NEC) (26), but the extent to which poor long-term outcomes reflect initial illness severity as opposed to subsequent sub-optimal nutritional management and nutrient deficiencies is unclear. Preterm infants with GF are also at increased risk of other complications such as the development of metabolic bone deficiency (27).

It has been shown that cumulative nutritional deficits may be drastically reduced in both extremely and very preterm born infants after optimising nutritional policy during the first weeks of life, and that GF could even be prevented (28-30). Early optimised and appropriate nutrition, both parenteral and enteral, together with adequate provision of own mother's milk may improve neurodevelopmental and visual outcome in very preterm infants (31-35). Improved growth is also associated with improved lung function (36), a lower risk of bronchopulmonary dysplasia and pulmonary hypertension (37). GF is though multifactorial, including genetics, and weight gain alone will not improve neurodevelopmental outcomes.

Regular growth monitoring and standardised approaches to the management of an infant with GF in the NICU are likely to be helpful, but these will need adapting to the specific individual circumstance. Potentially correctable nutritional explanations (that may also occur in combination) in decreasing likelihood of occurrence for GF in the NICU include:

1. Inadequate macronutrient intakes due to suboptimal or unbalanced fortification of breastmilk or use of artificial formula milk not designed for preterm infants
2. Electrolyte imbalance, especially sodium, but occasionally potassium and chloride
3. Acid base disturbances due to alkali imbalance or excessive renal osmolar load
4. Anaemia secondary to blood losses or iron-deficiency
5. Increased nutrient demands (e.g., energy intakes in infants with BPD), poor gastrointestinal absorption (e.g., fat malabsorption due to loss of bile-salt stimulated lipase in pasteurised breastmilk) or increased losses (e.g., ileal stoma losses post-NEC)
6. Inadequate or excessive energy-protein ratios
7. Inadequate micronutrient, mineral or vitamin intakes especially phosphate and zinc, but note that assessment of micronutrient status in acutely ill infants is challenging and can be misleading (38)
8. Hormonal imbalance e.g., thyroid or parathyroid deficiency, rarely due to low iodine intake, renal problems or poor exocrine pancreatic function

Catch-up growth in preterm infants

Catch-up growth or recovery growth is usually defined as a period with accelerated rates of growth following a period of growth faltering, in order to reach the growth of a normal foetus or infant (39). Catch-up growth in preterm infants may occur before or after hospital discharge, and is also considered to be a physiological and compensatory process following a period of restricted growth that aims to restore the infant to its optimal growth trajectory. However, there are concerns that rapid catch-up growth may increase the risk of cardiovascular and metabolic disease in later life especially when it is due to catch-up in weight without contemporaneous linear or head growth. Changes that occur during catch-up growth have shown to alter gene expression and may result in a higher risk of metabolic diseases in later life. This has been well demonstrated in animal studies (40), but also described in several long-term follow up studies in term as well as preterm or very low birthweight infants (41). There are no data that allow clinicians to determine the optimal degree or duration of catch-up growth in an individual infant. Careful consideration must be used in balancing the well-documented neurocognitive risks of nutrient deficiencies and slow growth in early life, against the theoretical risks of adverse metabolic programming in later life.

Conclusion

There is still insufficient data to determine the optimal pattern of growth for preterm infants in terms of both quantity and quality that balances the goal of achieving an optimal neurodevelopmental outcome with the theoretical risks of metabolic diseases in later life.

Conclusions:

C1: Based on current evidence, the optimal growth velocity that optimises outcomes in preterm infants remains unclear. LOE 2+

C2: According to WHO in-utero growth foetal weight gain decreases from slightly above 20 g/kg/d at 23-25 weeks of gestation to about 10 g/kg/d at term age (LOE2++).

Recommendations:

R1: Regular monitoring of weight, length and HC growth is strongly recommended. Ideally, weight should be measured at least once or twice daily during the first 1-2 weeks, followed by measurements 2-3 times weekly in the stable growing phase. Length and HC should be measured once weekly unless clinical conditions (e.g. hydrocephalus) indicate more frequent monitoring (GPP)

R2: After a typical acceptable initial weight loss of 7-10%, reaching a nadir at day 3-4, nutritional strategies should aim to regain birth weight by 7-10 days of age, followed by growth along a target centile and a gradual transition to the corresponding birth percentile on the WHO postnatal growth chart within the first weeks or months post term (GPP)

R3: Nutritional management and growth assessment for infants born IUGR/SGA should be the same as those born AGA, although initial weight loss is often less and acceptable up to 4-7% of birth weight. (GOR B)

R4: Postnatal growth trajectories (weight, length and HC) of each infant must be followed and evaluated to ensure nutrition is adequate; ideally using a growth chart based on a large robust dataset (GPP)

R5: In infants experiencing postnatal growth faltering, some catch-up growth should be allowed but rapid catch-up growth should be avoided. If catch-up growth is perceived as too rapid, ensure that nutrition is within recommended intake and not excessive (GPP)

R6: NICUs should adopt a standardised approach to the management of postnatal growth faltering. If growth faltering is recognized, ensure that nutrition is within recommended intake and not inadequate. Careful consideration must be used in balancing the well-documented neurocognitive risks of nutrient deficiencies and slow growth in early life, against the theoretical risks from rapid catch-up growth and adverse metabolic programming in later life (GPP)

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Supplementary Digital Content no. 16: Breast milk (Buccal colostrum, donor human milk, and pasteurisation of mother's own milk to reduce Cytomegalovirus transmission)

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants

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Short summary

While the administration of buccal colostrum to premature infants appears safe and theoretically attractive from both an emotional as well as immunological point of view, no clear clinical benefits have consistently been proven in high-resource settings. There is therefore no current data to recommend routine administration of buccal colostrum to premature infants in order to reduce morbidity or mortality.

Fresh mother's own milk (MOM) contains higher amounts of macronutrients, and immunoactive and trophic factors than Holder-pasteurised MOM or donor human milk (DHM). Nevertheless, fortified pasteurised DHM instead of preterm formula may reduce necrotising enterocolitis rates in preterm infants, whereas other neonatal morbidity and mortality rates are unaltered. We do strongly recommend MOM as the first choice of feeding in both preterm as well as term infants. In case of insufficient MOM availability, however, fortified DHM is conditionally recommended over preterm formula in preterm infants born <32 weeks gestation or with a birth weight <1500 g. When providing DHM, health care providers must continue to increase awareness of the benefits of MOM over both DHM and preterm formula.

The vast majority of Cytomegalovirus (CMV) seropositive women undergo CMV reactivation in breast tissue during lactation and excrete CMV in their breast milk, which may cause (sub)clinical CMV transmission in approximately 15-20% of very preterm infants, although rates are possibly higher in extremely preterm infants. Symptomatic postnatal CMV infection, presenting as thrombocytopenia, cholestasis, or sepsis-like illness occurs in less than half of infected infants; whereas associations with development of bronchopulmonary dysplasia, necrotising enterocolitis, and adverse neurological sequelae are less clear. Whilst Holder-pasteurisation will effectively eliminate CMV from breast milk, it also reduces or completely destroys many beneficial and important bioactive factors. There is insufficient evidence whether potential sequelae of postnatal CMV transmission are more harmful than potential adverse effects arising from providing pasteurised instead of fresh MOM. Thus, while we acknowledge the potential adverse consequences of postnatally acquired CMV, especially in the most immature infants, we do not recommend pasteurising MOM from CMV-positive women as pasteurisation simultaneously reduces the beneficial effects of fresh MOM.

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Extended version

Buccal colostrum

Human breast milk and amniotic fluid contain a vast array of immunoprotective, trophic, and other bioactive factors which stimulate the immune system and protect against infection. Ordinarily these fluids are in close contact with the oropharyngeal-associated lymphoid tissue, but after very preterm birth the oropharynx is bypassed through the use of oro- or nasogastric tubes. Oropharyngeal tissue however, may play a critical role in developing the immune system. Colostrum, especially that expressed from women who delivered preterm, has very high concentrations of immunological and other possible protective factors (1, 2). Therefore, small quantities of buccal or oropharyngeal colostrum could be protective by stimulating local lymphoid tissues, mucosal uptake, and local barrier protection against pathogens (3).

Several RCTs in the last decade have explored this issue. Typically, small volumes (0.2 – 0.5 mL) of mother's own colostrum are administered every few hours in the mouth/cheek during the first few days or week of life. This practice must be seen separately from the practice of early oral feeding, as discussed in SDC no 13. Trials on buccal colostrum have recently been summarised in several systematic reviews and meta-analyses (4-8). Despite slightly differing inclusion criteria (e.g. Panchal et al. also included cohort studies (6)), three of them concluded there were no clinical benefits of applying oropharyngeal colostrum to preterm infants, as mortality, sepsis, and NEC rates were all unaltered. However, this conclusion was based on 6 RCTs only including 335 infants in total in the Cochrane review as an example (5). Time to reach full enteral feeding appeared to be reduced, although there was high heterogeneity and this conclusion was considered as very low quality evidence (5). The most recent two meta-analyses included 3 additional studies published since 2019 so that up to 689 preterm infants were summarised (7, 8). With these additional data the relative risks (with 95% confidence intervals) for mortality, sepsis, and NEC were 0.63 (0.38–1.05), 0.78 (0.60–1.03), and 0.59 (0.33–1.06), respectively (7). However, a subgroup analysis by the authors revealed that the positive results were mainly derived from studies conducted in low- and middle-income countries. Very recently, a new RCT from India was published, where 260 infants (mean birth weight 1260 g) were randomised to receive buccal colostrum or not (9). In this trial, the largest to date, no clinical benefits of this practice were reported. On the other hand, no adverse effects, such as aspiration pneumonia, were reported in any of the studies, although the total number of studied infants remains relatively low. Multiple trials are currently underway which may change conclusions in the near future. Apart from potentially beneficial direct somatic effects, there are indications that oral care with mother's own milk (including colostrum) increases parental involvement, and thus may indirectly increase maternal milk

provision although this was studied in a different neonatal population (infants with congenital diaphragmatic hernia) (10).

C1: While the administration of buccal colostrum to premature infants appears safe and theoretically attractive from both an emotional as well as immunological point of view, no clear clinical benefits for the infant have consistently been proven in high-resource settings (LOE 1-).

R1: No recommendation can be made either for or against the use of buccal colostrum in preterm infants in order to reduce neonatal morbidities or mortality.

