Are Enteral Intakes of Protein, Lipid, Carbohydrate, Energy, Calcium, and Phosphorus in VLBW Preterm Infants Meeting Current Expert Recommendations?

by

Dawn Ving Yan Ng

A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Nutritional Sciences
University of Toronto

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Abstract

Research evidence supports the importance of nutrition to facilitate growth, reduce morbidity and enhance the neurodevelopment in very low birth weight (VLBW) infants. Local neonatal intensive care units have adopted more aggressive feeding guidelines to achieve nutrient goals. This study aims to examine the impact of major morbidity and birth weight (BW) on achieving expert nutrient recommendations in a cohort of VLBW preterm infants during initial hospitalization. Infants with ≥ 1 major morbidity/BW <1000g were less likely than their healthy/BW 1000-1499g counterparts, to achieve recommendations, with 53%, 45%, 46%, 48%, 45%, and 47%/53%, 44%, 45%, 49%, 45%, and 48% , vs. 33%, 25%, 34%, 38%, 32%, and 26%/36%, 28%, 38%, 39%, 33%, and 28%, respectively, failing to achieve minimum requirements for protein, lipid, carbohydrate, energy, calcium, and phosphorus. Discussion and further research is needed to identify obstacles and develop strategies to completely eradicate this deficit in nutrient delivery.
Acknowledgements

Four years ago, I walked into the office of an incredibly talented researcher and scientist, Debbie O’Connor. She interviewed me and somehow saw fit to take me under her wing, making me a part of the DoMINO research group as their newest research volunteer and the rest, as they say, is history. Fast forward to today, I am about to defend my masters of Science thesis and words cannot express how grateful I am to have been mentored by Debbie these past few years. I really admire your ability to warmly encourage us students while continually challenging us and holding us to excellent standards in everything whether it be in writing papers, presenting, or answering questions, of which there were many. You gave me the precious opportunity to be a part of this dedicated research team, and to continually better myself through the priceless experiences gained. I can only hope that I did not disappoint you and made your decision to have me as a Masters student a worthwhile one. So, from the bottom of my heart I want to say thank you, Debbie.

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Chapter 1 Introduction

1.0 Introduction

The optimal delivery of nutrition to preterm very low birth weight (VLBW; birth weight <1500g) infants in the neonatal intensive care unit (NICU) is undeniably a core element of neonatal healthcare. There is an inextricable link between nutritional adequacy and growth, neurodevelopment, as well as long term health outcomes.\(^1\)

Protein, lipid, and energy intakes during the first 2 postnatal weeks are significantly associated with improved neurodevelopment in VLBW infants.\(^2\)-\(^3\) Additionally, protein and energy intake during initial hospitalization significantly impact weight gain\(^4\),\(^5\) as well as head circumference growth in VLBW infants.\(^4\),\(^6\) Adequate head circumference growth rates are often associated with improved neurodevelopmental outcome.\(^7\)-\(^10\) Appropriate amounts of protein and concomitant energy levels are required to maintain a positive nitrogen balance and to maximize the utilization of protein.\(^11\)-\(^13\) Interestingly, energy from carbohydrate sources protects against amino acid oxidation and favours the deposition of lean mass.\(^14\) Lean mass accretion is crucial for better metabolic and health outcomes later in life including lower risks for type 2 diabetes, obesity and cardiovascular diseases.\(^15\),\(^16\) Both calcium and phosphorus are indispensable for bone mineralization and sufficient intakes prevent osteopenia of prematurity and fractures.\(^17\),\(^18\) Furthermore, ionic calcium and phosphorus are essential cellular components and play central roles in both intra- and inter-signalling mechanisms.\(^19\)-\(^21\)

Chronic lung disease (CLD), patent ductus arteriosus (PDA), late onset sepsis, severe retinopathy of prematurity, and necrotizing enterocolitis (NEC) collectively account for the leading causes of mortality and morbidity in the NICU.\(^22\)-\(^26\) Strongly associated with each other,
PDA often leads to CLD and there is a heightened risk for developing late onset sepsis and/or NEC with the diagnosis of CLD. Both CLD and late onset sepsis increase energy requirements and the management of CLD and PDA often involves the restriction of fluids. Combined with the general symptom of feeding intolerance, the management of CLD and PDA poses a challenge to the provision of optimal nutrition to the VLBW preterm infant.

Confirmed by clinical evidence, expert nutrient recommendations have traditionally taken into account a healthy preterm infant’s resting and growth needs, with the aim to achieve the gold standard of approximating in-utero growth rates and fetal composition. The recently published expert nutrient recommendations were revised to accommodate catch-up growth given the inevitable cumulative nutrient deficit experienced by the preterm infant during the first few postnatal weeks. While many NICUs have implemented more aggressive early nutrition protocols, few studies have evaluated the achievement of expert recommendations using prospective data, and none have done so with the newly published recommended guidelines. Furthermore, as infant morbidity increases nutrient and energy requirements, it would be valuable to evaluate their intakes against current recommendations as no separate guidelines currently exist for feeding through specific morbidities.

This study aims to answer the following research questions: 1) Do enteral intakes of protein, lipid, carbohydrate, energy, calcium, and phosphorus intakes in a cohort of VLBW infants during initial hospitalization meet current expert nutrition recommendations? 2) Do VLBW infants that have at least one major morbidity differ in achieving recommended nutrient intakes versus those that do not?
Chapter 2 Literature Review

2.1 Feeding the Preterm Infant

Preterm delivery occurs at ≤ 37 weeks gestational age, thus interrupting the nutrient supply otherwise received via the placenta. This traumatic transition to the extrauterine environment is further complicated as the preterm neonate is particularly susceptible to a whole host of illnesses including but not limited to necrotizing enterocolitis (NEC), chronic lung disease (CLD), late onset sepsis, brain injury, and patent ductus arteriosus (PDA). Many of these illnesses alter the energy and nutrient requirements of the preterm infant.\textsuperscript{32, 42} The preterm infant’s immature physiological systems further heighten their fragile condition.

As a further complication, the immaturity of the preterm infant’s gastrointestinal tract renders adequate provision of nutrients via the enteral route impossible, necessitating the use of parenteral nutrition (PN) to provide sufficient nutrients especially during the first few postnatal days until the transition to full enteral feeding is achieved.\textsuperscript{43, 44} In the past, reluctance to administer amino acid solutions was due to the resulting metabolic acidosis and hyperammonemia.\textsuperscript{45, 46} Also, lipid mixtures were linked to incidences of sepsis, lipid intolerance, chronic lung disease, hyperglycemia, and increased pulmonary resistance.\textsuperscript{47-50} Since then, changes in PN formulations such as the replacement of protein hydrolysates with crystalline amino acid solutions have allowed the consideration of early PN as a viable option.\textsuperscript{51} Randomized controlled trials have documented the safety of both amino acid and lipid PN administration to preterm infants immediately after birth and within the first 2 postnatal days respectively.\textsuperscript{52-54}
Kotsopoulos et al. found that preterm infants of gestational age < 28 weeks regained birth weight faster with earlier PN amino acid delivery. Typically, preterm neonates do not regain birth weight until approximately 2 weeks of age. Consequently, an increased growth rate is required to match the fetus’ growth in-utero and to prevent postnatal growth retardation. As preterm infants transition to enteral feeds, mothers’ own milk is the gold standard in spite of its lower nutrient content because of its protective effect over sepsis and NEC. Thus, to provide sufficient nutrients, human milk is fortified with a multicomponent human milk fortifier (HMF) to promote optimal growth during this important ‘catch-up’ period. ‘Catch-up’ growth occurring between birth and term has been associated with improved long term neurocognitive outcome. However, catch-up growth favouring fat mass over lean body mass has been associated with poorer metabolic and health outcomes later in life.

At term corrected age, preterm infants have significantly lower lean body mass but similar fat mass compared to term born infants, corroborated by a meta-analysis on studies published between 1947 and 2011. Such decreased relative proportions of lean body tissue to fat mass interferes with normal growth patterns and is associated with an elevated risk of cardiovascular disease, type 2 diabetes and adiposity in later years. However, the same meta-analysis was unable to assess whether these differences were due to poor intrauterine growth or to compromised postnatal tissue accretion. Nevertheless, increasing the proportion of carbohydrate as a source of nonprotein energy may better reduce protein oxidation and consequently support its utilization in lean mass accretion, suggesting that optimal postnatal nutrient proportions may have therapeutic advantages in achieving targeted growth.

Appropriate nutrition is crucial to achieve appropriate body mass composition, optimal growth, support normal physiology, and neurodevelopment. Nevertheless, when it comes to
feeding the preterm infant, there are a unique set of challenges and obstacles to overcome in order to achieve these goals.

2.2 Energy-Containing Macronutrients, Calcium, and Phosphorus

2.2.1 The Reference Fetus

Data from whole body chemical analyses enabled the creation of a representative body composition of the human fetus between gestational ages 22 to 42 weeks (and corresponding weights).68-70 This reference fetus allows the estimation of daily increments in the major components of the body including macronutrients, minerals, and trace elements.69 With the goal of achieving in-utero growth rates and fetal body composition in the preterm infant, this approach has been used to estimate the nutrient requirements of the preterm infant. Thus, the estimated daily net synthesis rates of protein required for growth is 2 g/kg/day.69 The increments of fat, calcium and phosphorus in body composition of the human fetus is estimated be approximately 1.76 g, 195 mg, and 105 mg respectively, to achieve a weight increase from 1.0 to 1.5 kg.69

2.2.2 Protein

2.2.2.1 Protein and the Preterm Infant

As zwitterions, all amino acids are in possession of an amino group on one end and a carboxyl group on the other. The biochemically defining feature of the amino acid is its amine group which relinquishes a hydrogen ion to form a covalent peptide bond with the carboxylic end of the next amino acid in a process that consumes energy and releases a molecule of water for every bond formed. Protein in the body exists in dynamic equilibrium with the ‘amino acid pool’, whereby tissue protein is continually broken down and synthesized in what is described as protein turnover.71,72 Pencharz et al. found no effect of diet or birth weight on total body nitrogen
turnover in a cohort of VLBW infants, but found an inverse correlation between protein intake and both endogenous protein breakdown as well as skeletal muscle protein breakdown.\textsuperscript{73} Fractional synthesis rates indicate huge variations in protein turnover depending on the organ and amino acid in question.\textsuperscript{72, 74} The fractional synthesis rates in rats for skin, bone, and liver are not uniform, at 60\%, 90\%, and 82\% respectively.\textsuperscript{72} Higher turnover rates are consistent with higher utilization of amino acids by the particular tissue. Stoll et al., reported a 61\% first-pass intestinal metabolism rate for threonine, coinciding with the fact that secretory mucins particularly secretory mucin 2 (MUC2), contain regions greatly enriched in threonine.\textsuperscript{72, 75-77} In preterm neonates, although lower birth weight infants (< 1500 g) have poorer protein absorption, they utilize protein significantly more efficiently, resulting in no difference in net nitrogen retention.\textsuperscript{73} Preterm infants are efficient at utilizing endogenous amino acids, with a reported 92.9 ± 2.6 \% of nitrogen flux being used for protein synthesis.\textsuperscript{73}