Mother's own milk and donor human milk

For obvious reasons, both ethical as well as practical, no RCTs compare mother's own milk (MOM) to artificial formula milk feeding (11). Human milk is considered the gold standard (12-14), especially for premature infants provided it is adequately fortified where required (see other sections). Compared to formula feeding, MOM is associated with lower rates of several neonatal morbidities including necrotising enterocolitis (NEC) and perhaps also bronchopulmonary dysplasia (BPD) (15-17). Additionally, there appears to be an independent beneficial positive effect on long-term neurodevelopmental outcome (18-21), despite slower in-hospital growth compared to artificial formula-fed infants, often termed the 'breast-feeding paradox' (22). In the last 15 years, donor human milk (DHM) has regained interest and is used to make up a shortfall in the supply of MOM. Since then, several new trials have been conducted (23-27). DHM, however, is usually expressed from women who delivered a term-born infant several months before, and therefore has lower macronutrient and bioactive factor contents compared to milk expressed at earlier stages (28-30). Besides, during DHM preparation, multiple plastic container changes are required, which probably forms the main reason in the DHM preparation process why fat content is about 10% lower when compared to expressed human milk (31-33). In addition, almost all donor milk banks (DMB) pasteurise donated milk to eliminate bacteria and inactivate viral shedding including maternal reactivated cytomegalovirus (CMV) or human immunodeficiency virus (HIV) infection, although some DMB provide raw donated milk after extensive maternal screening (34-36). Pasteurisation also inactivates several growth hormones and digestive enzymes such as bile-salt stimulated lipase, further impairing enteral fat uptake (37), and many (although not all) immunological and other bioactive factors are significantly lowered or completely abolished as well (38-40). It is unknown whether these differences explain why in several studies the faecal microbiome from infants that receive DHM does not resemble the microbiome of infants consuming MOM (41, 42), since other studies show more resemblances (43).

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A recent Cochrane review update, based upon 12 RCTs including 1879 infants, concluded that there was moderately certain evidence that the use of DHM compared to formula resulted in lower rates of NEC, whereas mortality and infection rates were similar (44). Growth, including head circumference, however, was slower in those fed DHM. Most of the 12 RCTs were conducted in the 1980s, and only 4 RCTs with 955 infants (born at <30 wks gestation or with birth weight <1250 or 1500 g as inclusion criteria) compared preterm formula (as opposed to term formula) with fortified DHM (instead of unfortified DHM). Based on only these latter trials, the main conclusions were unaltered (although less strong), with a lower risk ratio for NEC stage 2 or 3 of 0.61 (95% CI: 0.38 – 0.97) when feeding fortified DHM versus preterm formula, with a number needed to treat of 29 (95% CI: 15 – 355). Another meta-analysis assessed surgical NEC rates specifically in these 4 trials and although the risk ratio for surgical NEC was lower with DHM (RR: 0.45; 95% CI: 0.19 – 1.09), it did not reach statistical significance, probably also due to low power because of rarity of NEC events (45). In a meta-analysis that assessed both cohort and randomised studies, it appeared that DHM-fed infants had lower BPD rates in the 8 cohort studies, though this was not confirmed in the RCTs (46). Long-term follow-up was assessed in only one RCT (24), suggesting that at 18 months' corrected age cognitive composite scores were similar, even after adjustment for background confounders (mean difference donor vs formula: -2.0 (95% CI -5.8 to 1.8)). Currently, at least 9 new trials are being planned, conducted, or finalised as reviewed elsewhere (47), randomising 2387 infants to DHM or preterm formula in case of insufficient MOM. Hopefully, this will further determine whether DHM is truly beneficial in preventing NEC or other serious morbidity.

When the provision of DHM to (former) preterm infants may be stopped and replaced by preterm of post-discharge formula in case of insufficient MOM, is nearly impossible to answer from a scientific point-of-view. Obviously, risk of NEC and other morbidities decrease with advancing age, so that within the local setting DHM availability, supplies, and costs must be balanced against the advantages of DHM in the absence of MOM.

A 2016 systematic review assessed whether implementation of a DHM programme altered hospital rates of MOM usage (48). Availability of DHM could theoretically lead to reduced MOM rates due to introduction of an acceptable alternative but could also lead to increased awareness of the benefits of human milk. Based on limited data, no clear changes of MOM rates were seen (48). Later that year a very large data registry from over 40,000 preterm infants showed that MOM usage increased over time between 2007 and 2013, both in hospitals that implemented DHM and in hospitals that still used preterm formula in the absence of MOM (49). Additionally, it was shown that NEC rates decreased in all hospitals, irrespective of initiation of a DHM programme, although the lack of DHM in a hospital was a positive predictor of NEC (OR: 1.15; 95% CI: 1.03–1.28), after adjusting for confounders (49). Three recent smaller cohorts showed lactation success rates to either decrease over time or remain unaltered after implementing DHM on the NICU (50-52). To conclude, overall,

most studies reported either no or a positive impact of DHM availability on breastfeeding rates when DHM is an available alternative. It seems prudent to monitor MOM rates and increase awareness on the differences between MOM and DHM in both parents as well as health care providers. Recently, a decision tree tool was developed to promote and support lactation rates in departments who simultaneously offer DHM (53).

High quality DHM costs may vary considerably from milk bank to milk bank, even if produced non-profit, and amount on average ~€150 per litre, but may range from €70 to €300 per litre (54, 55). This may be due to variation in the handled volumes per time period and operational running and transportation costs. Artificial preterm formula may be cheaper per litre, but if there is a true reduction in NEC rates, total costs from a societal perspective do not differ or might even be lower when providing DHM as a supplement compared with preterm formula in a high MOM use setting (55-57). The European Milk Bank Association (EMBA) has published recommendations for the establishment and operation of donor milk banks (36, 58). Currently, there are no trials comparing the effect of banked donor preterm milk compared to banked term milk regarding growth and developmental outcomes in preterm infants (59). New methods of pasteurizing human milk, other than classical Holder-pasteurisation (30 min at 62.5°C), are currently being investigated, but are not yet recommended for routine practice (60). The optimal methods for handling both MOM and DHM (from expression, to storage and preparation in the local milk kitchen) for administration to preterm infants are out of the scope of these nutritional guidelines and are described elsewhere (61-64).

C1: Fresh MOM contains higher amounts of macronutrients, and immunoactive and trophic factors than Holder-pasteurised DHM (LOE 1++).

C2: Fortified pasteurised DHM instead of preterm formula milk reduces NEC rates in preterm infants, whereas other neonatal morbidity and mortality rates are similar (LOE 1+).

R1: We strongly recommend MOM as the first choice of feeding in both preterm as well as term infants (GOR A).

R2: In case of insufficient MOM availability, fortified DHM is conditionally recommended over preterm formula milk in preterm infants born <30 weeks gestation or with a birth weight <1500 g (GOR B).

R3: When providing DHM, health care providers must continue to increase awareness of the benefits of MOM over both DHM and preterm formula. Health care providers must support and facilitate mothers as much as locally possible in order to promote higher rates and volumes of MOM provision (e.g. through lactation consultants) (GOR A).

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Pasteurisation of own mother's milk to reduce cytomegalovirus transmission

Congenital cytomegalovirus (CMV) infection results in serious adverse outcomes but fortunately occurs infrequently. In contrast, postnatal CMV transmission occurs relatively frequently in preterm infants, mainly by transmission from their mother's own milk (MOM). The precise risks and clinical consequences of this route of CMV transmission for an individual preterm infant remains unclear. While pasteurisation of MOM may be effective to omit this transmission risk, this practice comes with disadvantages as well as outlined below, so that the pros and cons of pasteurizing MOM must be evaluated.

Approximately 50% of all women of childbearing age in Western-European countries are CMV-seropositive, but seroprevalence ranges from 30% to 90%, influenced by socioeconomic status and origin of the study population (65, 66). During lactation, for reasons that are not entirely clear, the vast majority of these women undergo virus reactivation in breast tissue and shed virus in their breast milk (66-68). Breast milk is thus an important vehicle for postnatal vertical CMV transmission, although simultaneously, breast milk itself, and especially colostrum, has considerable but variable antiviral activity against CMV thereby potentially reducing neonatal infection (69, 70). Prior placental transport of maternal CMV immunoglobulins-G (IgG) protects against symptomatic CMV infection in term-born infants but infants born preterm partially miss out on this period of passive immunisation and are thus more vulnerable.

A systematic review from 2010 based on 12 studies and 479 infants showed that CMV could be detected in breast milk in a median of 87% of CMV-IgG-positive mothers who had delivered preterm (71). In these studies, CMV could be detected in breast milk at the earliest at day 8 of life, although more recent studies detected CMV in one-third to half of colostrum or first-week milk samples from CMV-seropositive women (68, 72-75). CMV-shedding in milk peaks at around four weeks postpartum and diminishes after 2 to 3 months (66-68). CMV transmission rates (defined as urinary CMV-shedding in their infants) was around 20% in the systematic review from 2010 (71) and 16% in another meta-analysis from 2013 that included 17 studies and 695 infants (76).

Sequelae of CMV infection transmitted via breast milk are difficult to separate from other complications arising from premature birth. Postnatal CMV infection in preterm infants may be asymptomatic or present as thrombocytopenia, neutropenia, hepatobiliary complications (e.g. hepatitis or cholestasis (77)), and sepsis-like illness (78). The risks of symptomatic disease in the preterm infant were estimated at 3.7 and 6.6% of CMV-positive infants in the aforementioned systematic reviews (71, 76), of which sepsis-like symptoms constituted 0.7% and 2.7%, respectively. More recent cohort studies, however, showed either lower (66-68, 73, 79) or higher (80-82) transmission and disease rates, especially in infants born below 25

weeks gestational age. Moreover, postnatal CMV transmission has also been associated with development or worsening of BPD (83, 84), NEC (73, 85, 86), or even death (83), although not all studies are consistent (81, 87-91).

Several long-term follow-up studies of postnatal CMV transmission reported no adverse functional neurological outcomes including hearing loss up to age 6y (92-94) although other cohort studies described lower cognitive and motor scores at toddler age (95) and school-age (96), and lower IQ-scores in adolescence (97) compared to non-infected individuals. Importantly, in the small cohort of postnatal CMV cases in preterm infants <25 weeks gestation, there was a trend towards more hearing problems (80). Although two other reports did not detect statistical differences from neurological clinical assessment, MRI brain-scanning showed microstructural changes in the occipital white matter (98) and differences in activation using functional MRI of key brain regions, as well as differences in grey matter volumes within the activated brain regions (99).

To conclude, postnatal CMV transmission may occur in 15-20% of preterm infants born to CMV-seropositive women after breastfeeding. Although not all data are consistent, infection may complicate neonatal clinical course, and possibly also affect later neurodevelopmental outcomes as also recently summarised in a systematic review (100). Risks for adverse effects might be highest in the most immature infants, perhaps because they are the least protected by placental CMV-IgG transport. Contrary to the many benefits of MOM, fresh MOM thus simultaneously poses a potential risk for subclinical or symptomatic CMV infection, with possibly long-term adverse effects in some infants. This has prompted many NICUs to process MOM in order to reduce CMV transmission rates. Freezing milk for at least 3 days at -20°C might reduce CMV titres (72), although this strategy may not be fully effective (101). Similarly, a reduction in CMV transmission was seen after freezing in a retrospective cohort (79). On the other hand, in a small RCT comparing provision of only freeze-thawed MOM versus a combination of both raw and freeze-thawed MOM, transmission rates of those with CMV-DNA-positive milk (n=66 infants in total) were 8% and 6% respectively, and none of the infants developed clinical disease (67). Currently, freeze-thawing of MOM does not appear to be an effective strategy to avoid CMV transmission.

A more rigorous approach has been advocated or employed in several countries for very or extremely premature infants, by providing only fresh colostrum and pasteurising all subsequent MOM from CMV-seropositive women (62, 64, 102). However, there is a lack of studies comparing either fresh or routinely pasteurised MOM of either all women or just women who are CMV-seropositive. Only 1 RCT and 4 cohort studies have compared these practices (see Table 1). In summary, there are no clear clinical advantages of Holder-pasteurising MOM in order to prevent neonatal morbidity despite the potential reduction in postnatal CMV transmission. Although Holder-pasteurisation (30 min at 62.5°C) is effective for CMV elimination it also reduces or completely destroys many beneficial and important bioactive factors, although other beneficial molecules seem less effected (38-40). As an

alternative to Holder-pasteurisation, short-time pasteurisation (5 to 15 sec) at 62.5 to 72°C has also been advocated as this approach might be as effective in CMV reduction, while being less harmful for beneficial bioactive factors and nutrient content (60, 74, 103). However, this practice has not been systematically evaluated in the setting of preventing CMV-related disease and ameliorating outcome. It is important therefore to balance potential benefits from a reduction in CMV transmission against the risk of decreasing the many proven benefits of fresh MOM. In addition to the considerable economic and resource costs of pasteurisation, there may also be effects on maternal perceptions and behaviours that negatively impact on continued expressed breast milk provision when addressing their milk as potentially contagious.

Table 1: summary of studies comparing provision of either fresh MOM or Holder-pasteurised MOM to premature infants and their outcomes

First author, year, country, study type (ref)	**Control group**	**Intervention group**	**Main outcomes**
Cossey, 2013, Belgium, RCT (104)	Fresh MOM; n=151	Holder-pasteurising all MOM; n=152	No clear benefits or adverse effects (trend towards less late-onset sepsis (p=0.23), less BPD (p=0.15), and shorter length of stay (p=0.14) in group with fresh MOM). Fresh MOM which was contaminated with pathogenic bacteria was discarded and replaced by formula. CMV was not demonstrated, nor systematically assessed in women, their milk or infants
Stock, 2015, Austria, cohort study (2 epochs) (105)	Holder-pasteurising all MOM; n=159	Fresh MOM; n=164	Similar morbidity rates, although there was a trend towards more NEC in the pasteurised milk group 4.4 vs 2.4% (p=0.25). Postnatal CMV was screened in 52 infants, who were all non-symptomatic: transmission was seen in 11/28 infants who received unpasteurised milk, and 1/24 in those fed pasteurised milk (p=0.008).
Yoo, 2015, Korea, cohort study (2 epochs) (106)	freeze-thawing all MOM (3 days at -20°C); n=301	Holder-pasteurising all MOM; n=56	In the control group, 9% of infants were screened CMV-seropositive. Most of these infants had laboratory abnormalities and higher odds for BPD, although they also had a gestational age of 2 weeks less than uninfected infants. At 2 years corrected age, neurodevelopmental outcome including hearing loss was not different between infants with CMV transmission or not
Dicky, 2017, France,	NICUs with policy of fresh	NICUs with policy of Holder-	The odds for developing BPD were more than halved after adjustment for background variables if fresh MOM is provided. Incidences of other morbidities were similar.

cohort study (107)	MOM; n=636)	pasteurising all MOM; n=290)	No info on CMV transmission or whether it was tested.
Sun, 2019, China, prospective cohort (108)	Donor milk or frozen MOM which was also Holder-pasteurised in CMV-seropositive women; n=109	At least 1 daily portion of fresh MOM (upon maternal agreement); n=98	None of the infants were infected with CMV, though no information was given on whether infants were routinely tested or by which methods. Besides, information is missing how many women in both groups were actually CMV-seropositive and of whom had their milk pasteurised in the control group or how many infants received DHM. Clinical outcomes included improved in-hospital growth and a lower incidence of several neonatal diseases upon receiving fresh MOM, although baseline characteristics differed between groups (e.g. more antenatal steroids in intervention group).
De Halleux, 2019, prospective cohort (109)	Holder-pasteurised MOM; n=41	Raw MOM; n=15	The indication of pasteurisation of MOM was either a CMV-positive mother or bacterial contamination of MOM. Mean weight gain was higher in the infants that received predominantly raw MOM (21.1 (SD 1.6)) versus those that predominantly received pasteurised MOM 19.1 (SD 1.8).

C1: Nearly all CMV-seropositive women undergo CMV reactivation in breast tissue during lactation and excrete CMV in their breast milk, which may cause (sub)clinical CMV transmission in approximately 15-20% of very preterm infants, although rates may be higher in extremely preterm infants (LOE 1+).

C2: Symptomatic postnatal CMV infection, presenting as thrombocytopenia, cholestasis, or sepsis-like illness occurs in a minority of infected infants although associations with BPD, NEC, and adverse long-term neurological sequelae are less clear (LOE 1-).

C3: Whilst Holder-pasteurisation will effectively eliminate CMV from breast milk, it also reduces or completely destroys many beneficial and important bioactive factors (LOE 1++).

C4: There is insufficient data to determine whether the potential sequelae of postnatal CMV transmission are more harmful than potential adverse effects arising from providing pasteurised instead of fresh MOM (LOE 2+).

R1: While we acknowledge the potential adverse consequences of postnatally acquired CMV, especially in the most immature infants, there is insufficient evidence to recommend routine pasteurisation of MOM from CMV-positive women as pasteurisation simultaneously reduces the activity of many bioactive factors (GOR B).

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Supplementary Digital Content no.17

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Osmolarity and hydrolysed protein (February 2022)

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1 Osmolality

In 1974 Linda Brook et al. suggested that the hypertonicity of a new elemental formula (650 mOsm/l) may have contributed to an increased incidence of necrotizing enterocolitis (NEC)[12]. Then main cause of the osmolarity was the replacement of disaccharides by glucose which increased the osmolarity by 221 mOsml/l and accounted for 488 mOsml/l of total osmolality. In the same year, Robert de Lemos et al. published data suggesting that 1.5 times concentrated mothers milk induced experimental NEC in newborn goats[3]. Finally, Santulli et al. suggested that hyperosmolarity may be an additional factor in causing NEC based on a case series involving 64 surgical and autopsy specimens[4]. Prompted by these studies in 1976 the American Academy of Pediatrics (AAP) recommended that the osmolarity of infant formula should not exceed 400 mOsm/l (approximately equivalent to an osmolality of 450 mOsm/kg)[5]. However, a recent review on milk osmolarity by Freya Pearson et al. found[6]: 1) no causal relation between the osmolality of nutrients and the development of necrotizing enterocolitis; 2) a delay in gastric emptying if osmolality exceeded 450 mOsm/kg, and 3) that the osmolality of commonly used milk feeds was below the AAP recommendation threshold (<450 mOsm/kg).

In 2010, the ESPGHAN Committee on Nutrition position paper on the composition of the enteral nutrient supply of preterm infants[7] suggested higher protein intakes could be met using fortification of human milk. However, an editorial questioned the safety of these recommendations because of the increase in osmolarity or osmolality above the recommended AAP threshold[8]. In general, breast milk fortification increases the osmolality of the milk immediately after addition (by up to more than 50%), followed by additional smaller

increases in osmolarity (up to 10%) after storage at 4°C for up to 24 h[9-13]. Target fortification of breast milk can increase osmolality up to 376 +/- 66 mOsm/kg[14] and 436 +/- 13 mOsm/kg[15] without documented adverse events. Osmolarities >400 mOsm/l or osmolalities >450 mOsm/kg i.e. exceeding AAP recommendations, have occurred when extra protein or extra HMF has been added to standard fortified breast milk[10 11 13 16]. High osmolality measurements have also been reported with certain NICU practices such as adding powdered preterm formula or a glucose polymer solution to breast milk[17]. Recently it was reported that several common feeding formulations used in the US exceeded 450 mOsm/kg[18]. Given the lack of evidence of adverse effects it was concluded that the current AAP maximum osmolality guidelines should be reevaluated in order to enable optimal nutrition for preterm infants[18]. A recent systematic review on milk feed osmolality and adverse events in newborn infants and animals did not find any consistent evidence that differences in feed osmolality in the range 300–500 mOsm/kg are associated with adverse gastrointestinal symptoms in neonates[19]. However, significant differences in other aspects of feed composition occurred alongside the differing osmolality levels making the interpretation of results regarding the independent impact of osmolality challenging[19]. Linear relationships between the amount of macronutrients added to human milk and the final osmolality have been reported[20]. One forward approach could be for individual NICUs to know the osmolality of the final milk they give to preterm infants.[20].

Routine additives and medications can significantly increase the osmolality of breast milk to levels that exceed current guidelines and may also pose a risk in preterm infants[11 21 22]. Of note, not all osmolality created is equal. Molecules that contribute to osmolality measured by an osmometer but cross cellular membranes and do not set up an osmotic gradient, are less likely to create epithelial stress that may be a potential risk factor for NEC[23]. However, we consider it prudent to dilute additives in the largest possible volume of feed, to use multi component breast milk fortifiers in preference to multiple individual supplements, and to avoid simultaneous addition of multivitamins, electrolyte solutions, or other high osmolar substances where possible.

2 Hydrolyzed protein

Hydrolyzed protein has increasingly been made available for preterm infant feeding over the last 20-30 years. Epidemiological data suggest that human milk feeding reduces the risk of NEC, and many NICUs use donor human milk where there is shortfall in supply of mother's own milk (MOM) but some NICUs use formula to make up any shortfall. There is, however, no agreement as to whether avoiding whole cow milk protein milks and using a hydrolysed product is beneficial. Human milk evolved over millennia and is primarily a whole-protein milk although it also contains some peptides and free amino acids[25]. Human milk is whey predominant, whilst some preterm formula milks contain more casein, although many breastmilk fortifiers are composed of hydrolyzed whey.