Additionally, protein quality deserves considerable attention when it comes to feeding the VLBW preterm infant. Whey-predominant formulations contain a lower methionine to cysteine ratio and cause less metabolic stress.\textsuperscript{69} The preterm infant’s plasma amino acid profile is dependent upon what they are fed.\textsuperscript{78} Further, preterm infants fed a casein-predominant formula were more likely to develop acidosis.\textsuperscript{79} Whey-predominant formulations were based on the pattern of human breast milk compared to the bovine-based casein-predominant formulations.\textsuperscript{69}

\textbf{2.2.2 Function of Protein}

The only energy-containing macronutrient to carry amino groups, protein is a major dietary contributor of nitrogen, an element crucial for the deposition of lean body mass, particularly in the preterm infant.\textsuperscript{80, 81} Apart from supporting growth, the accretion of lean mass in the preterm infant during the early postnatal period is a major contributor to satisfactory health
outcome over the lifespan, evidenced by a decreased risk for type 2 diabetes, childhood insulin insensitivity, and adiposity which are associated with cardiovascular diseases and hypertension.\textsuperscript{15, 82}

Key biological roles are dominated by protein, as evidenced by its ubiquitous presence in the composition of enzymes and transporters.\textsuperscript{83-85} A plethora of enzymatic processes catalyze thousands of biochemical reactions, ensuring the body functions properly in various systems including digestion, respiration, circulation, homeostasis, and excretion.\textsuperscript{86, 87} As an example, the pancreatic triglyceride lipase breaks down triglycerides in the digestion process.\textsuperscript{88} Proteins associated with plasma membranes are pivotal in transporting necessary atoms and molecules across the selectively permeable phospholipid bilayer according to cellular, mitochondrial, and nuclear needs.\textsuperscript{89} For example the Niemann Pick C2 (NPC2) protein mediates the transfer of cholesterol between membranes.\textsuperscript{84} Working in tandem with signalling proteins to regulate cell function, protein is indispensable in the minutiae processes of cellular metabolism, growth, and function.\textsuperscript{87}

Additionally, protein and amino acids play especially important biological roles as neurotransmitters and signalling molecules.\textsuperscript{90} Contributing to the regulation of sleep, consciousness, and arousal within the brain, the amino acid glutamate behaves as an excitatory neurotransmitter whereas glycine is an inhibitory one.\textsuperscript{91} Other amino acids such as valine, isoleucine, leucine, and tryptophan modulate the formation of 5-hydroxytryptamine receptors, implicated in the regulation of satiety.\textsuperscript{92} Further, amino acids act as signaling molecules to regulate numerous biochemical processes including the activation of the mammalian target of rapamycin complex (mTORC1), a complex closely involved in neuron modulated protein
translation and metabolic homeostasis through stimulating biosynthesis, and repressing autophagy.  

The ubiquitous presence of protein and amino acids in the body and their myriad functions, both structurally and biochemically, emphasise that protein is a critical macronutrient for life and for the preterm infant.

2.2.3 Lipid

2.2.3.1 Lipid and the Preterm Infant

Lipids encompass a class of biomolecules that are characterised by their amphiphilic and hydrophobic nature. In 2005, taking into account eukaryotic and prokaryotic sources, the International Lipid Classification and Nomenclature Committee (ILCNC) published a comprehensive classification system that organizes lipids into 8 well-defined categories, fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides. In preterm infant nutrition, the lipid classes largely discussed in the literature are fatty acyls, glycerolipids, glycerophospholipids, and sterol lipids.

As the predominant building blocks for complex lipids, fatty acyls are a fundamental category of biolipids. The glycerolipid category includes all glycerol-containing lipids, mostly populated by the mono-, di-, and tri-substituted glycerols. The esterification of 3 molecules of fatty acids to 1 molecule of glycerol yields a triglyceride, commonly known as fat, the primary form of energy storage for humans. Fatty acids are typically described as short chain, medium chain and long chain fatty acids. Glycerophospholipids, also known as phospholipids possess a hydrophilic head and 2 hydrophobic tails, and are thus able to form a bilayer, typically creating a biological barrier to act as cell membranes, which are largely impermeable to hydrophilic molecules. Glycerophospholipids function as binding sites for intracellular and
extracellular signalling molecules involving the activation or deactivation of other biomolecules through phosphorylation/dephosphorylation of lipids triggering downstream cascades in various metabolic, muscoskeletal, and immunoregulatory processes. Within the sterol lipid class, cholesterol and its derivatives are the most well-known and most widely studied. Cholesterols contribute enormously to the regulation of permeability and structural integrity of cell membranes and also intensely support endocrine system function as the precursors to all steroid hormones in the body, regulating important biological functions such as reproduction, metabolism, growth, and blood pressure.

In human development, long chain polyunsaturated fatty acids (PUFAs), particularly arachidonic acid (AA; 20:4n6) and docosahexanoic acid (DHA; 22:6n3) are exceptionally important. These omega-6 and omega-3 PUFAs play pivotal roles in neuro- and visual development. The placenta preferentially transfers DHA to the fetus compared to other PUFAs, with accretion in fetal tissues doubling during the third trimester highlighting its physiological importance for development. Significant accretion of DHA and AA occurs in the fetal brain during the third intrauterine trimester, emphasizing the importance for preterm infants to obtain these essential fatty acids from dietary sources during the first postnatal weeks. O’Connor et al demonstrated in a randomized controlled clinical trial that supplementation of AA and DHA in preterm infants resulted in improved visual acuity and cognition. Furthermore, a separate study by dit Trolli et al. reported that cumulative lipid intakes in preterm infants at 2 postnatal weeks were significantly associated with improved neurological outcome at 1 year corrected age.

Lipid and its derivatives are integral to modulating important physiological processes, including growth, immune response, visual and neurodevelopment particularly during the vital
early postnatal weeks with short and long-term impacts on health.\textsuperscript{36,107} The many indispensable roles of lipid highlight the necessity for adequate intakes especially for the rapidly developing preterm infant. Although PUFAs are transferred to the fetus via the placenta throughout gestation, 90\% of in utero fat deposition occurs during the third trimester.\textsuperscript{108,109} At birth, a 500 g preterm infant at 24 weeks has <1\% total body weight of fat stores.\textsuperscript{110} The preterm infant is thus exceptionally vulnerable to lipid deficits if early postnatal intakes from both parenteral and enteral sources are insufficient.

\subsection*{2.2.4 Carbohydrate & Energy}

\subsubsection*{2.2.4.1 Carbohydrate and the Preterm Infant}

Glucose is the predominant source of energy in the body, particularly for the brain, whereby energy is needed to maintain neuronal membrane potentials, and the heart.\textsuperscript{111} In preterm infants, whole-body glucose utilization is twice the rate in term infants.\textsuperscript{111} Prolonged hypoglycemia in preterm infants has been shown to compromise neurodevelopment at 18 months corrected age.\textsuperscript{112} On the other hand, an extended state of hyperglycemia promotes excess fat deposition, which is associated with higher risks of obesity, type 2 diabetes, and cardiovascular disease later in life.\textsuperscript{113} The conversion of excess glucose into fat for storage in adipocytes costs energy and results in increased $O_2$ usage and $CO_2$ production, potentially augmenting the burden on the preterm infant’s already fragile respiratory system.\textsuperscript{111} A combination of factors enable the occurrence of hyperglycemia such as high intravenous glucose infusion combined with the presence of stress-reactive hormones in the preterm infant that promote gluconeogenesis and decrease glucose utilization.\textsuperscript{114} It is therefore imperative that glucose is provided at adequate levels to the preterm infant to facilitate optimal lean mass accretion while avoiding excess fat gain.
**2.2.4.2 Energy and the Preterm Infant**

Protein, lipids, and carbohydrate all provide energy in addition to their unique roles in preterm infant growth and development. Energy is required to maintain basal metabolic processes and fuel the synthesis of new tissues. When energy needs are not met from lipid and carbohydrate intakes, amino acids may be oxidized to release energy instead of being utilized for tissue synthesis.\textsuperscript{111} Sufficient energy intake is necessary for successful protein and fat accretion in the preterm infant.\textsuperscript{12} While optimal energy balance is necessary to achieve a composition of weight gain that mimics the weight gain of a fetus, the energy source is also an important factor in maximizing amino acid accretion. When the ratio of non-protein energy supply was 65% to 35% from carbohydrate and fat respectively, the rate of protein oxidation was lower compared a reversed ratio.\textsuperscript{14} Thus, non-protein energy from carbohydrate (versus fat) appears to exert a protective effect against protein oxidation, thereby encouraging growth that consists primarily of lean mass accretion instead of fat mass deposition.\textsuperscript{14}

**2.2.5 Calcium & Phosphorus**

**2.2.5.1 Bone Mineralization**

An obvious physiological role of calcium and phosphorus is bone mineralization, a process in which calcium and phosphorus salts are deposited into the bone matrix to build strong and hard bones. Bone mineral accretion occurs throughout pregnancy, with a majority of in-utero deposition occurring during the third trimester of pregnancy.\textsuperscript{17, 115, 116} Approximately 80% of calcium and phosphorus at term is accumulated after 24 weeks gestation in-utero.\textsuperscript{36, 68, 117, 118} Consequently, the very preterm infant misses a significant portion of this in-utero mineral accretion phase. It is therefore crucial that the preterm infant obtain calcium and phosphorus at levels sufficient to promote bone health and prevent osteopenia of prematurity. Indeed, it has
been demonstrated that calcium and phosphorus supplementation is required to achieve adequate intakes, particularly for preterm infants fed human milk. Calcium absorption depends on calcium intake, whereas phosphorus absorption replies upon both phosphorus intake and ratios of calcium to phosphorus.

The dynamic process of bone remodeling involves bone resorption and formation which releases and stores calcium and phosphorus as needed via the action of osteoclasts and osteoblasts respectively; this regulates calcium and phosphorus levels in the extracellular fluid. Briefly, various inputs including the presence of factors like the insulin growth factor –1 (IGF-1), tumour necrosis factor-α (TNF- α), and parathyroid hormone activate lining cells that upregulate their expression of Receptor Activator of Nuclear κB Ligand (RANKL) which interacts with the Receptor Activator of Nuclear κB (RANK) on “pre-osteoclasts” that trigger the dissolution of bone through acidification and the use of lysosomal enzymes. Resorption releases factors stored in the bone matrix including bone morphogenetic proteins, fibroblast growth factors (FGF), and transforming growth factor-β (TGF-β) that attract osteoblasts to produce new bone matrix at the site followed by mineralisation.