In term infants hydrolyzed protein formulas have been used for prevention or therapy of allergic disease but strong data on efficacy are lacking. Protein hydrolysis alters amino acid

kinetic, reabsorption, first pass, utilization by the gut, and oxidation. Studies conducted in the 1990s showed that protein hydrolysates were associated with reduced nutrient utilization especially for nitrogen[26] but these formulas are generally regarded as safe and no adverse effects on growth or development have been demonstrated in term infants. Furthermore, the degree of protein hydrolysis varies between commercially available milk formula. For definition, formula where the predominant size of residual peptides are between 3000 and 10,000 kDa (kilo Daltons) are termed 'partially hydrolyzed', whereas 'extensively hydrolyzed' refers to formula containing peptides between 1500 and 3000 kDa. Breastmilk fortifiers often use hydrolysed whey protein. It is possible that the degree and range of Dalton sizes affects outcomes, but no data exist in preterm infants. Furthermore, in preterm infants there are no good data suggesting benefit for allergy prevention[27].

In the 1980s and 1990s, hydrolyzed protein infant formula milk designed for preterm infants were introduced primarily because many thought early feeding tolerance might be better in small preterm infants. Whilst some hoped they might be associated with lower rates of other complications e.g., NEC, no adequately powered studies were conducted[28]. Several well conducted metabolic studies using timed stool collection periods, and randomized controlled trials, consistently show faster gastrointestinal transit with hydrolyzed protein formulas[29 30 31]. Equivalent nitrogen and mineral retention were observed when the protein concentration of the hydrolyzed formula was increased by 10%[30], and more recent randomized trials have shown improved protein utilization perhaps due to other changes in formulation or production in contrast to historical products using protein hydrolysates[32]. Virtually identical plasma amino acid profiles without obvious imbalances have been reported when comparing hydrolyzed and nonhydrolyzed protein preterm infant formula[30 33]. In the late 1990s the potential benefits of earlier introduction of enteral nutrition were increasingly recognized and one of the first randomized trials specifically designed to study this used a hydrolyzed protein formula if human milk was not available[34]. The trial had initially been designed to determine whether replacement of lactose by maltodextrin would further increase very early enteral feeding tolerance in these infants, but the data did not support this.

In VLBW infants on a strict standardized feeding protocol protein hydrolysis significantly accelerated the time of achieving full feeds (150 ml/kg/day, primary study outcome)[35]. The data of a more recent similar RCT in significantly more mature preterm infants suggest the same trend, however this trial was underpowered with regard to this particular outcome[36]. Owing to the concern of reduced nutrient bioavailability as suggested by earlier studies, Florendo et al. randomized healthy preterm infants already on whole protein formula feeding (>120 kcal/kg/d) to either hydrolyzed or non-hydrolyzed preterm infant formula and found no difference in growth[40].

Three additional observational trials all conducted in China and published in Mandarin were identified in our systematic search using pubmed. In the largest trial (n=692) extensively hydrolyzed protein formula (eHPF) was associated with a decreased incidence of NEC and fasting feeding advancement[41]. The second paper used eHPF when feeding intolerance was given or anticipated (n=328[42]) and feeding advancement was delayed in eHPF infants. The third trial (n=86[43]) concluded that standard protein formula accelerates feeding advancement in contrast to eHPF or human milk.

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Whilst hydrolyzed protein milks generally appear safe, the long-term health effects are unknown, and any modification of milk protein may be associated with potential health risks in susceptible infants. For example, in vitro cow's milk protein-derived amino acid modification products present in infant formulas can induce cellular stress[44] perhaps contributed to by faster gastrointestinal transit and absorption[29]. Some have also suggested that certain peptides created by artificial hydrolysis may have diminished or even harmful functional activities[28 45]. Another theoretical long-term risk is created by the presence of relatively high levels of "advanced glycation end-products" in most hydrolyzed formulas which have been associated in other settings with development of chronic inflammatory, metabolic, or neurodegenerative diseases[28 46]. Altogether however, the clinical relevance of these theoretical concerns is unknown. Finally, the more complex processing required to create safe hydrolyzed protein products adds substantially to costs.

On the other hand, faster achievement of full enteral feeding[35] may be associated with less nosocomial infections, less metabolic consequences of prolonged exposure to parenteral nutrition, and last but not least, lower costs. Therefore, more studies are needed[28]. In summary, protein hydrolysis increases the osmolality and may reduce nutrient bioavailability but may accelerate gastrointestinal transport and early enteral feeding advancement. Whilst the use of hydrolyzed preterm infant formulas appears generally safe, there is insufficient evidence to recommend routine use, no data to determine the optimal degree of hydrolysis (if indeed hydrolyzed formula are associated with clinical benefit) and no data to show routine use decreases the risk of NEC [28]. Further high quality, trials with long-term follow up are required[47].

3 Recommendations and Conclusions

C 1: The available evidence does not allow the definition of an upper safety osmolality threshold for enteral feeding of preterm infants. **LOE 2+**
C2: Commercial ready-to-feed milk formula with an osmolality that is at the upper end of the intake range may create challenges for clinicians who want to use additional supplements (e.g., iron, vitamins, sodium etc.) but avoid excess feed osmolality. **LOE 4**
C 3: Commercial milk formula differ in the degree of protein hydrolysis (range of Dalton sizes) which may be associated with differing functional effects. **LOE 3**
C4: In preterm infants hydrolyzed protein formula accelerates gastrointestinal transit and enteral feeding advancement but there are no data to show routine use improves long-term outcome. **LOE 1+**

R 1: Where supplements or other feed additives are given, these should be added to the largest possible volume of milk feed **GPP**
R2: Where breastmilk fortification is required, multi component fortifiers should be used in preference to multiple individual nutrient supplements. **GPP**
R 3: Hydrolyzed protein may be used for early enteral feeding in preterm infants if human milk is not available. **GPP**

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Supplementary Digital Content no.18

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Supplemental bionutrients, Lactoferrin, Choline, Milk Fat Globule Membrane, Human Milk Oligosaccharides, Bile-salt stimulated lipase, Lutein, Nucleotides. February 2022

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Introduction

Multiple components present in human breast milk (of which many are also present to varying degrees in bovine milk) have been explored as potential 'bionutrients' or 'immuno-nutrients' that could be added to enteral feeds in preterm infants. The list of potential milk bionutrients continues to increase, and includes proteins (lactoferrin), vitamin-like compounds or carotenoids (choline and lutein), enzymes (bile-salt stimulated lipase, BSSL), sugars (human milk oligosaccharides, HMOs) nucleotides or 'bio-nutrient complexes' such as milk-fat globule membrane (MFGM) which consists of lipids and proteins . These bionutrients may be synthesised (e.g. recombinant BSSL or lactoferrin) or derived from bovine milk (e.g. lactoferrin or MFGM) or other sources (lutein). We systematically searched the literature for studies, randomised controlled trials (RCTs) and meta-analyses of these nutrients exploring their clinical effect in preterm infants.

Choline

Choline is a conditionally essential water-soluble nutrient with vitamin-like qualities and is found in a wide variety of foods including breastmilk and infant formula. Choline has

multiple physiological functions many of which also require an adequate supply of phospholipids and docosahexaenoic acid, DHA (1,2)(3). Functions include structural roles in cell membrane and myelin synthesis, cell signalling, and neuro-transmitter functions as a precursor of acetylcholine synthesis. Choline is also involved in DNA methylation, via its metabolite betaine, which is involved in the production of S-adenosyl-methionine. Adults can produce choline in the liver, but de-novo synthesis in preterm infants may be limited. There are theoretical risks of toxicity as choline is metabolised to trimethylamine oxide, high levels of which may cause liver damage and are associated with cardiovascular disease in adults. EFSA recommends that infant formula contain a minimum choline concentration of 25mg/100kcal(4).

We conducted a review of literature to determine the evidence base in infancy. No studies have adequately defined minimum or maximum intakes in preterm infants, and no deficiency state has been described. However, a recent study showed improved measures of neural function in the first year of life in healthy term infants born to mothers who received additional choline supplementation during the 3rd trimester (930mg v 480mg/day) highlighting the possible importance for preterm infants who miss this period of accretion(5). We did not identify any published meta-analyses or RCTs of supplemental choline that include clinical or functional outcomes in very preterm infants, but a recent small study showed that 30mg/kg/day additional choline normalizes plasma concentrations when administered in addition to the estimated 24mg/kg/day received from breastmilk (3). This suggests that some preterm infants may benefit from choline supplements. Limited data also suggest there may be a role for choline as part of a multi-nutrient supplement given to infants at elevated risk of abnormal developmental outcomes in the post-discharge period (6,7).

Conclusions

C1. Dietary intakes in preterm infants must include choline because de novo synthesis may be limited or compromised **LOE 2**

C2. Defining minimum and maximum intakes is challenging due to a lack of RCTs in preterm infants. **LOE 2**

C3. There is no current evidence that preterm infants primarily fed with breastmilk benefit from routine choline supplements. **LOE 2**

Recommendations

R1. There are no data to support any change in the previous recommended daily intake of 8-55mg/kg/day, although higher intakes appear safe. **GOR C**

R2. Milk formula designed for preterm infants should include choline in a concentration designed to meet recommended intakes. **GOR B**

R3. Routine choline supplementation in preterm infants is not recommended. **GPP**

Lactoferrin

Lactoferrin is a glycoprotein in the whey fraction of mammalian milks and is present in human colostrum in much higher concentrations than mature human milk or bovine based milk formula (8). Numerous basic scientific studies have explored potential mechanisms of actions of lactoferrin which include anti-bacterial, viral, and fungal activity, as well as effects on immune and brain development. These are of relevance in preterm infants. Initially the anti-infective properties of lactoferrin were thought to be due to its iron-binding capacity which would deprive certain pathogenic bacteria of iron for growth. However, subsequent studies showed that lactoferrin was also cytotoxic and may promote the growth of 'beneficial' bacteria such as Bifidobacteria.

Most clinical studies in preterm and term infants have explored the effect of supplemental bovine lactoferrin which is widely available and cheap, however a small number of studies have tested recombinant human lactoferrin. Studies suggest that although the in-vitro activity of human and bovine lactoferrin may be similar important structural and functional differences exist. In clinical studies, investigators have used different manufacturers (and therefore different methods of pasteurization and processing) and a range of doses, all of which may impact on bioactivity and functional outcomes. In preterm infants most clinical studies have determined the impact of supplements on late onset sepsis (LOS). A Cochrane meta-analysis (MA) in 2017 of six trials including 886 participants (9) provided low certainty

of evidence for a reduction in LOS (RR 0.59 95% c.i. 0.40-0.87) and necrotising enterocolitis, NEC (RR 0.46 95% c.i. 0.29-0.74). However, the largest trial to date included 2203 very preterm infants randomised to supplemental lactoferrin or placebo and showed no significant impact on LOS or NEC(10). Although an updated MA (11) concludes that a significant impact of lactoferrin on LOS reduction is still present, there are too many uncertainties regarding dose, bioactivity of different manufacturers preparations and publication bias to recommend routine supplementation.

Conclusions

C1. Lactoferrin is present in higher quantities in human breast milk compared to formula, but a recommended lower or upper intake cannot be adequately defined. **LOE 2**

C2. Trials of supplemental bovine lactoferrin in preterm infants show inconsistent results on key neonatal outcomes especially late onset sepsis. **LOE 2+**

C3. The bioactivity and functional effect of supplemental bovine lactoferrin may differ depending on dose and manufacturer preparation. **LOE 2**

Recommendations

R1. There is insufficient evidence to support the routine use of supplemental bovine lactoferrin in very preterm infants. **GOR B**

Milk fat globule membrane (MFGM)

The milk fat globule is formed inside lactating mammary gland cells and is principally composed of triglyceride. When the globule is secreted into the milk ducts it acquires a portion of the mammary gland cell membrane to form a tri-layer structure around the globule which is called the milk fat globule membrane (MFGM). The MFGM contains numerous protein and lipid components with a range of functional activity including anti-infective, immune and cognitive effects. Whilst all mammalian milks have a MFGM, the structure is highly species specific. In addition, most artificial infant milk formula (including those designed for preterm infants) are produced from milk powder and whey protein concentrates where the milk fat is discarded and replaced with a blend of vegetable oils. Infant milk

formula therefore does not routinely contain many of the MFGM-associated proteins and lipids. Several clinical trials have explored the effect of MFGM supplementation in infants and children with positive effects on reducing infective episodes and improving cognitive function(12–14). Supplemental MFGM may have a similar effect in preterm infants, and may impact on NEC, LOS, and longer-term cognitive outcome but we did not identify any clinical RCTs. Similar challenges to those described for other supplemental milk proteins or components will need to be addressed including differences in bioactivity between commercial products and determining the optimal dose and mode of delivery. Whilst there are no trials of MFGM in preterm infants, the available data suggest that supplementation may be safe, and further trials could be seen as an important priority for neonatal research.

Conclusions

C1. MFGM is a key component of human breast milk with a range of important functions including anti-infective, immune, and cognitive development. **LOE 2**

C2. The bioactivity and functional effect of supplemental bovine MFGM may differ depending on dose and manufacturer preparation. **LOE 3**

Recommendations

R1. There is insufficient evidence to support the routine use of supplemental bovine MFGM to preterm infants. **GOR 2**

Nucleotides

Nucleotides represent up to 20% of the non-protein nitrogen in human milk which is much higher than in other species. There are several different nucleotides in human milk, and studies suggest they may be involved in a range of metabolic, immune, and nutritional processes(15). However, human data are limited and their presence in breastmilk may simply reflect metabolic processes in the mammary gland rather than specific functions for the infant(4). Several small trials have explored supplementation in infants with outcomes including antibody titres, incidence of infections and growth. A recent systematic review of growth and nucleotide supplementation(15) including 720 infants between 1988 and 2010, but only 3 studies involved preterm infants(16–18). Meta-analysis of the 8 studies showed

inconsistent effects. Growth (including head growth) in some studies was greater in supplemented infants which may be due mechanisms involving intestinal health and/or gut microbiota, but this effect was not seen in the studies of preterm infants(17,18). Studies exploring infectious outcomes and antibody titres were also inconsistent, but no harm has been noted in infants fed with formula supplemented with levels up to 5mg/100kcal(4). A recent trial focused on the post-discharge period in infants at high-risk of brain injury, including infants born preterm <31 weeks GA, provided a supplement of 1.8mg/kg/day of both uridine of cytidine monophosphate, along with additional choline and docosahexaenoic acid. The pilot trial was small and not powered for 2-year developmental outcome, but no adverse effects were seen(6,7).

C1. Nucleotides represent a significant proportion of non-protein nitrogen in human milk. **LOE 2-**

C2. Studies in preterm and term infants do not show consistent effects on growth or other health outcomes. **LOE 2**

R1. Routine supplementation of nucleotides in preterm infants is not recommended. **GOR B**

Inositol

Inositol is a six-carbon sugar alcohol present in biological systems primarily as myo-inositol and is required for mammalian cell growth and survival. Mature human breastmilk contains around 130-325 mg/L (20-50 mg/100 kcal) although levels in colostrum are higher(19) and EFSA recommends a minimum concentration of 4mg/100kcal in infant formula(4).

Inositol and its derivatives are involved in a multitude of processes including signalling pathways, enzyme activation and lipid synthesis, and inositol is specifically relevant for preterm infants because it is involved in maturation of several components of surfactant(20). Animal studies correlate changes in surfactant composition with plasma levels of inositol and supplementation studies increased phospholipid levels to normal(21). Early studies in infants suggested supplementation might be beneficial in infants with respiratory distress syndrome

(RDS) and that levels in early breastmilk from mothers delivering preterm had particularly high concentrations(21,22).

Clinical trials conducted in the 1980s and 1990s(21) suggested possible reductions in ROP, and non-significant decreases in rates of BPD and neonatal death at 28 days of age. Multicentre RCTs conducted since the previous ESPGHAN position paper have now provided considerably more data including a pharmacokinetic study(23) (n=74 preterm infants of 23-29w GA), and two larger studies exploring the impact of supplementation on ROP: (n=122 preterm infants 23-29w GA) and (n=638 preterm infants <28w GA)(24). Of note for this position paper, some trials provided i.v. as well as oral supplementation. The first 4 studies formed the basis of a Cochrane review published in 2015(25) which suggested significant reductions in death, IVH higher than grade 2, and ROP stage 3 or greater, and no impact on BPD, but emphasised that additional high-quality studies were needed. However, the possible benefits from the previous review have not been supported by the two most recent RCTs, the largest of which was stopped prematurely after n=638 of the planned 1760 infants were recruited, due to significantly higher mortality in the myo-inositol intervention group. These 6 trials form the basis of the most recent meta-analysis(20) which demonstrates no overall benefit of inositol supplementation for any key neonatal outcome.

C1. Inositol is an essential nutrient in early life and concentrations are higher in breastmilk from mothers delivering preterm. **LOE 2**

C2. Trials conducted 20-30 years ago suggested possible benefit from supplementation in preterm infants, but these have not been confirmed in more recent RCTs, and there may be a risk of higher mortality in infants receiving supplements. **LOE 2**

R1. Routine inositol supplementation for very preterm infants is not recommended. **GOR A**

Human milk oligosaccharides (HMOs)

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants 2022: Lactoferrin, Choline, Milk Fat Globule Membrane, HMOs, BSSL, Lutein, Nucleotides

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Human milk oligosaccharides (HMOs) are non-digestible carbohydrates and are the third largest solid component in human milk after lactose and lipids(26). There are more than 200 unique HMOs present in human milk and their structure is different to oligosaccharides in other mammalian milks. HMOs are resistant to pasteurization and freeze-drying(27,28) and their concentrations in breastmilk change during lactation, as well as differences reflecting genetic and environmental factors. HMOs appear to have significant impacts on immune function in-vitro and in animal models, and may protect against necrotising enterocolitis and sepsis, as well as improving brain development. Effects are likely to involve multiple mechanisms and benefits may be modulated through signalling pathways of enterocytes and/or by modulating the gut microbiota. Human milk is especially rich in fucosylated HMOs, especially 2-FL, and these HMOs appear to be key in promoting a Bifidobacteria-dominated gut microbiota(29). Recently, oligosaccharides structurally identical to those in human milk have been synthesised artificially. A recent study in term infants showed that a formula supplemented with 2-FL and LNnT was well-tolerated and supported age-appropriate growth, and was also associated with softer stools, and a lower incidence of reported lower-respiratory tract infections and anti-pyretic use(30). No RCTs have been conducted in preterm infants but observational studies have associated specific HMO profiles with a lower incidence of NEC(31,32).

C1. HMOs are unique components in human breastmilk contributing to a range of beneficial effects especially on gut health in preterm infants. **LOE 2**

C2. Differences in HMO profiles may be associated with differences in the rate of necrotising enterocolitis in preterm infants. **LOE 2**

C3. HMOs are different in structure and function to other oligosaccharides. **LOE 1**

C4. There are no RCTs of providing additional HMO supplements in preterm infants **LOE 2**

R1. Routine supplementation of HMOs in preterm infants is not recommended. **GOR C**

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants 2022: Lactoferrin, Choline, Milk Fat Globule Membrane, HMOs, BSSL, Lutein, Nucleotides

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Lutein

Carotenoids are natural pigments synthesised by plants and bacteria, and found in a variety of fruits and vegetables, of which lutein and zeaxanthin are two of the major compounds. Lutein is considered essential as it is not synthesised by humans but is secreted into breastmilk. Lutein accumulates in the human eye, especially in the macula, giving the yellowish color and it performs dual functions as an antioxidant and a high-energy blue light filter(33). Lutein also accumulates in the brain(34), and studies in primates indicate that a diet containing xanthophylls is important for ocular and brain function. There is placental lutein transfer in-utero, and supply to the infant occurs via breastmilk or infant formulas containing lutein. Plasma concentrations of lutein correlate with intake and are like the breastfed infant for selected infant formula containing lutein supplements(35,36). In a study of autopsied brain samples from infant who died, lutein was detectable in all samples from 22 term infants, but low-to-absent brain lutein concentrations were found in 8 preterm infant brains(34). Regulatory bodies have not raised concerns about the safety of lutein in various products. In 2008, the European Food Safety Authority (EFSA 2008; 823, 1-24) released a scientific opinion on the suitability of lutein in infant formula and follow-on formula; in the opinion EFSA stated that the proposed use of 250 μg lutein/L in infant formula products raised no safety concerns. In 2010, EFSA re-evaluated lutein as a food additive and derived an acceptable daily intake of 1 mg/kg/day, based on a no-observed-adverse effect level of 200 mg/kg/day in a 90-day study in rats, with an additional 200-fold safety factor (EFSA 2010;8:1678). The average westernized diet is low in lutein, but the health consequences of low lutein intakes in infancy, childhood or adulthood are not clear.