Insufficient levels calcium or phosphorus in the body result in the commonly occurring osteopenia of prematurity whereby the risk of fracture is elevated. As the bone matrix is the primary reservoir of calcium and phosphorus in the body, inadequate intakes compromises bone health. Osteopenia in preterm infants is chiefly related to deficits in the 2 essential bone minerals, calcium and phosphorus, instead of vitamin D. Balance studies in preterm infants indicate that mineral absorption is a linear function of mineral intake and that vitamin D supplementation does not increase serum calcium or phosphorus levels.
2.2.5.2 Non-bone related functions of Calcium

Apart from contributing to bone mineralisation, calcium is an essential intracellular and extracellular signalling messenger in the body, regulating muscular contraction and dilation, action potential transmission, and hormonal secretion. Approximately 98% of total body calcium is stored in the skeleton, making it the reservoir of calcium in the body while the rest circulates within the intracellular and extracellular fluid. Approximately 50% of extracellular fluid levels of calcium exist as ions and are biologically active, 40% is bound to protein, and 10% forms reversible complexes with anions such as phosphate, bicarbonate, sulphate, and citrate.

Calcium ions act through differential potential gradients regulated by protein channels, and is active in extracellular, cytoplasmic and nucleic spaces. With the ability to bind to various binding proteins, ionic calcium either activates or inhibits downstream signalling pathways. Calcium is extensively involved in both intracellular and extracellular signalling, regulating a staggering number of biological phenomena including muscle contraction, gene expression, neurotransmitter release, secretory processes, cell proliferation, cell apoptosis, cell-cell interaction, maintenance of structural integrity, and tissue repair. Reflective of the extensive biological role of calcium, a MEDLINE search done in 1997 yielded 24,000 articles published just within the 2 previous years. Thus, while a detailed review of each of these functions is outside the scope of this thesis, it suffices to acknowledge the widespread use of calcium in the body for important physiological processes.

2.2.5.3 Non-bone related functions of Phosphorus

Like calcium, the large majority of total body phosphorus (85%) is deposited in the skeleton. When in circulation throughout the body, phosphorus exists either in its inorganic
form, bound to lipids, or esters. Phosphorus functions within numerous biologically active molecules including adenosine triphosphate or ATP, the body’s primary energy currency; nucleic acids which encompass DNA and RNA, the blueprints of life; creatine phosphate, a high energy reserve found primarily in the brain and skeletal muscles; phospholipids, a key component in cell membranes, and the various intermediary phosphorylated components of cell metabolism and signalling.

Phosphate is highly involved in the metabolism of proteins, lipids and carbohydrates, regulating the intermediary steps as well as several key enzyme-dependent reactions such as glycolysis, 2,3diphosphoglycerate synthesis, and ammoniagenesis. Also, within the cell, phosphate is exchanged for malate, as it is transported across the mitochondrial membrane in gluconeogenesis, a key metabolic process that regulates blood glucose levels.

Apart from being an indispensable component of major metabolic processes, phosphorus has an interdependent relationship with calcium in the regulation of absorption and bioavailability. Phosphorus absorption is dependent on the relative amounts of calcium and phosphorus, making it impossible to study one without the other.

2.3 Nutrition, Growth and Neurodevelopment

The provision of nutrients as soon as possible after birth at sufficient levels and proportions will support appropriate growth, neurodevelopment, and better health outcomes later on in life for the preterm infant. Numerous studies have found associations between intakes of particular nutrients and multiple growth anthropometrics and/or neurodevelopment in preterm infants, as summarised in Table 1. While protein, lipid, carbohydrate, and energy intakes have each been positively associated with weight, length, and head circumference measurements, protein and energy are most often implicated in promoting growth. Of the studies published
between 1985 and 2014 investigating this relationship, 3 were randomized controlled trials, 2 of which report both protein and energy to be significantly associated with head circumference gains.\textsuperscript{6, 141, 142}

In 1985, Georgieff et al. reported that energy deficits in preterm infants were associated with poorer than average neurodevelopment scores at 1 year corrected age.\textsuperscript{143} Recent studies confirm that protein intakes during the first postnatal week are significantly associated with improved neurodevelopmental outcomes in preterm infants at 18 months corrected age; each 1g/kg/day of protein intake during this time corresponded to an 8.2-point increase in the Mental Development Index on the Bayley Scales of Infant and Toddler Development (Bayley).\textsuperscript{2} Another study found cumulative lipid intakes at 2 postnatal weeks to be significantly associated with improved neurological development at 1 year corrected age.\textsuperscript{3} Further, enteral protein intakes during the first 2 weeks were identified to be associated with improved cognitive and motor scores on the Bayley III test.\textsuperscript{144} Findings from a few studies did not support this association. Poindexter et al. examined protein and energy intake for the first 5 and first 20 days after birth in a secondary analysis of an early vs late PN study and did not find significant associations with neurodevelopment at 18 months corrected age.\textsuperscript{145} A study published by Cester et al. did not find higher protein intakes to be significantly associated with improved neurodevelopment at 2 years corrected age, however, the authors acknowledged that the study was underpowered to detect a clinically significant improvement in Bayley-III scores.\textsuperscript{146} To date, these are the only studies directly investigating and linking nutrient intakes (enteral or combined enteral and PN) to improved neurodevelopment.

However, the association between impaired growth and neurodevelopment is well documented. Table 2 summarizes the studies reporting the association between multiple growth
anthropometrics and later neurodevelopment in very low birth weight (VLBW) infants. Only included in the table are anthropometrics measured postnatally up to a maximum of 2 years of age, as rapid brain growth occurs between the third trimester and 2 years of age\textsuperscript{147}. In 6 of the 8 studies, head circumference was reported to be significantly associated with neurodevelopment.\textsuperscript{7-10, 148, 149} Nutritionally influenced physical changes in the caudate nuclei volume are significantly correlated with verbal IQ in adolescent males born preterm.\textsuperscript{37} In addition to head circumference, both weight and length have been associated with improved neurodevelopment. It follows that insufficient nutrients for proper head growth would also adversely impact weight gain and overall growth.
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<td>Retrospective cohort</td>
<td>&lt;1000g</td>
<td>&lt; 33</td>
<td>First week PN lipid</td>
<td>NA</td>
<td>Yes</td>
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<tr>
<td>Burattini et al., 2013&lt;sup&gt;142&lt;/sup&gt;</td>
<td>131</td>
<td>Italy</td>
<td>RCT, single blinded Prospective cohort</td>
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<td>NA</td>
<td>NS</td>
<td>Protein</td>
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<tr>
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<td>New Zealand</td>
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<td>&lt;1000g</td>
<td>≤ 31</td>
<td>Protein</td>
<td>Protein</td>
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<tr>
<td>Sjostrom et al., 2013&lt;sup&gt;152&lt;/sup&gt;</td>
<td>531</td>
<td>Sweden</td>
<td>Retrospective cohort</td>
<td>&lt;1500g</td>
<td>&lt; 27</td>
<td>Protein, lipid, carbohydrate, energy</td>
<td>Protein, carbohydrate, energy</td>
<td>Yes†</td>
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<tr>
<td>dit Trolli et al., 2012&lt;sup&gt;3&lt;/sup&gt;</td>
<td>48</td>
<td>France</td>
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<td>Protein</td>
<td>Cumulative lipid</td>
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<tr>
<td>Maas et al., 2012&lt;sup&gt;153&lt;/sup&gt;</td>
<td>224</td>
<td>Germany</td>
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<td>&lt; 32</td>
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<td>NA</td>
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<tr>
<td>Miller et al., 2012&lt;sup&gt;154&lt;/sup&gt;</td>
<td>92</td>
<td>Australia</td>
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Table 1. cont. Impact of nutrition on growth and neurodevelopment in preterm infants

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Location</th>
<th>Study Design</th>
<th>Birth weight</th>
<th>Gestational Age (wks)</th>
<th>Major outcomes</th>
<th>Nutrients Examined</th>
<th>Daily data</th>
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<tbody>
<tr>
<td>McLeod et al., 2012</td>
<td>63</td>
<td>Australia</td>
<td>Prospective</td>
<td>&lt;2580g</td>
<td>&lt; 33</td>
<td>Protein</td>
<td>Protein, energy</td>
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<td>Senterre &amp; Rigo, 2011‡</td>
<td>84</td>
<td>Belgium</td>
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<td>Cormack et al., 2011‡</td>
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<td>Australia &amp; New Zealand</td>
<td>Retrospective</td>
<td>&lt;1500g</td>
<td>NA</td>
<td>Enteral protein intake in first 2 weeks</td>
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<td>Stephens et al., 2009²</td>
<td>148</td>
<td>USA</td>
<td>Retrospective</td>
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<td>Protein</td>
<td>First week protein &amp; energy</td>
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<tr>
<td>Martin et al., 2009¹</td>
<td>1187</td>
<td></td>
<td></td>
<td>&lt; 28</td>
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<td>First week protein, lipid &amp; carbohydrate</td>
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<td>Valentine et al., 2009</td>
<td>440</td>
<td>USA</td>
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<td>Tan &amp; Cooke 2008</td>
<td>142</td>
<td>UK</td>
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<td>NA</td>
<td>&lt; 29</td>
<td>Cumulative protein &amp; energy</td>
<td>Protein, energy</td>
<td>Yes</td>
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</table>
Table 1. cont. Impact of nutrition on growth and neurodevelopment in preterm infants

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Location</th>
<th>Study Design</th>
<th>Birth weight</th>
<th>Gestational Age (wks)</th>
<th>Major outcomes</th>
<th>Nutrients Examined</th>
<th>Daily data</th>
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</thead>
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<tr>
<td>Collins et al., 2008</td>
<td>138</td>
<td>Australia</td>
<td>Prospective</td>
<td>NA</td>
<td>&lt; 33</td>
<td>Lipid&lt;sup&gt;≥&lt;/sup&gt;, Carbohydrate&lt;sup&gt;≥&lt;/sup&gt;</td>
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<td>Poindexter et al., 2006</td>
<td>1018</td>
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<td>Secondary analysis</td>
<td>&lt;1000g</td>
<td>NA</td>
<td>Protein (Day1-5), energy (Day1-20)</td>
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<td>Ernst et al., 2003</td>
<td>69</td>
<td>USA</td>
<td>Retrospective chart review</td>
<td>&lt;1000g</td>
<td>&lt; 30</td>
<td>Protein, energy</td>
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<td>Trotter &amp; Pohlandt 2002</td>
<td>20</td>
<td>Germany</td>
<td>Balance study on RCT subset</td>
<td>&lt;1000g</td>
<td>&lt; 29</td>
<td>Ca &amp; P retention&lt;sup&gt;≤&lt;/sup&gt;</td>
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<td>NA</td>
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<td>Olsen et al., 2002</td>
<td>564</td>
<td>USA</td>
<td>Retrospective</td>
<td>&lt;1500g</td>
<td>&lt; 30</td>
<td>Protein, energy</td>
<td>Protein, energy</td>
<td>No&lt;sup&gt;§&lt;/sup&gt;</td>
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<td>Embleton et al., 2001</td>
<td>105</td>
<td>UK</td>
<td>Prospective</td>
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<td>≤ 34</td>
<td>Cumulative Energy</td>
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<td>Berry et al., 1997</td>
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<td>Canada</td>
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<td>Study</td>
<td>n</td>
<td>Location</td>
<td>Study Design</td>
<td>Birth weight</td>
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<td>Major outcomes</td>
<td>Nutrients Examined</td>
<td>Daily data</td>
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<td>Polberger et al., 1989&lt;sup&gt;16&lt;/sup&gt;</td>
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<td>Sweden</td>
<td>RCT, double-blinded</td>
<td>&lt;1500g</td>
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<td>Protein, lipid, carbohydrate, energy, calcium, phosphorus</td>
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<td>Protein, energy</td>
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<tr>
<td>Georgieff et al., 1985&lt;sup&gt;14&lt;/sup&gt;</td>
<td>73</td>
<td>USA</td>
<td>Prospective</td>
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<td>≤ 34</td>
<td>Energy</td>
<td>Protein, lipid, carbohydrate, energy, calcium, phosphorus</td>
<td>Yes</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Energy</td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup>RCT: Randomized controlled trial; <sup>b</sup>NA, not studied; <sup>c</sup>Over the first 10 days; <sup>d</sup>Daily until Day 28, thereafter weekly until death or hospital discharge; <sup>e</sup>Daily until Day 7, thereafter weekly until Day 28; <sup>f</sup>Data collected on days of life 0, 3, 7, 14, 21, 28; <sup>g</sup>Prospective intervention compared against a retrospective cohort as control group; <sup>h</sup>Ca & P retention were significantly correlated with Ca & P intake; <sup>i</sup>Energy proportion from lipid intakes were negatively associated with weight, length, and head circumference; <sup>j</sup>Holding total energy constant, the energy contribution of protein, lipid, and carbohydrate to growth was examined.
<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Location</th>
<th>Study Design</th>
<th>Birth weight</th>
<th>Gestational Age</th>
<th>Growth measure associated with neurodevelopment</th>
<th>Time period growth measure was studied</th>
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<tbody>
<tr>
<td>Sammallahti et al., 2014&lt;sup&gt;10&lt;/sup&gt;</td>
<td>103</td>
<td>Finland</td>
<td>Retrospective</td>
<td>&lt;1500g</td>
<td>&lt; 37</td>
<td>Head circumference*</td>
<td>Between birth and term</td>
</tr>
<tr>
<td>Ramel et al., 2012&lt;sup&gt;162&lt;/sup&gt;</td>
<td>62</td>
<td>USA</td>
<td>Retrospective review</td>
<td>&lt;1500g</td>
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<td>Length</td>
<td>4 and 12 months corrected age</td>
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<tr>
<td>Nash et al., 2011&lt;sup&gt;163&lt;/sup&gt;</td>
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<td>Canada</td>
<td>Retrospective review</td>
<td>&lt;1500g</td>
<td>NA</td>
<td>Weight gain</td>
<td>Between 6 months and 2 years corrected age</td>
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<tr>
<td>Franz et al., 2009&lt;sup&gt;148&lt;/sup&gt;</td>
<td>219</td>
<td>Germany</td>
<td>Prospective follow-up</td>
<td>&lt;1500g</td>
<td>30</td>
<td>1) Weight gain 2) Head circumference</td>
<td>1) Between birth and discharge 2) Postdischarge</td>
</tr>
<tr>
<td>Ehrenkranz et al., 2006&lt;sup&gt;9&lt;/sup&gt;</td>
<td>495</td>
<td>USA</td>
<td>Prospective</td>
<td>&lt;1000g</td>
<td>NA</td>
<td>Weight, head circumference</td>
<td>Between regaining birth weight and discharge/transfer/age 120 days/achieving weight of 2000g 2 years of age</td>
</tr>
<tr>
<td>Latal-Hajnal et al., 2003&lt;sup&gt;149&lt;/sup&gt;</td>
<td>219</td>
<td>Switzerland</td>
<td>Prospective</td>
<td>&lt;1250g</td>
<td>≤ 36</td>
<td>Weight, length, head circumference</td>
<td></td>
</tr>
<tr>
<td>Stathis et al., 1999&lt;sup&gt;7&lt;/sup&gt;</td>
<td>124</td>
<td>Australia</td>
<td>Not specified</td>
<td>&lt;1000g</td>
<td>NA</td>
<td>Head circumference</td>
<td>8 months</td>
</tr>
<tr>
<td>Hack et al., 1991&lt;sup&gt;8&lt;/sup&gt;</td>
<td>249</td>
<td>USA</td>
<td>Prospective</td>
<td>&lt;1500g</td>
<td>NA</td>
<td>Head circumference</td>
<td>8 months</td>
</tr>
</tbody>
</table>

*after controlling for neonatal complications
2.4 Recommended Nutrient Intakes for Preterm Infants

The goal of nourishing infants has been to achieve in utero rates of growth and body composition. With improvements in neonatal care around the world enhancing survival rates in preterm infants, the focus has shifted from ensuring immediate survival of the infant toward facilitating optimal development and future quality of life. Accumulating evidence points to the crucial role of adequate nutrition on optimal growth and long-term neurodevelopment. Importantly, ‘too much’ growth is undesirable as rapid catch-up growth consisting predominantly of fat mass deposition has been connected to poor metabolic outcomes later in life.

Sufficient energy is needed to supply basal metabolic needs and to support tissue synthesis, a highly energy intensive process. The synthesis and deposition of lean tissue requires an optimal protein to energy ratio to maximize the utilization of protein and amino acids. Data indicate that an energy intake of 120 kcal/kg/day ensures a close-to-maximal utilization of protein delivered at 3.6 g/kg/day. Given the inevitable nutrient deficit incurred during the transition of the preterm infant to the extraterine environment, the newest recommendations range from 3.5–4.5 g/kg/day to compensate for accumulated deficit. The calcium and phosphorus requirements to support in-utero bone mineralisation rates are 120 mg/kg/day and 75 mg/kg/day respectively. Also, calcium and phosphorus balance studies reveal that phosphorus retention is dependent upon calcium retention. Recommended intake levels decrease the risk of fracture and osteopenia of prematurity in preterm infants.

Expert committees have revised nutrient recommendations according to findings in the literature that indicate a higher protein intake would be beneficial. Table 3 highlights the various nutrient intake recommendations published by major expert nutrition committees between 1987
and 2014 to illustrate the adjustments made in response to research findings. There is a clear increasing trend of recommended protein intakes from 2.9-3.6 g/kg/day in 1987, to 3.5-4.5 g/kg/day in 2014.

### 2.4.1 Meeting Nutrient Requirements in Preterm Infants

The vulnerable state of the preterm infant predisposes it to various illnesses and medical complications that interfere with the provision of adequate nutrition. The periodic evaluation of the success in meeting these recommendations is important especially with the publication of updated recommendations. Table 4 briefly outlines the achievement of these expert nutrient recommendations. In spite of the increasing recommendations, newer studies indicate successful achievement of expert guidelines compared to the older ones, suggesting an improvement in efficient and safe delivery of nutrients to the preterm infant early in life. The newest studies reveal that target protein intakes of 3.5-4.5 g/kg/day are not achieved until at least 2 postnatal weeks or not at all, over a monitored duration of 28 and 70 days respectively.\textsuperscript{144,152} Only 2 of the most recent studies evaluate intakes against the ESPGHAN 2010 recommendations and none use the latest guidelines published in 2014 by Koletzko et al.\textsuperscript{38,164}
Table 3. Recommended enteral intakes for very low birth weight infants over time

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>2.9-3.6</td>
<td>3.0-4.0</td>
<td>3.4-4.3</td>
<td>3.5-4.0</td>
<td>3.4-4.2</td>
<td>3.5-4.5</td>
<td>3.5-4.5</td>
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<tr>
<td>Lipid</td>
<td>5.3-7.2</td>
<td>4.5-6.8</td>
<td>5.3-6.8</td>
<td>5.4-7.2</td>
<td>5.3-7.2</td>
<td>4.8-6.6</td>
<td>4.8-6.6</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>8.4-16.8</td>
<td>7.5-15.5</td>
<td>11.5-15.0</td>
<td>10.0-14.0</td>
<td>11.5-17.0</td>
<td>11.6-13.2</td>
<td>11.6-13.2</td>
</tr>
<tr>
<td>Energy</td>
<td>110-130</td>
<td>105-135</td>
<td>110-141</td>
<td>105-130</td>
<td>110-130</td>
<td>110-135</td>
<td>110-130</td>
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<tr>
<td>Calcium</td>
<td>84-168</td>
<td>160-240</td>
<td>148-222</td>
<td>210</td>
<td>100-220</td>
<td>120-140</td>
<td>120-200</td>
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<tr>
<td>Phosphorus</td>
<td>60-108</td>
<td>78-118</td>
<td>98-131</td>
<td>110</td>
<td>60-140</td>
<td>60-90</td>
<td>60-140</td>
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</tbody>
</table>

†ESPHGAN: The European Society for Pediatric Gastroenterology Hepatology and Nutrition; ‡LSRO: Life Science Research Office; *Formula-fed only
Table 4. Studies that have evaluated the achievement of nutrient recommendations for preterm infants

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Study Design</th>
<th>Birth Weight</th>
<th>GA $^1$</th>
<th>Recommendations used</th>
<th>Protein</th>
<th>Lipid</th>
<th>Carbohydrate</th>
<th>Energy</th>
<th>Calcium</th>
<th>Phosphate</th>
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</thead>
<tbody>
<tr>
<td>Christmasmann et al., 2014$^{177}$</td>
<td>79</td>
<td>Prospective</td>
<td>NA</td>
<td>&lt; 34</td>
<td>ESPGHAN 2005, Tsang 2005$^{36}$</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>Sjostrom et al., 2013$^{152}$</td>
<td>531</td>
<td>Retrospective</td>
<td>&lt;1500g</td>
<td>≤ 27</td>
<td>ESPGHAN 2010$^{164}$, Tsang 2005$^{36}$, AAP 2008$^{178}$</td>
<td>×</td>
<td>✓</td>
<td>✓</td>
<td>✓$^†$</td>
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<td>N/A</td>
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<td>Watts et al., 2013$^{179}$</td>
<td>31</td>
<td>Retrospective</td>
<td>&lt;1860g</td>
<td>&lt;32$^1$</td>
<td>ESPGHAN 2010$^{164}$</td>
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<td>N/A</td>
<td>N/A</td>
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<td>Huston et al., 2012$^{180}$</td>
<td>34</td>
<td>Prospective</td>
<td>&lt;1000g</td>
<td>NA</td>
<td>Tsang 2005$^{36}$</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<td>✗</td>
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<tr>
<td>Cormack et al., 2011$^{144}$</td>
<td>80</td>
<td>Retrospective</td>
<td>&lt;1500g</td>
<td>NA</td>
<td>Tsang 2005$^{36}$ &amp; ESPHGAN 2010$^{164}$</td>
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<td>N/A</td>
<td>N/A</td>
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<td>Martin et al., 2009$^{41}$</td>
<td>1187</td>
<td>Prospective</td>
<td>NA</td>
<td>&lt; 28</td>
<td>AAP 2004$^{176}$, Tsang 2005$^{36}$</td>
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<td>✓</td>
<td>✗</td>
<td>✗</td>
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<tr>
<td>Ernst et al., 2003$^4$</td>
<td>69</td>
<td>Retrospective</td>
<td>&lt;1000g</td>
<td>&lt; 30</td>
<td>Tsang 1993$^{181}$, AAP 1998$^{182,**}$</td>
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<td>Tsang 1993$^{181}$, AAP 1998$^{182,**}$</td>
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<td>N/A</td>
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Table 4. cont. Studies that have evaluated the achievement of nutrient recommendations for preterm infants