Several small trials explored supplementation of lutein plus zeaxanthin and have been summarised in a recent systematic review(37). Three small trials in very preterm infants enrolled n=63 in a single NICU, n=114 from 5 NICUs, and n=229 from 3 NICUs and were all conducted in Italy, but no trials showed any benefit in key neonatal outcomes including ROP.(38)

C1. Lutein and zeaxanthin are essential nutrients and dietary requirements are usually met via human breastmilk or supplemented infant formula. **LOE 2**

C2. Trials of additional supplements do not show benefit in preterm infants. **LOE 2+**

R1. Routine additional supplements of lutein and zeaxanthin to very preterm infants are not recommended. **GOR B**

Bile Salt Stimulated Lipase (BBSL)

Bile salt dependant lipase (BSDL) is a lipolytic enzyme expressed in all mammalian species in the exocrine pancreas and secreted into the intestinal lumen to facilitate digestion and absorption of dietary fat along with other enzymes. However, many other enzymes are important in fat digestion. BSDL is also found in the blood, but its role in the circulation is less clear(39). In the gastrointestinal (GI) tract, BSDL has broad specificity and hydrolyzes a variety of different substrates including tri-, di-, and monoglycerides, cholesteryl and retinyl esters, phospholipids, and ceramides(40–42). Exocrine pancreatic function is not fully developed at birth, and BSDL production may be insufficient to fully support fat absorption. In some species, including humans, a very similar enzyme BSSL is secreted by the lactating mammary gland which may partially compensate for the lower endogenous production(42,43). Breastmilk-derived BSSL, once activated by endogenous bile salts in the upper small intestine, contributes to efficient use of milk fat in breast-fed infants,(39,44) and BSSL may therefore be important for healthy growth and development in preterm infants(40,45,46).

A recombinant human BSSL (rhBSSL) has been developed and produced as an oral therapeutic strategy to improve lipid absorption. Two studies explored the effect of rhBSSL supplementation in preterm infants. In the first cross-over study, preterm infants were fed pasteurized breast milk or formula, and the effect of rhBSSL supplementation on growth and fat absorption were compared to placebo. One week of rhBSSL supplementation significantly improved mean growth velocity by 2.93g/kg/day ($P < 0.001$) and the absorption of the long-chain polyunsaturated fatty acids docosahexaenoic acid (DHA), and arachidonic acid

(ARA) but there were no significant effects on total fat absorption(47). In a follow up RCT, 415 preterm infants were enrolled: there were we significant effects on growth or several other secondary endpoints although possible adverse effects were higher in the rhBSSL treated group(48). However, in a predefined sub-group, small for gestational age infants demonstrated a significant benefit for rhBSSL on growth. No other RCTs in preterm infants are available.

C1. BSDL produced by the exocrine pancreas is important in fat digestion and absorption, but secretion may be lower in preterm infants. **LOE 2**

C2. BSSL produced by the mammary gland and secreted into human breastmilk is important in fat digestion, but concentrations are dramatically reduced by pasteurization. **LOE 2**

C3. Trials of supplemental recombinant BSSL do not show growth or other health benefits in preterm infants. **LOE 2**

R1. Routine use of supplemental recombinant BSSL in enterally fed very preterm infants is not recommended. **GOR B**

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Supplementary Digital Content no. 19

ESPGHAN Committee of Nutrition (CoN) Position Paper on Enteral Nutrition for Preterm Infants: Breast milk fortification (February 2022)

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Preterm formula /BM fortifiers/supplements

MOM - mothers own milk

DM - donor milk, pasteurized human milk from other women than the mother

BM - breast milk – both donor milk and mother's own milk

BMF - Bovine milk based fortifier – fortifier based on cow's milk

HMF - Human milk based fortifier – fortifier based on human milk

NEC – Necrotizing enterocolitis

SF - standard fortification

AF - adjustable fortification

TF - target fortification

EMBA - European Milk Bank Association

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1 Background

Breast milk is considered as the natural and best source of nutrition for newborn infants. This also holds true for preterm infants because breast milk is tolerated best, allows fast introduction of enteral feeds with low sepsis rates, and provides important substances besides macro- and micronutrients such as human milk oligosaccharides that promote a NEC preventing bifido microbiome, growth factors, immunoglobulins and also cellular components [1]. The effect of the latter three ones is yet not fully understood and is actually a highly active area of research [2].

Previous studies have shown that neonatal growth rates are linearly and closely related to protein and to energy intakes [3, 4]. Term infants have growth rates of 5-10 g/kg/d during their first weeks of life and therefore require an enteral protein intake of 1.6 to 2.5 g/kg/d and a matching energy intake of 85 to 110 kcal/kg/d. To ensure adequate supply, “Mother Nature” had adjusted the nutrient content of mature breast milk as – from an evolutionary perspective – breast milk was the only source to cover the needs for total fluid intake in this population. Because of immature renal concentration capacity, total fluid intake during the first three months post-term is the highest ever in life time and is on average 165 ml/kg per day [5]. Healthy term infants are able to adapt to fluid intakes in the range of 140 to 200 ml/kg/. As a consequence, breast milk produced within the first two weeks of lactation contains on average up to 2.0 (IQR: 0.3:3.7) g protein/dl followed by a decline over the first 28 days of life/lactation. Mature (> 4 weeks of lactation) breast milk contains on average 1.2 (IQR: 1.0;1.4) g/dl of protein and 67 (IQR: 62;72) kcal/dl of energy. In addition, there is significant variability of macronutrient composition - not only between mothers, but also within the same mother (intra- and interindividual variability). Published nutrient contents range from 0.5 – 2.0 g/dl for protein and from 45 - 90 kcal/dl for energy content (see also paragraph #6) [6-9].
Additionally, recent research found that protein, CHO and fat are secreted and controlled via different pathways and, therefore, native breast milk does not seem to show a strong correlation of these three nutrients [10]. The teleological meaning of this variability actually remains unclear, however, term infants are somewhat competent in self regulation of the volume of milk ingested and may therefore cope with varying composition of mother’s milk.
Preterm infants, however, experience significantly higher growth rates of up to 17 - 23 g/kg/d up to 32 weeks PCA. Such growth rates require a protein intake of 3.5 to 4.5 g/kg/d and a corresponding energy intake of 115 to 165 kcal/kg/d. While neonatal care – at least in some places of the world (Europe) – allows higher fluid intakes for selected and stable very low birth weight (VLBW) infants beyond 150 or 160 of up to 180 and 200 ml/kg/d, it is very unlikely to meet these protein and energy needs by feeding solely native breast milk [11]. Even if a protein content should reach 2 g/dl, the infant still must be able to tolerate an enteral intake of at least 200 ml/kg/d which not all, but only a few ones will do. And, at 1.0 g protein per 100 ml of native breast milk, a VLBW preterm infant on full enteral feeds would need to digest up to 400 ml/kg/d of native breast milk to reach a protein intake of 4 g/kg/d, a fluid volume that is unlikely to be tolerated. In addition, such high volume also would cause an unbalanced energy-to-protein intake: energy intake would be as high as 180 - 240 kcal/kg/d which is far beyond the average needs of preterm infants (i.e. 115 - 140 kcal/kg/d) and would most likely cause metabolic stress and also contribute to an unfavorable body composition [12].

While under certain conditions a very few preterm infants may receive their required nutrient intake from native breast milk alone it may be wise - from a nutrition and growth physiology perspective of preterm infants - to adapt the nutritional content of breast milk for the population of VLBW infants.

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During the last decades commercially available single or multi-component fortifier products have been developed and/or their composition is still being refined. Their fortification recipes are based on two assumptions, (i) individual breast milk composition can be represented by an average macronutrient content of breast milk and (ii) the average enteral fluid intake of preterm infants is 150 - 160 ml/kg/d. The following chapter reviews the evidence of this approach as well as its limitations in light of new data, namely that (i) macronutrient content of human milk is subject to high variability and (ii) that many NICUs nowadays accept higher enteral fluid volumes of up to 180 or even 200 ml/kg/d [12].

2 Effect of fortification of human milk on growth: evidence from clinical trials

Though from a scientific perspective the physiological basis supporting the use of fortifiers for human milk is quite strong [4] there is only limited evidence from clinical trials. With regards to **multi-component** fortifiers there is one review including eight trials with a total of 505 VLBW infants [13a]. Weight gain was better by 2.18 (CI 25-75: 1.54-2,81) g/kg/d, length gain by 0,16 (CI: 0.11-0.20) cm/week and head circumference by 0.07 (0.03 -0.11) cm/week with fortification. The trials cover a period of 30 years, include only a small numbers of subjects and are thus of limited generalisability. There are two Cochrane reviews investigating the effect of **protein** supplementation. The first one includes six trials with a total of 204 subjects: an increase in weight gain by 3.8 g/kg/d, in length by 0.12 cm/week and in head circumference by 0.06 cm/week was found [14]. The second, more recent one includes nine studies with a total of 861 infants born before 32 weeks or having a birth weight below 1500g. In summary, evidence is low to moderate that increased protein concentration improves weight gain [15].

With regards to **fat** supplementation there are two trials showing conflicting results. The first one performed in either 14 preterm infants added 1 gm human milk fat per 100 ml [16,17], but did not find an effect on weight gain, length or head circumference. The other one was an unmasked RCT in 78 VLBW infants that exclusively were fed breast milk (either mother's own milk or donor milk) routinely fortified with a human milk derived cream. Human milk fat was added in case that measured energy content of native breast milk samples was low. Extra-fat lead to better weight (14.0 vs 12.4 /kg/d, P = .03) and length (1.03 vs 0.83 cm/wk, P = .02) gain [18] and shortened the length of stay [19]. However, postnatal weight gain was still compromised and there was no body composition assessment to check that increased weight gain was not related to increase in fat mass.

For **carbohydrate fortification**, only one trial investigated the effect of extra carbohydrates as part of a probiotic intervention in 75 preterm infants [20,21]. A mild effect on weight gain (MD +160.4 grams, 95% CI 12.4 to 308.4 grams) was observed on study day 30.

There is indirect evidence from two recent trials from limited resource countries how human milk fortification affects growth. These studies were initiated because WHO does not recommend BM fortification in such countries due to the significant financial burden: The first trial (n = 148 infants) found that routine fortified infants (randomized once enteral intake was at 150 ml/kg/d) grew better compared to subjects without fortification (weight: mean difference (MD) 2.0 g/kg/day; 95% CI 1·03 to 2·92; p<0·001;length: MD 0·09cm/week; 95% CI 0·02 to 0·2;p=0·02) [22]. The second trial in n = 52 VLBW infants compared routine fortification starting at 100mlkg/d vs. a group (selective fortification) with an enteral intake of up to 200 ml/kg/d followed by additional fortification if needed. Growth

rates were not statistically significantly different (routine: 10.8 vs. selective: 8.4 g/kg/d; p=0.6), but were below expected values mostly because of low nutritional content of local breast milk [23].

No trials were done that compare the effect of current concepts of fortification of human milk on neurodevelopmental outcome in VLBW infants at 18 months or 5 years of age [24]. However, with regards to side effects fortification is considered to be safe with respect to incidence of NEC, feeding intolerance and osmolality [13].

In conclusion, taking the evidence from clinical trials as well as current data of nutritional physiology into account it seems to be reasonable to enhance nutrient content of BM fed to VLBW infants using contemporary fortifier products.

3 Time point of introduction of contemporary fortifier products

Actually, there is no uniform recommendation on when to start fortification of human milk [25]. The practice of first introducing enteral feedings to 150 ml/kg/d and then starting with half strength followed by full strength fortification two days later was well accepted but nowadays maybe outdated. Tillman et al reported that BM fortification is tolerated already from day one, leads to reduced parenteral intake, but does not necessarily increase postnatal growth, but improves bone metabolism [26]. One recent review quoted two trials comparing 20 vs 100 ml/kg/d and “first feeding” vs. 75 ml/kg/d as time points to introduce HMF [27-29]. There were no differences in growth rates, but also not for adverse outcomes suggesting that early fortification may be as safe as delayed fortification, but the level of evidence remains low.

In conclusion there is no evidence about the best time point to start fortification of human milk. Early fortification seems to be as safe as delayed fortification.

4 Composition of fortifier products

Nutrient composition of contemporary multi-component fortifier products varies between brands and manufacturers. For a detailed summary with a list of ingredients see Table 1 [8, 30-35]. Most products add 1 to 1.1 g of protein per 100ml of human milk (donor milk and/or MOM). Recent publications, however, show that protein levels of standard fortified MOM using contemporary fortifier products may result in lower than recommended protein intakes. This is mostly due to the fact that protein content of human milk is decreasing with lactational age and may frequently fall below assumed levels by 0.3 to 0.5 g/dl [5-10, 36-38]. For example, recent publications show that protein content of native 12-hour batches is on average as low as 1.1 - 1.2 g/dl, with individual concentrations being as low as 0.5 g/dl [36]. Standard fortification increases the protein content of native breast milk from 1.2 g/100 ml to 2.2 g/100 ml which results in a protein intake of 3.3 g/kg/d (at 150 ml/kg/d) which is below the recommended intake. Some authors suggested to use percentile charts to account for the decrease during the lactational period [39]. However, the interindividual variability is always larger than the variability over time making precise predictions impossible. There are also no corresponding data available for other macronutrients. Overall, there are no clinical data that this approach will really improve intake.

Higher feeding volumes of up to 180 ml/kg/d may improve protein intake and, nowadays, a higher proportion of VLBW infants may be able to tolerate such fluid intakes. However, it may not be wise

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to base recommendations for the composition of fortifiers on extreme conditions as it would put those infants at risk for growth failure that are unable to tolerate such intakes. Overall, it is important for clinicians to use appropriate figures for average milk protein content when prescribing HMF. HMFs with a higher protein content, e.g. adding1.4 g of protein per 100ml may be useful if the milk protein concentration is low and the volume of enteral feeds need to be kept at 150 ml/kg/d.

There is also compositional heterogeneity with regards to the quality of energy supply: a group of fortifiers (mostly from European companies) adds no or only small amounts of fat (0 - 0.02 g/100ml) but delivers most of the calories by adding 2.7 to 3.4 g/100ml of lactose (fat:CHO ratio below 10:90). A second group of products add energy mainly as fat (0.36-1.0 g / dl) , but only low amounts of carbohydrates (0.4 – 1.8 g/dl) (range of fat:CHO ratio between 30:70 and 80:20). Of interest to note that the amount of total energy provided by these fortifiers is somehow in a similar range (14.4. – 17.8 kcal/100 ml). So far, it is not well understood why and how different isocaloric fat-to-CHO ratio are able to affect the quality of growth. Results from previous clinical trials providing identical amounts of protein with an isocaloric design suggest that a more fat based (66% fat:33% CHO) energy intake increases amino acid oxidation thus impairing protein synthesis [40,41]. More recent product seem to favor a higher protein content and a more equal distribution of energy intake between fat and carbohydrates [31,35]. Assuming a fluid intake of 160 ml/kg/d the addition of 1.7 g of protein, 0.5 g of fat, and 1.2 g of carbohydrates (CHO) per 100 ml would maximize the number of BM samples that comply with ESPGHAN recommended intakes [8]).

Routine Fortifier	Fat [g/dl]	Protein [g/dl]	CHO [g/dl]	Energy [kcal/dl]	Ref
Milupa – Aptamil HMF	0	1.1	2.7	15.2	[30]
Mead Johnson - EnfaCare	1	1.1	0.4	15.0	[32]
Nutricia - Nutriprem HMF	0	1.1	2.7	15	[33]
Abbott – Similac Advance Liquid HMF	0.36	1.0	1.8	14.4	[34]
Nestle - PreNan HMF	0.72	1.4	1.3	17.4	[31]
Abbott – Similac Human Milk Fortifier Hydrolized Protein Concentrated Liquid	0.7	1.7	2.5	15	[35]
Native human milk	3.4 - 4.0	1.0 - 1.3	7.0 - 8.0	65	[7,8,11]
Recommended nutrient composition (assuming a TFI	4.4	2.7 (-3.0)	8.8	86	ESPGHAN

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of 150 ml/kg/d)					

Table 1: Macronutrient composition of (i) fortifier products available in Europe and North America (rows 2 - 6), (ii) human milk composition (ranges) (row 7) and (iii) targeted levels according to ESPGHAN (row 8). Contents of (ii) and (iii) are given per 100 ml ready-to-feed product

In conclusion, there is some variation in the macronutrient composition of contemporary fortifier products. There is some evidence that optimizing the protein content alone might not be sufficient, but needs to encompass quantity and quality of energy intake as well to achieve proper growth patterns. In future, research and refinement to provide the optimum non-protein make-up needs to be done. Clinicians should be aware of the macronutrient composition of the fortifier product that they are using.

5 Other sources of fortification: the role of high-density preterm formula

There were some recent activities to achieve fortification of human milk by using highly concentrated preterm formula (30 kcal/oz = i.e. 99 kcal/100ml) [42-44]. The study by Pillai was an observational study in 27 infants reporting weight gain increasing from 12.5 to 15.9 g/kg/d after switching to the 30kcal-formula. Willeitner reported in a group of 70 premature infants that human milk fortification by adding a 30kcal/oz preterm formula was not inferior to adding a powdered fortifier. Similar findings were reported by Chinnapan when comparing human milk fortification adding either powdered fortifier or powder for preterm formula in n=123 infants.

In conclusion, there are reports that BM can also be fortified using concentrated high-caloric special care products instead of contemporary powdered or liquid industrial fortifiers (mostly US). However, the use in clinical routine cannot be recommended because of the extremely low level of evidence and also missing EU safety data.

6 Individualized fortification

To ensure adequate postnatal growth preterm infants must be provided with adequate amounts of amino acids/protein as building blocks for accrual of lean mass. In addition, preterm infants also need to receive an adequate amount of energy to cover (i) basal and movement induced metabolism, (ii) energy stored in the newly build-up tissue, but also (iii) energy needed to synthesize lean mass (see chapter #2 of these guidelines). Unmatched (i.e. mostly too low) energy intake can become a limiting factor for protein synthesis. “Excess” dietary amino acids are not incorporated into new lean mass, but are oxidized instead so that the carbohydrate backbone can be used for synthesis of CHO’s or other metabolites. The remaining NH_3-groups are toxic and need to be removed from the body. By forming water soluble urea, a strong osmolyte, they are excreted via urine, however, which is a water and energy consuming pathway. Increased amino acid oxidation (either as a consequence of too high protein intake or as of a relatively too low energy supply) may cause BUN levels rising to critically high levels. Protein and energy intakes, therefore, need to be balanced for optimum growth. Deviations thereof have the potential to lead to either poor growth

(i.e. lean mass accretion too low), growth of poor quality (unfavorable body composition), excess of fat (due to too high energy supply) and/or metabolic disturbances (i.e. increased BUN formation leading to “cosmetic” edema or mimicking sepsis).

The preceding chapter delineated the concept of BM fortification which assumes an average content of macronutrients. With this approach it was possible to develop multi-component fortifiers so that actual recommendations for macronutrient intakes are being met for most preterm infants [45]. Previous research on breast milk analysis however has revealed that macronutrient content varies significantly within the same mother, but more so between mothers [46,47]. Recent research in larger sample sizes confirmed this variability, but also showed no correlation between macronutrient contents in human milk [6,10]. In other words, a significant proportion of preterm infants will receive enteral diets of unbalanced composition, for example, high or normal protein, but low energy content or a low protein but high energy contents [10]. Unbalanced diets of such extent are not compatible with appropriate growth rates and quality [48]. These findings might explain the observation that still more than 50% of VLBW infants fed standard fortified breast milk experience postnatal growth faltering [49].

Potential ways out here are to overcome the natural variation of breast milk composition by 1) target pooling of breast milk or by 2) introducing individualized fortification. Target pooling of breast milk has been applied, but unfortunately failed to be efficient [50,8]. The authors state “…Target-pooling DBM to meet a caloric minimum alone does not meet recommended protein intake for VLBW infants. Infants fed calorically target-pooled donor breast milk still demonstrate a disproportionate negative change in length z-score over time and would likely benefit from more aggressive and earlier fortification strategies which target protein as well. Whether target-pooled DM offers improved growth compared to random-pooled donor milk remains unknown…”.

Individualized fortification may be applied as “adjustable or “targeted” fortification. “Adjustable” describes the practice to adapt fortifier strengths on the course of blood urea nitrogen (BUN) levels [51]. It is assumed that during the phase of stable growth BUN levels reflect protein utilisation: high BUN levels may indicate significant amino acid oxidation and thus represent a tentative saturation point for protein intake. Low BUN levels, on the other hand, may indicate a metabolic potential for increased protein intake [51]. Recommendations for BUN level are discussed in the section on protein. The advantage of BUN levels is the simplicity of the approach; however it requires a blood sample to be taken, it also needs some time for the body to metabolically react to changes in intakes – delays which could cause a cumulative growth deficit. More so, cut-off levels for BUN to trigger fortifier in- or decrease currently are arbitrary and not adjusted to gestational or postnatal age but reported a significant benefit.

“Target” fortification analyses macronutrient content of selected BM samples at the bedside and adds nutrients to achieve a macronutrient intake that meets ESPGHAN recommendations for stable preterm infants <1800g birthweight. It is more labor-intensive, has been shown to be feasible but has the potential to prospectively correct nutritional deficiencies before either biochemical parameters or poor growth signal insufficient supply [52,53]. This may be especially more effective in infants with mothers that produce BM with an unbalanced dietary composition [52,54].