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Study Design</th>
<th>Birth Weight</th>
<th>GA</th>
<th>Recommendations used</th>
<th>Protein</th>
<th>Lipid</th>
<th>Carbohydrate</th>
<th>Energy</th>
<th>Calcium</th>
<th>Phosphate</th>
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<td>Olsen et al., 2002(^5)</td>
<td>564</td>
<td>Retrospective</td>
<td>&lt;1500g</td>
<td>&lt; 30</td>
<td>AAP 1985(^{184}), ESPGHAN 1987(^{173}), Tsang 1993(^{181})</td>
<td>(\times)</td>
<td>N/A</td>
<td>N/A</td>
<td>(\times)</td>
<td>N/A</td>
<td>N/A</td>
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<td>Loui et al., 2002(^{185})</td>
<td>10</td>
<td>Prospective, balance study</td>
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<td>(\leq 27)</td>
<td>AAP 1985(^{184}), ESPGHAN 1987(^{173}), Tsang 1993(^{181}), CPS 1995(^{174})</td>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>(\times)</td>
<td>(\times)</td>
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<tr>
<td>Embleton et al., 2001(^{40})</td>
<td>105</td>
<td>Prospective</td>
<td>(\leq 1750g)</td>
<td>(\leq 34)</td>
<td>Tsang 1993(^{181}), AAP 1998(^{182})* (\ast)</td>
<td>(\times)</td>
<td>N/A</td>
<td>N/A</td>
<td>(\times)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

\(^1\)GA, gestational age; \(^2\) N/A, not examined or not reported in study; *Protein recommendations met by week 3; † ESPGHAN energy recommendations met by majority of infants between day of life 29-70, but not Tsang and AAP recommendations; ‡Protein recommendations met by week 2, remained at lower limit of the recommended range; **3.0 g/kg/day for protein and 120 kcal/kg/day for energy; \(^3\)3.0-4.0 g/kg/day for protein and 120-165 kcal/kg/day for energy; \(^\dagger\)Minimum recommendations met by week 2 by most infants (24 of 31).
2.5 Major Morbidities in the NICU

2.5.1 Increased energy requirements

Postnatal growth failure is especially evident in preterm infants with morbidities including necrotizing enterocolitis (NEC), chronic lung disease (CLD), and late onset sepsis. Both late onset sepsis and chronic lung disease (CLD) in preterm infants have been associated with concomitant increases in energy expenditure. Patent ductus arteriosus (PDA), a condition whereby the ductus arteriosus remains open or reopens in preterm infants, is associated with a higher risk for NEC and particularly CLD.

An often-used definition of CLD by the Canadian Neonatal Network is the need for oxygen at 36 weeks postmenstrual age. A major cause of mortality and morbidity in the NICU, CLD is inversely associated with gestational age. Also, NEC and sepsis may increase the risk of CLD by predisposing the preterm lung to inflammation, edema, and injury. Infants with CLD have an elevated basal metabolic rate alongside higher oxygen consumption and their energy needs were estimated to be 15-25% higher than their healthy counterparts. This elevated levels of nutrient and energy needs for adequate growth continue even after recovery from CLD.

Closely associated with and often leading to CLD, PDA affects approximately a third of all VLBW infants delivered, with 70% of preterm infants with gestational age < 28 weeks requiring a medical or surgical intervention for PDA. Altered blood flow from increased pulmonary circulation leads to decreased organ perfusion, a major contributory factor in the complications arising from PDA that include NEC and feeding intolerance. Additionally increased pulmonary flow combined with low oncotic pressure and increased capillary permeability often observed in preterm infants with respiratory distress creates conditions conducive to the loss of pulmonary compliance, increasing the risk of CLD.
Both low gestational age and low birth weight are major risks factors for the development of retinopathy of prematurity (ROP), a significant cause of morbidity in preterm infants.\textsuperscript{194, 195} Often occurring alongside NEC, late onset sepsis, and CLD, ROP is characterised by improper vascularisation of the retina and may lead to blindness in the most severe cases.\textsuperscript{195-197} Oxygen supplementation and duration are significantly associated with severe ROP.\textsuperscript{194, 198} The pathogenesis of ROP in the very preterm infant is strongly related to oxygen exposure, which halts retinal vessel growth through the down-regulation of oxygen-regulated angiogenic growth factors including the vascular endothelial growth factor (VEGF).\textsuperscript{195, 199} Further, hyperglycemia in VLBW infants is significantly associated with ROP.\textsuperscript{200} Also, Wallace et al., report inadequate postnatal weight gain in the first 6 weeks after birth to be a risk factor for ROP.\textsuperscript{197} Thus appropriate nutrition in VLBW infants is important in preventing ROP.

Late onset sepsis is a significant cause of mortality and morbidity amongst preterm infants. In a large scale study involving 6956 VLBW infants, approximately 70\% of first-episode late onset sepsis cases were caused by gram-positive organisms.\textsuperscript{31} The highest death rates from sepsis involve infections by \textit{Pseudomonas aeruginosa}, \textit{Candida albicans}, \textit{Serratia marcescens}, and \textit{E. coli}.\textsuperscript{201} As with CLD, the rate of late onset sepsis infections is inversely related to gestational age.\textsuperscript{30} Total energy expenditure increases by 40-70\% in preterm infants with sepsis compared to healthy controls.\textsuperscript{202}

\textbf{2.5.2 Nutrient management of sick infants}

It necessary to tightly regulate fluid intake in the management of PDA and CLD.\textsuperscript{24, 203} High fluid intakes (>170 ml/kg/day) during the first few postnatal days have been shown to increase the risk of PDA.\textsuperscript{204} Unfortunately, limiting total fluid intake restricts the provision of nutrients and energy to the preterm infant. Paradoxically, adequate nutrient intakes are vital to reducing the risk
and severity of CLD. Further, while there is evidence suggesting that a minimum amount of enteral nutrition is necessary to lower the risk of CLD, a larger prospective sample or randomized controlled trial is needed to confirm this finding. The needs of specific nutrients in infants with CLD and PDA are poorly defined, and require further investigation. Most NICUs do not have a separate feeding protocol for sick infants. Likewise, the nutrient requirement for preterm infants with NEC or sepsis is not established. The current published expert recommendations are for healthy growing preterm infants.
Chapter 3 Achieving Enteral Nutrient Recommendations in Preterm Infants

3.1 Rationale for Study

Optimal intakes of nutrients in VLBW preterm infants during the early postnatal period is vital to support optimal growth, reduce morbidity and promote later neurodevelopment, and health. Protein, lipid, carbohydrate, and energy intakes during initial hospitalization have been associated with improved weight, length, and head circumference measurements. In the literature, head circumference is significantly associated with improved neurodevelopment. Importantly, protein, lipid, and energy intakes in preterm infants during this critical time are significantly associated with improved neurodevelopmental outcome.

The correct proportions and amounts of nutrients early in life, particularly during hospitalization are essential for optimal growth in VLBW infants. Expert recommendations for healthy, growing preterm infants are designed to approximate in-utero growth rates and fetal mass accretion. It is crucial that weight gain includes the correct proportion of lean mass to fat mass deposition to reduce the future risks of metabolic syndrome and related cardiovascular diseases. Additionally, sufficient intakes of the minerals calcium and phosphorus ensures adequate bone mineralization thus protecting against osteopenia of prematurity, a common condition among the preterm infant population.

However, various major co-morbidities make it challenging to feed infants at a level that meets their needs. In addition to ensuring the adequacy of nutrient intake in accordance with expert recommendations, clinicians have to manage clinical complications that commonly affect preterm VLBW infants, which sometimes interfere with the nutrient delivery. Infant morbidities such as CLD, NEC, late onset sepsis and PDA are associated with each other. A recent retrospective
study found that infants with CLD had significantly higher rates of postnatal growth failure. Furthermore, CLD and late onset sepsis increase energy requirements in preterm infants, while the management of PDA and CLD involves fluid restriction, often hindering the provision of adequate nutrition.

Although many NICUs have implemented more aggressive early nutrition protocols, few studies have evaluated the achievement of expert recommendations using prospective data, and none have done so with the newly published recommended guidelines. Further, it is important to periodically evaluate the success at achieving these guidelines. As no separate guidelines currently exist for feeding through specific morbidities, data from this study is valuable in providing an assessment of the nutrient adequacy of infants with at least 1 major morbidity. Study findings would hopefully start meaningful discussions on the feasibility of achieving current recommendations in very sick preterm infants and the development of strategies to overcome these obstacles.

3.1.1 Research Questions

1) Do enteral protein, lipid, carbohydrate, energy, calcium, and phosphorus intakes in a cohort of VLBW infants during initial hospitalization meet current expert nutrition recommendations? Specifically do enteral intakes achieve recommended goals of:

i. 3.5-4.5 g/kg/day for protein,

ii. 4.8-6.6 g/kg/day for fat,

iii. 11.6-13.2 g/kg/day for carbohydrates,

iv. 110-130 kcal/kg/day energy,

v. 120-200 mg/kg/day for calcium,

vi. 60-140 mg/kg/day for phosphate.
2) Do VLBW infants that have at least one major morbidity (e.g. late-onset sepsis, NEC, CLD, PDA) differ in achieving these recommended nutrient intakes versus those that do not?

3.1.2 Research Hypotheses

1) Current enteral intakes of protein, lipid, carbohydrate, energy, calcium, and phosphorus in a considerable proportion of VLBW infants do not meet current expert nutrient recommendations.

2) VLBW infants with at least one major morbidity are less likely to achieve current nutrient recommendations.