7 Evidence for efficacy of different fortification strategies

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European Milk Bank Association (EMBA) quotes that both methods of individualized fortification (i.e. "adjustable" and "targeted" may be applied in daily routine [55]. So far, four trials studied the effect of adjustable vs. standard fortification [51, 56-58]. They found that adjustable fortification improved growth rates for mostly all anthropometric parameters. For the critical appraisal of adjustable fortification it is important to note that BUN cut-off levels to increase or decrease fortification strengths were arbitrarily chosen and were not optimized or validated in clinical studies, although they showed benefit. It is also important to note that adjustable fortification is able to increase intakes of protein and/or energy, but does not have the potential to fix imbalances in macronutrient composition associated with impaired protein utilisation.

For target fortification two pilot studies [52, 53] and several further randomized clinical trials have been performed [54,56,59-63]. While some earlier RCTs did not identify differences in growth rates between target and standard fortification, more recent trials consistently found target fortification to be superior to standard fortification. This might be explained by the fact that the early trials did not use validated human milk analysers and frequently did not report to adhere to "Good Clinical and Laboratory Practice" (GCLP). Earlier studies tended to focus more on adding solely protein whereas the more recent trials also add other macronutrients like fat and carbohydrates to adjust energy intake. Furthermore, most of the studies have other methodological differences that makes evidence synthesis challenging. This practice reflects the more recent understanding that it is key to provide an energy intake that matches the protein intake of the body. Thus, the concept of target fortification is to provide a constant and coherent dietary intake of macronutrients. The potential of target fortification to efficiently reduce the considerable random variation of macronutrient composition of native human milk was recently quite nicely illustrated [67]. The coherent dietary intake may also be the reason why target fortification seems to be superior to adjusted fortification as found in three out of four studies addressing this question [56,65,66]. The fourth study did not find a difference between both approaches, although a relatively detailed protocol was used to adjust protein and energy intake according to growth [67].

Overall, there is only one Cochrane analysis so far that reports moderate- to low-certainty evidence suggesting that individualized (either targeted or adjustable) fortification of enteral feeds in very low birth weight infants increases growth velocity of weight, length, and head circumference during the intervention compared with standard non-individualized fortification [68].

In summary, there is emerging evidence that targeted fortification is more appropriate to compensate/eliminate nutritional deficiencies/imbalances and leads to better growth when compared to standard fortification. Although target fortification directly incorporates nutritional physiology and real-life data of nutrient contents of ingested breast milk to create the most appropriate dietary regime for any given day, there is no strong evidence to support a significant clinical benefit when compared to adjustable fortification. In this context it is important to mention that actually three more protocols of RCTs on target fortification were published [69-71]. First results are to be expected within the next 12-36 months.

8 Osmolality

There are concerns about critically high osmolality levels when additional fortification is being provided to human milk for VLBW infants. In this context it is of importance that the scientific basis

and evidence for osmolality limits or cut-off levels are very weak and is not confirmed by recent analyses [72-74]. While the subject will be discussed in detail in a different chapter, some papers relevant to individualised fortification shall be reviewed [75-77]. All authors state that individualised fortification increases osmolality already during the first hour, but generally stay below 480 [75] or 460 mosm/l [76] during the next 12 to 24 hours which is considered to be a safe practice.

In conclusion, unbalanced dietary intake of human milk may compromise postnatal growth and poses a significant risk for optimal development of VLBW infants. Individualised fortification strategies have the potential to improve nutrient intake in such subjects and improve growth rates. Although current evidence from clinical trials is still limited, individualised fortification strategies including adjusted and targeted approaches may be appropriate for clinical use..

9 Fortification strategies for donor milk

Preterm infants fed unfortified donor milk may be at increased risk for suboptimal growth and neurodevelopmental performance [78-80]. This has been attributed to an insufficient intake of protein: most donor milk originates from mothers with excess and/or prolonged milk production and therefore is typically obtained later in lactation. Indeed, some donor milk programs even exclude mothers to donate before their offspring at least reached four months of age - this is put into place to ensure sufficient MOM supply to their own child before beginning to donate.

It is well known that protein content drops during lactation, and donor milk typically contains average protein levels of 0.8 to 1.2 g/100 ml [9]. This amount is by far below the average of 1.4 to 1.5 g protein/100 ml which is considered as the basis to perform proper standard fortification (see paragraphs 4 and 6). The Delta in protein intake of approximately 0.5 g/100 ml translates into a growth deficit of 3 to 5 g/kg/d which is clinically significant. To overcome this nutritional deficiency it has been suggested (and also applied in clinical routine) to routinely add extra protein to batches of donor milk. A reasonable amount has been suggested to be 0.5 (0.3 - 0.7) g protein per 100 ml [8,65, 81-83]. This practical approach, however, has not yet been validated in independent clinical studies, but from a nutritional physiology perspective it seems to make sense to add extra protein.

An alternative approach to increase the nutritional quality of donor milk would be to disclose the nutritional contents of each donor milk batch to the customer [84]. This is current practice in diverse milk banks in Poland (personal communication by A Wersolowska, Warsow, Poland). It allows neonatal staff to adequately fortify donor milk beyond standard fortification to ensure sufficient intake for all MN for each individual preterm infant fed DM. A third option to reduce the number of donor milk batches with critically low protein content would be by pooling several batches from different donations [8,85]. Pooling can be done by randomly picking batches, by combining batches obtained early and late in lactation or by combining batches with complementary macronutrient profiles - strategies that are currently under investigation [8, 86]. The last one seems to be the most promising one, but obviously would require prior knowledge of nutritional content - for example by performing bedside analysis.

In conclusion, donor milk is at increased risk for insufficient protein content that cannot always be compensated with standard fortification. If fluid intake is limited, clinicians should consider to provide extra protein of approximately 0.3 - 0.7 g/100ml. Quality of donor milk could be improved by introducing donor milk pooling algorithms - which seems to be a promising area of research.

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10 Exclusive human milk diet

Feeding human milk as MOM or donor milk is considered a preventive measure for NEC (Quigley) [80]. The exact mechanism still is unclear, on one hand HM oligosaccharides promote a gut-protecting bifido-dominant microbiome. On the other hand, exposing the preterm gut to bovine protein - amongst other factors - may be associated with higher NEC incidence. While rates of formula fed preterm infants are decreasing fortification frequently still is provided by commercially available bovine based multi-component fortifier products [24]. From studies feeding VLBW infants with different amounts of cow's milk based formula and human milk there seems to be some dose-response relationship between risk for NEC and the amount of bovine protein ingested [87,88]. Also, interventional donor milk trials with reduced exposition to preterm formula have found significant reductions of NEC rates and sometimes also of sepsis rates. From this observation a concept of cow's milk free diet including fortification was suggested for clinical use in VLBW infants to reduce NEC rates and was also claimed to be a cost-effective measure [89,90]. The concept is also known as "exclusive human milk diet" (eHMD). Contemporary fortifier products come as liquids (after extensive lacto-engineering) or as powder (after lyophilisation of native or partly defatted human milk). Production applies similar processes as known from bovine fortifier industry and usually exceeds the technical means of non-profit human milk banks.

11 Evidence from clinical trials

There is only one Cochrane review on the effect of exclusive human milk diet on outcome [91]. A single RCT in 127 VLBW infants was identified being the first to compare the effect of human vs. bovine milk fortifiers in BM fed VLBW infants [92]. There was no difference in feeding intolerance and NEC. Neonatal short-term morbidity showed a decrease in ROP, and most other parameters showed a trend ($p = 0.07$) towards less incidence in the exclusively human milk fortifier group, for oxygen at 36 weeks and the morbidity/mortality index, but missed a statistical level of significance of 0.05. Weight gain was better in the bovine formula group, however again not reaching a significance level of $p<0.05$.

There are a number of studies that also investigate the introduction of exclusively human milk fortifiers on NEC rates and consistently find a reduction by a factor of 2. However, the designs of some of these studies had confounders due to the dietary setup of the control group (not exclusively on breast milk, not randomized, re-analysis of other studies etc.) and because of funding sources [93-101].

The experience with lyophilized human milk fortifier products is limited to some preclinical studies [102-104].

12 Growth and long-term follow-up

An observational study in n=51 preterm infants on eHMD found less postnatal growth retardation than previously reported bovine fortifiers, whereas body composition and neurodevelopmental outcome were in the range of previously reported figures [105]. In contrast, the O'Connor trial reported growth differences during the 7 week intervention period that were in favor of bovine milk

fortifiers (15%, i.e. weight gain of 1303 vs 1124 g; head growth 6.8 vs 6.2 cm/7 weeks) [92] . Similar observations were made in a recent study in 192 VLBW infants [106] which found reduced growth rates until the end of the intervention period at $32^0/_7$ weeks of gestation in the group fed exclusively human milk fortifier when compared to the group fed bovine fortifier(16.5 vs. 18.9 g/kg/d). This difference disappeared until term age. Such observations are of concern because they may indicate that macronutrient intake by exclusively human milk diet may be lower than with contemporary fortifier products and hence affect growth and potentially neurodevelopment as well. Indeed, the follow-up analysis of the “OptiMoM” study did not show differences in neurodevelopmental follow-up.

However, it can be assumed that the previously described concept of individualized fortification - once applied to exclusively human milk diet - may overcome growth related disadvantages.

In conclusion, there seems to be a potential to reduce NEC rates by following a protocol of exclusive human milk diet. However, the current evidence is too low to recommend the introduction into clinical routine as a strategy to reduce NEC and sepsis rates. Further research including clinical trials without confounding dietary intake of bovine origin is needed

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Conclusions

C1: The protein content of some fortifiers might be insufficient to increase protein concentrations to recommended intake levels if the volume of enteral feeds is limited **LOE 2**

C2: The optimal time to start fortification is not clear, but early fortification seems to be as safe as delayed fortification, may reduce cumulative nutrient deficiencies, and positively influence bone metabolism **LOE 2+**

C3: There is variation in the nutrient content of commercially available fortifiers and this may affect growth and health outcomes **LOE 2**

C4: Adjustable and target fortification strategies may be employed to compensate for variation in human milk macronutrient composition, but the optimal strategy is uncertain. DHM may require higher levels of fortification compared to MOM **LOE 2+**

C5: Fortifiers derived from human milk may reduce the risk of NEC but there are insufficient data from adequately powered studies to determine the optimal strategy **LOE 2+**

Recommendations

R1: We recommend the use of multi-component fortifier products to enhance the nutrient content of human milk fed and to promote growth in preterm infants **GOR A**

R2: We recommend starting fortifier when enteral intakes reach 40 – 100 ml/kg/d. **GOR C**

R3: Individualised fortification strategies including adjustable and targeted approaches may be appropriate. **GOR A**

R4: There is insufficient evidence to recommend the routine use of human milk derived fortifiers until further high-quality data is available. **GOR C**

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