3.2 Methods

3.2.1 Study Design & Subjects

This study was nested within the DoMINO (Donor Milk for Improved Neurodevelopmental Outcomes, ISRCTN35317141) double-blind randomized control trial whose primary research objective was to determine whether the use of pasteurized donor milk (instead of preterm formula) as a supplement to mother’s own milk (MOM) for 90 days or until hospital discharge, whichever comes first, improves neurocognitive development at 18 months corrected age among infant born of VLBW. The methods of this trial have been published in detail previously and prospective collection of daily nutrient intakes was pre-planned. Enrolment of VLBW infants occurred with the first 96 hours after birth between October 2010 and December 2012 at four level III NICUs in Toronto and Hamilton, Canada: Mount Sinai Hospital, The Hospital for Sick Children, Sunnybrook Health Sciences Center, and McMaster Children’s Hospital. The inclusion criteria for participation were: 1) day 1 to 4 of life; 2) birth weight; BW < 1500 g; 3) expected initiation of enteral feeding within the first 7 postnatal days. The exclusion criteria were: 1) any serious chromosomal or congenital anomaly that may affect developmental outcome; 2) severe asphyxia at birth; 3)
enrolment in another clinical study affecting nutritional management; 4) likely transfer to an NICU where study protocol could not be continued. For this sub-study, infants that died before the provision of any enteral feeding were excluded from analyses. The study was approved by the Research Ethics Committee at each participating hospital in accordance with the policies outlined in the Tri-council policy statement on the ethics of research involving human subjects. Whenever infants met the eligibility criteria, permission was requested from their attending physician to approach families and written consent was obtained for participation.

According to the feeding protocol for DoMINO, all infants received MOM first if it was available. Infants were randomized to receive a supplement of pasteurized donor milk or preterm formula whenever MOM was unavailable. The feeding intervention for DoMINO lasted for 90 days after enrolment into DoMINO or until hospital discharge, whichever occurred first. Of note, the study continued after infants were transferred to one of 19 local community NICUs in the greater Toronto and Hamilton area prior to discharge home. Provision of MOM was not blinded in the study. All study feeds (e.g. donor milk, preterm formula) were prepared by one of our research diet technicians upon receipt of a feeding order using a laminar flow cabinet in a designated milk preparation room at the Hospital for Sick Children. MOM was prepared by either the research dietitians or according to local hospital policy (e.g. milk preparation area, bedside).

Feeding protocols in each NICU recommend the initiation of PN within the first 24 hours after birth at 80 ml/kg/day and advanced at a rate of 20 ml/kg/day as tolerated. For the stable infant, enteral feeds were initiated within the first week and advanced at a rate of 15-20 ml/kg/day up to 140-160 ml/kg/day. The most recently published recommendations for enterally fed preterm VLBW infants advise a target of 3.5-4.5 g/kg/day for protein and 110-130 kcal/kg/day energy. Intakes are recommended to be 4.8-6.6 g/kg/day for lipids, 11.6-13.2 g/kg/day for carbohydrates,
120-200 mg/kg/day for calcium, and 60-140 mg/kg/day for phosphate.\textsuperscript{38} Nutrient fortification with a bovine-based human milk fortifier commenced at an enteral intake of \textgeq 120 ml/kg/day, and central lines were removed once tolerance was observed. A whey protein module (Beneprotein, Nestle) was added to donor milk upon commencement of nutrient fortification to increase the protein concentration of donor milk (0.9 g/dl) to the average estimated protein concentration of MOM expressed after 30 days (1.2 g/dl).\textsuperscript{36, 210} More than 95\% of our donor milk was purchased and shipped from the Mother’s Milk Bank of Ohio while a backup supply was obtained from Calgary Mother’s Milk Bank; both are members of the Human Milk Banking Association of North America, HMBANA. Donor milk from Ohio consisted of milk that was collected and pooled from at least 3 mothers who had expensed their milk for donation within 3 months of delivery. At the time of the study, donor milk was not used as part of routine care in all but one recruiting hospital; and in this unit only for a limited time in smaller babies. None of the participating NICUs had written feeding protocols for infants that were ill.

3.2.2 Data Collection & Calculating Nutrient Intakes

Daily recipes used in preparing feeds and the corresponding daily volume of milk consumed by each infant were collected prospectively. Each feeding recipe and the daily volumes of milk actually consumed were entered into separate password protected sections of the DoMINO study database to ensure that research staff not involved in milk preparation remained blinded throughout the duration of the study. After unblinding the study, to calculate the daily amounts of nutrients received, both sections of the database were merged according to date and caloric strengths to reveal feed types. The nutrient composition of MOM was estimated using literature values of milk expressed by mothers of preterm infants, weighted according to sample size of the studies they were drawn from (Table 5). To account for the variability of MOM composition over time, weekly
cut-offs were used in my literature review to obtain weighted literature values for week 1, week 2, week 3, week 4, and >week4. As values levelled out into a consistent number matching the dietary reference intakes for each nutrient beyond week 4, “>week4” represents all subsequent days in a separate category. Donor human milk values from the Ohio Milk bank typically indicated they contained 0.9 g/dl protein. Donor milk from Calgary Mother’s Milk Bank were not analyzed for protein content. All other nutrients of interest were estimated using reported literature values for donor milk.\textsuperscript{211-222} Nutrients received from preterm formula were calculated using product monographs. In our study, both Enfamil A+ and Similac Special Care preterm formulas were used; at 20kcal/30ml, the concentrations of protein (g/ml), lipid (g/ml), carbohydrate (g/ml), energy (kcal/ml), calcium (mg/ml), and phosphorus (mg/ml) were 0.020, 0.034, 0.074, 0.68, 1.12, and 0.56 respectively, for Enfamil A+ and 0.020, 0.037, 0.070, 0.68, 1.22, and 0.68 respectively, for Similac Special Care.

Infants receiving MOM placed at the breast to facilitate initially non-nutritive suckling and then transitioned to full breastfeeds, usually around 35 weeks post-conception as discharge approached. The number of times an infant was fed at the breast was recorded. When it was observed that an infant was breastfed at least 3 times and there was a significant decrease in volume of enteral feeds, the estimated nutrient intakes were considered inaccurate and not included in the present analysis. Test weights for feeding at the breast were unavailable.

Also collected prospectively were infant death and major morbidities including NEC, late onset sepsis, CLD, PDA, and severe ROP. NEC was defined as stage 2 or 3 according to the Modified Bell Staging Criteria.\textsuperscript{223} A confirmed case of late onset sepsis was defined as a positive culture from blood, cerebrospinal fluid, catheter or suprapubic urine at ≥ 5 postnatal days. For the current analyses CLD was defined as the need for oxygen at 36 weeks postmenstrual age.\textsuperscript{188,189} The
incidences of NEC, late onset sepsis, CLD, and severe ROP are associated with each other.\textsuperscript{27-29, 196} The association of late onset sepsis and CLD with elevated energy expenditure warrants their inclusion in the morbidity composite for this study alongside other associated comorbidities.\textsuperscript{32, 42} There is evidence that appropriate nutrition reduces the risk of incidences of CLD and ROP.\textsuperscript{187, 200} For this study, due to its significant association with CLD, cases of PDA were included in this composite for our morbidity group, defined as infants with at least one case of NEC, late onset sepsis, CLD, severe ROP or PDA.\textsuperscript{24, 27, 28, 187} All data collected was entered and contained in a Postgres database, a secure web based custom built PHP application. The database server is located behind the Sickkids firewall and is supported by the SickKids Research Information Technology Group.

Table 5. Weighted estimates of mother’s own milk nutrient composition

<table>
<thead>
<tr>
<th>Nutrient Unit</th>
<th>Protein, g/dl</th>
<th>Lipid, g/dl</th>
<th>Carbohydrate, g/dl</th>
<th>Energy, kcal/dl</th>
<th>Calcium, mg/dl</th>
<th>Phosphorus, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1\textsuperscript{†} (Days 1-8)</td>
<td>2.38</td>
<td>3.00</td>
<td>6.21</td>
<td>61.31</td>
<td>29.96</td>
<td>15.58</td>
</tr>
<tr>
<td>Week 2\textsuperscript{†} (Days 9-15)</td>
<td>2.03</td>
<td>3.44</td>
<td>6.34</td>
<td>64.41</td>
<td>28.14</td>
<td>15.87</td>
</tr>
<tr>
<td>Week 3\textsuperscript{†} (Days 16-22)</td>
<td>1.94</td>
<td>3.36</td>
<td>6.52</td>
<td>64.12</td>
<td>25.64</td>
<td>12.82</td>
</tr>
<tr>
<td>Week 4\textsuperscript{†} (Days 23-29)</td>
<td>1.79</td>
<td>3.43</td>
<td>6.90</td>
<td>65.65</td>
<td>25.64</td>
<td>12.82</td>
</tr>
<tr>
<td>&gt; Week 4\textsuperscript{†}</td>
<td>1.17</td>
<td>3.97</td>
<td>7.69</td>
<td>71.21</td>
<td>25.64</td>
<td>12.82</td>
</tr>
</tbody>
</table>

\textsuperscript{†}References used: Atkinson et al., 1981,\textsuperscript{211} Atkinson et al., 1980,\textsuperscript{224} Atkinson et al., 1987,\textsuperscript{225} Atkinson & Anderson 1983,\textsuperscript{226} Anderson et al., 1981,\textsuperscript{227} Anderson et al., 1983,\textsuperscript{222} Bauer & Gerss 2011,\textsuperscript{228} Butte et al., 1984,\textsuperscript{229} Chan et al., 1982,\textsuperscript{230} DRI guide 2006,\textsuperscript{74} Gross et al., 1981,\textsuperscript{231} Lemons et al., 1982,\textsuperscript{232} Moltó-Puigmartí et al. 2010,\textsuperscript{233} Paul et al., 1997,\textsuperscript{234} Sann et al., 1981,\textsuperscript{235} Saarela et al., 2005,\textsuperscript{236} Schanler & Oh 1980.\textsuperscript{220}
3.2.3 Statistical Analysis

Descriptive statistics were calculated for all variables of interest. Continuous measures were summarized using means and standard deviations whereas categorical measures were summarized using counts and percentages. Nutrient intakes were displayed using box and whisker plots indicating the median and quartile values. Whiskers were calculated as the lower quartile -1.5 IQR (interquartile range) for the lower whisker and as upper quartile +1.5 IQR for the upper whisker (outliers not shown). Differences in infant characteristics between the healthy and major morbidity groups such as birth weight and gestational age were assessed using a two sample t test for continuous variables. Categorical data (i.e. sex) were analyzed by Cochran Mantel Haenszel Statistics. Repeated measures linear models (using PROC MIXED) were used to evaluate change in nutrient intake over time (across weeks 1-10) controlling for site. The models included in the analyses were healthy vs major morbidity status, birth weight group (<1000g and 1000-1499g), a site by time interaction term, and birth weight/major morbidity stratum by time interaction terms. To determine whether the progression of time influenced the association between variables of interest and nutrient intakes, interaction terms with site and major morbidity status were added into the model. All analyses were two-tailed and a p-value of 0.05 was considered to denote statistical significance. Data was analysed using SAS Version 9.3 for Windows (SAS Institute, Cary, North Carolina, USA).

3.3 Results

3.3.1 Infant Characteristics

Between October 2010 and December 2012, out of 840 eligible infants whose families were approached, consent was obtained for 363 infants to participate in DoMINO. (Figure 1) Of the families of infants that declined participation, 189 were not interested in participating, 12 had
personal circumstances that precluded participation in DoMINO, 49 preferred the feeding regimen of current clinical practice, 178 did not want donor milk to be used as a supplement, and 11 did not want preterm formula to be used as a supplement. One infant died before any source of enteral nutrition was provided, and of the remaining 362 infants included in this analysis, 144 had no major morbidity (healthy) and 218 were diagnosed with any one of the following: NEC, late onset sepsis, CLD, severe retinopathy of prematurity, and PDA (Table 6). Infants in our cohort had a mean birth weight of 997 ± 272 g and gestational age of 28 ± 3 weeks. Approximately 27% of infants had a score of ≥20 on the Score for Neonatal Acute Physiology II (SNAP-II), a robust measure of illness severity.\textsuperscript{237, 238} Infants in the major morbidity group had significantly lower birth weights (p <0.0001) and gestational ages (p<0.0001) than infants in the healthy group. There were significantly more male infants in the major morbidity group (p=0.04). Among the anthropometric z scores, only weight z-scores on study day 1 were significantly different between the 2 groups (p=0.04); neither length (p=0.5) nor head circumference z-scores (p=0.6) on study day 1 differ significantly between our healthy and major morbidity groups.
Figure 1. Consort Statement

- Infants approached to participate in DoMINO (n=840)
- Infants Randomized (n=363)
- Withdrawn or died before any nutrition was received (n=1)
- Infants included in this analysis (n=362)

Declined (n=477)
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, mean ± SD (n), g</td>
<td>997 ± 272 (362)</td>
</tr>
<tr>
<td>Gestational Age, mean ± SD (n), wks</td>
<td>28 ± 3 (362)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>195 (54)</td>
</tr>
<tr>
<td>Female</td>
<td>167 (46)</td>
</tr>
<tr>
<td>Small for Gestational Age (SGA)&lt;sup&gt;a&lt;/sup&gt;, n (%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45 (12)</td>
</tr>
<tr>
<td>No</td>
<td>317 (88)</td>
</tr>
<tr>
<td>SNAP-II, n (%)</td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>179 (49)</td>
</tr>
<tr>
<td>10-19</td>
<td>83 (23)</td>
</tr>
<tr>
<td>≥ 20</td>
<td>96 (27)</td>
</tr>
<tr>
<td>5- Minute APGAR Score, n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 6</td>
<td>84 (23)</td>
</tr>
<tr>
<td>≥ 6</td>
<td>275 (76)</td>
</tr>
<tr>
<td>Days to full enteral feeding, mean ± SD (n), days</td>
<td>22 ± 15 (326)</td>
</tr>
<tr>
<td>Morbidity counts, n (%)</td>
<td></td>
</tr>
<tr>
<td>Patent ductus arteriosus (PDA)</td>
<td>172 (48)</td>
</tr>
<tr>
<td>Necrotizing Enterocolitis (NEC)</td>
<td>15 (4)</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>100 (28)</td>
</tr>
<tr>
<td>Severe Retinopathy of Prematurity</td>
<td>15 (4)</td>
</tr>
<tr>
<td>Chronic lung disease (CLD)</td>
<td>81 (22)</td>
</tr>
</tbody>
</table>

<sup>a</sup>An infant was considered SGA when birth weight according to gestational age fell below the 10th percentile on the Fenton Growth Chart.<sup>239</sup>
3.3.2 Nutrient Intakes During Initial Hospitalization

The newest expert recommendations for enterally fed, healthy, growing, preterm infants suggest a protein intake of 3.5-4.5 g/kg/day. In our cohort, the median intakes of infants weighing between 1000-1499 g (n=168) at birth achieve this protein goal on week 3 (n=165) and continue to do so until week 5 (n=153) (Figure 2). The median protein intake of these infants subsequently falls below this recommended range for weeks 6-10 (n=140, n=127, n=98, n=69, n=56). The median intakes of ELBW infants (BW < 1000g; n=194) approach this recommended protein intake at week 8 (n=159) but do not meet it until postnatal week 10 (n=147) (Figure 2).

Similarly, healthy infants in our cohort achieved median intakes within this recommended range on week 3 (n=141), 4 (n=137), and 5 (n=130), whereas infants in the major morbidity group do not until postnatal week 10 (n=161) (Figure 3).

Figure 2. Enteral protein intake according to birth weight group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).
The median lipid intake for heavier babies (BW 1000-1499g) in our cohort meet the recommended intake range of 4.8-6.6 g/kg/day\textsuperscript{240} by week 3 (n=165), whereas ELBW infants achieve this goal on week 5 (n=167) (Figure 4). Median enteral lipid intakes continue to fall within the recommended range until postnatal week 10 for both birth weight groups. This trend is also observed in the healthy vs morbidity analysis, whereby healthy infants achieve median lipid intakes within recommended levels at week 3 (n=141), whereas infants with at least one major morbidity meet these nutrient goals on week 5 (n=190) (Figure 5).
Figure 4. Enteral lipid intake according to birth weight group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).

Figure 5. Enteral lipid intake for infants in the healthy vs. major morbidity group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).
Median enteral carbohydrate intakes of infants weighing 1000-1499g at birth fell within the recommended range of 11.6-13.2 g/kg/day\textsuperscript{240} by week 4 (n=159) (Figure 6). This goal was achieved by our ELBW infants on week 6 (n=165). The median carbohydrate intake of healthy infants and infants with a major clinical morbidity met this recommendation on week 3 (n=141) and week 6 (n=187) respectively (Figure 7).

Figure 6. Enteral carbohydrate intake according to birth weight group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).
Figure 7. Enteral carbohydrate intake for infants in the healthy vs. major morbidity group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).

Enteral energy recommendations of 110-130 kcal/kg/day\textsuperscript{38} were achieved by week 3 (n=165) and 6 (n=165) according to birth weight group (1000-1499g and <1000g) respectively (Figure 8). The median intakes of healthy infants met this goal on week 3 (n=141), whereas infants with at least one major morbidity did so by week 6 (n=187) (Figure 9).
Figure 8. Enteral energy intake according to birth weight group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).

Figure 9. Enteral energy intake for infants in the healthy vs. major morbidity group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).
Recommended enteral calcium and phosphorus intakes are 120-200 mg/kg/day and 60-140 mg/kg/day respectively.\textsuperscript{38} Median calcium intakes met this recommended range by week 3 (n=165) and 6 (n=165) according to birth weight group (1000-1499g and <1000g respectively) (Figure 10). Similarly, phosphorus intakes in our cohort approximated recommended values by week 3 (n=165) and 5 (n=167) according to birth weight group (1000-1499g and <1000g respectively) (Figure 12). Comparing infants in the healthy vs morbidity groups, median enteral intakes achieved this calcium goal on week 3 (n=141) and 6 (n=187) respectively (Figure 11). Median phosphorus intakes similarly approximate recommended values by week 3 (n=141) for healthy infants and week 5 (n=190) for infants with at least one major morbidity (Figure 13).

Figure 10. Enteral calcium intake according to birth weight group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).
Figure 11. Enteral calcium intake for infants in the healthy vs. major morbidity group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).

Figure 12. Enteral phosphorus intake according to birth weight group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).
Figure 13. Enteral phosphorus intake for infants in the healthy vs. major morbidity group. Whiskers were calculated as ±1.5IQR (Tukey whiskers).

The results of the repeated measures linear models found a statistically significant effect of the interaction term healthy*time on intakes of all nutrients (all interaction terms p <0.0001) except protein (p=0.2) (Figures 3, 5, 7, 9, 11, and 13). Thus, over time, intakes of each nutrient with the exception of protein varied significantly according to their healthy vs morbidity status. Similarly, over time, intakes for each nutrient significantly varied based on site (all site*time interaction terms p<0.0001). Infants in the healthy group had statistically significantly higher protein intake compared to their counterparts in the major morbidity group (healthy main effect, p<0.0001) (Figure 3).

When examining the interaction between birth weight strata and time on nutrient intake, a statistically significant interaction was found for only protein (p<0.0001) and calcium (p<0.0001) (Figures 2 and 10). Intakes for all nutrients examined varied based on site over time at a level that
was statistically significant (all site*time interactions terms p<0.0001). A statistically significant main effect of BW strata was observed for intakes of all nutrients (BW < 1000g main effect, p<0.0001). (Figures 2, 4, 6, 8, 10, and 12).

3.4 Discussion

Our data indicate that the introduction of more aggressive feeding guidelines in our NICUs have resulted in higher nutrient intakes compared to those reported by Embleton et al. during a time when the standard of care was more conservative. We found that a majority of infants belonging to the higher BW (1000-1499 g) and healthy groups received enteral nutrition during hospitalization at a level that met current expert recommendations for protein, lipid, carbohydrate, energy, calcium, and phosphorus. Nevertheless, when median nutrient intakes of these groups achieved current expert recommendations (week 4 for carbohydrate; n=159, and week 3 for all other nutrients; n=165), the proportion of infants in the higher BW group whose nutrient intakes still did not meet recommendations were 36%, 28%, 38%, 39%, 33%, and 28% for protein, lipid, carbohydrate, energy, calcium, and phosphorus respectively. Similarly, the proportion of infants in the healthy stratum that had intakes below recommended levels for this time frame were 33%, 25%, 34%, 38%, 32%, and 26% respectively. Suboptimal nutrient intakes in these infants remain a concern. Individually, protein, lipid, and energy have each been associated with significantly improved growth as well as neurodevelopment. Importantly, cumulative protein and energy deficits are significantly associated with poor postnatal growth, with reported energy deficits accounting for up to 45% of variation in weight z-scores. Further, all 3 anthropometric growth measures (weight, length and head circumference) evaluated between birth and 2 years have been associated with improved long term neurodevelopmental outcome (Table 2).
Additionally, for the ELBW (BW <1000 g) and morbidity groups, when median intakes of these groups met recommendations, the proportion of infants with nutrient intakes below recommended levels were 53%, 44%, 45%, 49%, 45%, and 48%, for protein (week 10; n=147), lipid (week 5; n=167), carbohydrate (week 6; n=165), energy (week 6; n=165), calcium (week 6; n=165), and phosphorus (week 5; n=167), respectively in the ELBW group, whereas these proportions were 53%, 45%, 46%, 48%, 45%, and 47%, respectively in the morbidity group. In the landmark Embleton study published in 2001, the targeted intake of protein at the time was 3.0 g/kg/day, a level that was not achieved in the Embleton cohort. In our study, median protein intakes of the higher BW and healthy groups reached this level of protein intake by week 3, whereas infants in the ELBW and morbidity groups achieved this 3.0 g/kg/day mark by week 6, clearly showing that while there is room for improvement, current feeding guidelines are more successful at providing higher amounts of nutrition to our preterm infants than in the past.

Median enteral intakes of infants in both the higher BW and healthy groups achieved current expert recommendations by week 3 for all nutrients except carbohydrate; the median carbohydrate intake very closely approached recommended levels on week 3 and exceeded them by week 4. Given that the average number of days to reach full enteral feeds in our cohort was 22 ± 15 days, or approximately 3 weeks, our data indicate that the achievement of recommended protein, lipid, energy, calcium, and phosphorus intakes in the healthy and higher BW infant groups coincide with achieving full enteral feeding. Full enteral feeding was also achieved in approximately 3 postnatal weeks in a cohort of ELBW infants examined by Ernst et al. A retrospective study of the same cohort showed that they achieved the then current protein goal of 3.0 g/kg/day (vs. most recently published goals of 3.5-4.5 g/kg/day) also by week 3. However this cohort never achieved energy targets of 120 kcal/kg/day, which is within the range of current recommendations used in
our study (110-130 kcal/kg/day). In comparison, our infants in the higher BW and healthy groups achieved energy goals by week 3 and the median nutrient intakes of the ELBW and morbidity groups met this energy target on week 6.

Protein intakes in our study better approximated recommendations than those in a cohort of Swedish VLBW infants. Their recently published study found protein intakes to be below recommended levels throughout the entire study (days 1-70). Nevertheless, only 3 time frames were used in this study; days 1-7, days 8-28, and days 28-70, as data for only one day a week was collected after day 28. To the best of our knowledge, our study is the first to examine the achievement of recommended intakes for protein, lipid, carbohydrate, energy, calcium, and phosphorus using daily data collected prospectively.

Nutrient recommendations at the time of the Embleton study considered 3 g/kg/day to be acceptable but did not factor in nutrient needs for optimal growth or the minimization of postnatal growth failure after the inevitable failure to achieve minimal requirements to support metabolic needs in the days right after birth. The revised expert recommendations used in our study makes such provisions, approving protein intakes of up to 4.5 g/kg/day to support optimal growth. However, in our study, median protein intakes never reach 4.5 g/kg/day in all 10 weeks examined. On the other hand, after reaching recommended levels on week 3 for the higher BW and healthy groups, energy intakes remain solidly within recommended ranges thereafter. Energy intake is thus in excess when considered alongside suboptimal protein intake, creating an imbalanced protein-energy ratio that would theoretically favour the deposition of fat over lean mass. Compromised protein intake places infants at greater risk of postnatal growth failure. Although outside the scope of this study, a crucial next step would be to investigate growth trends alongside these nutrient intakes.
Median nutrient intakes of infants in our morbidity composite group consistently achieved recommended nutrient intakes 2 weeks later compared to infants in our healthy cohort. Eighty-one infants (23%) in the morbidity composite group were diagnosed with CLD. The lack of fat-free mass deposition in preterm infants diagnosed with CLD strongly suggest that the provision of nutrients at a level that supports catch-up growth is warranted. However, not only were protein intakes of 4.5g/kg/day never achieved, 53% of infants in the morbidity group did not meet minimum expert recommendations (3.5 g/kg/day) during the week median protein intake achieved recommended levels. Similarly, minimum carbohydrate requirements (11.6 g/kg/day) were not met by 45% of infants in the morbidity group, notably concerning because energy from carbohydrate (versus fat) favours lean mass accretion. Additionally, infants with CLD have been shown both to have higher energy needs and display postnatal growth retardation, compounding their risk for poorer metabolic outcomes later in life. The provision of proper nutrition to infants with CLD is challenging to the medical team as there are valid concerns surrounding the aggressive advancement of enteral feeds. Higher fluid intake in a cohort of ELBW infants during the first 10 postnatal days was found to be significantly associated with an increased risk of CLD. Often, fluid is restricted as a preventative measure against pulmonary edema to reduce mortality of CLD. Infants with CLD receive significantly lower levels of enteral nutrition compared to infants without CLD; however, data suggest that a vital amount of enteral nutrition is required to prevent the development of CLD. The issue is further complicated by the strong associations between incidences of CLD, late onset sepsis, NEC, and PDA.

Closely associated with and identified as a risk factor for CLD, PDA results in increased pulmonary flow, shunting additional fluid to the lungs. In order to reduce the fluid build-up in the lungs, the management of PDA (and CLD) include a tight regulation and sometimes even
restriction of fluid intake. Fluid intakes during the first postnatal week in preterm infants have been associated with significantly increased odds of developing PDA. 204 There is legitimate concern that fluid restriction due to the management of CLD and PDA leads to compromised nutritional intake. Indeed, energy and macronutrient intakes were found to be below minimum requirements in a cohort of Swedish preterm infants before, during, and after surgery for PDA. 242 The challenges in providing higher levels of nutrients to sick preterm infants under restricted fluid intake suggests there is a need to develop new products and/or protocols that can both facilitate and support the delivery of adequate nutrition in spite of limited fluid intakes.

As with CLD, septic episodes increase total energy expenditure in preterm infants, as determined using the doubly labelled water method. 202 Feeding intolerance is one of the general symptoms of late onset sepsis, forcing clinicians to strike a challenging balance between managing clinical complications and providing optimal nutrition. The use of PN to supplement feeds enhances the supply of nutrition to support growth and subsequent recovery. However the prolonged presence of central lines increase the risk for late onset sepsis. 31 In a study on first week nutritional intakes in a subsample of our current VLBW cohort, incidence of late onset sepsis decreased the likelihood of reaching full enteral feeds by 80%, 243 indicating a possible bi-directional causal relationship between poor nutrition and sepsis.

In a study by Ehrenkranz et al., the incidences of CLD, NEC, and late-onset sepsis significantly decreased across increasing weight gain quartiles, indicating an inverse relationship or that the sickest infants had the most compromised growth velocity. 9 As the sicker infants in our study experienced poorer intakes of protein, lipid, carbohydrate, energy, calcium, and phosphorus compared to their healthy counterparts, we expect to find greater postnatal growth failure when growth data is included in the analysis. In a double blinded randomized controlled trial, infants with
CLD that were fed a formula enriched in protein, calcium, phosphorus and zinc had significantly faster catch-up growth, higher lean and bone mass compared to those fed a standard formula. The long term effects (beneficial or otherwise) of such accelerated catch-up growth at term corrected age in infants who had CLD were outside the scope of the study but warrants further research.

Amino acid availability influences calcium and phosphorus metabolism; high protein levels promote lean mass deposition which necessitates the concomitant increase in phosphorus utilization, as phosphate ions are a major component of the cell cytoplasm and function. Conversely, inadequate protein intake impedes tissue synthesis rendering the utilization of phosphorus in cellular growth suboptimal at best despite intakes at recommended levels. Calcium and phosphorus exist in a ratio of 2:1 in human milk as well as in the skeleton, forming the logical basis for current expert enteral recommendations. It is also considered appropriate to take into account the need for phosphorus intake to compliment protein levels during lean mass accretion. This avoids the hypophosphatemia and hypercalecemia observed when high protein intakes deplete circulatory phosphorus, requiring the utilization of phosphorus stored in bone, simultaneously releasing calcium causing the observed hypercalecemia.

As only enteral intake data are presented here, a limitation of this study is that these findings cannot be used to assess nutrient intake adequacy prior to the enteral feeding period, when PN plays an important role in nutrient delivery. Nevertheless, our findings provide valuable data to analyse the achievement of enteral feeding recommendations. Further, PN data has already been obtained and will, in the near future be incorporated into these analyses for preparation to publish. Acknowledging the complexity and size of both the PN and enteral nutrition databases, a complete independent audit for data quality will be complete prior to publication. Another limitation to this study is that day of birth was defined as day 1 and thus did not always represent a complete 24-hour
period. Consequently, in order to capture seven complete 24-hour periods, nutrient information from day 1 to day 8 was used to represent week 1, days 8-15 represented week 2 and so on. Lastly, the nutrient composition of MOM and DM was not analyzed but estimated using literature values that were weighted according to sample size of the study. To account for the variability of MOM composition over time, weekly cut-offs were used in the literature review for weeks 1-4. After 4 postnatal weeks, estimates consistently approximated the dietary reference intake values, which were used for each nutrient beyond postnatal week 4.
Chapter 4 Conclusion

Although great strides have been made in feeding VLBW infants, in the present study 33% (protein), 25% (lipid), 34% (carbohydrate), 38% (energy), 32% (calcium), and 26% (phosphorus) of infants without at least one major morbidity had intakes below nutrient goals during the week. Median intakes reached recommended levels during initial hospitalization. Sicker infants had even poorer attainment of nutrient recommendations during this time, with 53%, 45%, 46%, 48%, 45%, and 47%, respectively failing to achieve minimum requirements. Similarly, 36%, 28%, 38%, 39%, 33%, and 28% (higher BW) vs. 53%, 44%, 45%, 49%, 45%, and 48% (ELBW) infants did not achieve recommended intakes for protein, lipid, carbohydrate, energy, calcium, and phosphorus respectively during initial hospitalization. Since the provision of adequate levels and optimal proportions of protein, lipid, carbohydrate, energy, calcium, and phosphorus to VLBW infants is crucial for proper growth, neurodevelopment and good metabolic outcomes later in life, it is important to periodically evaluate the achievement of these goals and to identify challenges associated with achieving them. There are currently no separate guidelines to feed infants that are ill despite documentation of elevated nutrient needs compared to healthy infants. There is insufficient research on the safety and ability to provide more nutrients as many sick infants are failing to achieve current recommendations for healthy infants. Thus, more research and discussion on this issue is warranted.

4.1 Future Directions

The provision of adequate nutrition prior to the achievement of full enteral feeding depends upon PN as a supplement to enteral feeds. Combining PN data with the enteral data currently presented in this study will enable the evaluation of the achievement of nutrient recommendations for this time period.
Current expert recommendations allow a protein intake of up to 4.5 g/kg/day for infants that require catch-up growth. Although, median protein intakes never reach 4.5 g/kg/day in our study, some infants receive protein at and above this level. Thus, evaluating growth in association with the nutrient intake data in this study will be a crucial next step. The relationship between specific growth measures and nutrient intakes will be investigated. Particularly interesting will be the relationship between head circumference and various nutrients, as head circumference has been linked to neurodevelopment in preterm infants. In addition, the relationship between nutrient intakes neurodevelopment will be investigated, taking into account growth and other variables.

The American Academy of Pediatrics section on breastfeeding states that MOM is the gold standard for feeding preterm infants given its many benefits including protective effects on NEC. Research is currently being conducted on the benefits of using human-milk based human milk fortifier instead of a bovine-based product. Feeding VLBW infants human milk versus preterm formula confers neurodevelopmental benefits as evidenced by higher BSID (Bayley Scales of Infant and Toddler Development) scores. Additionally, protein intake has been associated with improved neurodevelopment scores. Thus it would be interesting to evaluate whether and how the sources of specific nutrients (eg. protein from MOM vs DM or formula) impact these same outcomes of growth and neurodevelopment.
References


