Enteral diet and necrotizing enterocolitis in neonates: a scoping review and parent outcome prioritization project

by

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Abstract

Necrotizing enterocolitis (NEC) is a potentially life-threatening inflammatory disease of the gut affecting newborns, especially preterm infants. This complex disease is affected by numerous risk factors including genetics, intestinal microbiota, immune system responses, cardiac or respiratory conditions, and diet. Several studies have investigated the effect of diet on NEC, and many have found that bovine milk-based products are linked to more NEC cases than an exclusive human milk diet. Reasons for this effect are unclear. Further, the effect of other nutrition products like protein-hydrosylated formulas and fortifiers is largely unknown. Obtaining reliable evidence on diet and NEC is difficult, given the rarity of the disease, confounding in observational studies, and the costs of nutrition products and coordinating large studies. In order to advance the field of NEC and nutrition, a comprehensive evaluation of the literature, and development of sufficiently powered, high-quality studies relevant to researchers, clinicians and families are needed.

This thesis includes three studies: 1) a narrative review providing background on NEC, preterm nutrition and an overview of the association between diet and NEC in infants; 2) a scoping review mapping clinical research on different enteral diets and NEC, and systematic review of hydrolyzed nutrition products; and 3) a parent cross sectional survey on outcome prioritization that identified outcomes most important to parents, as well as compared parent priorities to commonly reported outcomes in the literature.

The scoping review identified 76 studies, mostly observational studies comparing a predominantly or exclusively human milk diet with an exclusive or partial bovine milk-based formula diet. The majority of these studies suggest that human milk may be protective against NEC, but sample size was often inadequate to detect significant differences between feeding groups. Only 1/5 RCTs, 6/21 cohort studies and 11/16 case control studies found that an

exclusive or predominantly human milk diet resulted in significantly fewer NEC cases than a diet containing a higher proportion of bovine milk-based products. Two randomized controlled trials comparing protein-hydrosylated fortifiers to intact-protein bovine milk-based fortifiers found no significant difference between the fortifiers and the development of NEC; however, the small sample size and imprecision of the effect estimates identified a low grade of evidence.

The parent outcome prioritization study included 15 participants from across Canada and found that infant death and NEC were the most important outcomes to parents. Parents prioritized outcomes involving parents (e.g. quality of life) more than the reflected quantitative literature; whereas, nutrition-related outcomes (e.g. duration of parenteral nutrition) were considered lower parent priorities but were often reported as primary outcomes in the literature. In order to understand the true effect of protein-hydrosylated products compared to human milk and bovine milk-based products on NEC, a large clinical trial is needed. We recommend collaborating with a parent advisory committee during the planning stages of future trials to ensure family-relevant outcomes are included when evaluating NEC and treatment interventions.

Preface

This thesis is an original work by Jocelyn Shulhan. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name “Parent outcome prioritization for neonatal nutrition and necrotizing enterocolitis”, No. 00068447, 3 November 2017.

Chapter 2 of this thesis has been published as Shulhan J, Hartling L, Dicken B, and Larsen BMK, “Current knowledge of necrotizing enterocolitis in preterm infants and the impact of different types of enteral nutrition products,” *Advances in Nutrition*, vol. 8, 80-91. I was responsible for the concept formation, data collection and analysis, and manuscript composition. B.M.K. Larsen was the supervisory author involved with concept formation, interpretation of study results and manuscript edits. L. Hartling assisted with critical appraisal and interpretation of study results, and contributed to manuscript edits. B. Dicken provided expert opinion and references, and contributed to manuscript edits. T. Fenton (external examiner for oral thesis defense) provided post-publication edits.

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Dedication

To my amazing parents, Carmen and Lorne Shulhan, for showing me the meaning of hard work, integrity and compassion.

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List of Abbreviations

CI	Confidence interval
DHM	Donor human milk
HM	Human milk
HMF	Human milk fortifier
IQR	Interquartile range
MOM	Mother's own milk
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
OR	Odds ratio
PICU	Pediatric intensive care unit
PTF	Preterm formula
RCT	Randomized controlled trial
RR	Risk ratio
SD	Standard deviation

Chapter 1: Introduction

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a complex and potentially life-threatening inflammatory disease of the gut affecting newborns, especially preterm infants. NEC is characterized by a pro-inflammatory response and injury of the gut wall barrier that may advance to necrosis and, potentially, perforation of the gut (1). An immature gastrointestinal tract and immune system, abnormal microbial colonization, genetic polymorphisms, excessive volume and type of enteral nutrition, and insults such as hypoxia may contribute to the development of NEC (1, 2). In Canada, 5.1% of infants born <33 weeks gestation develop NEC (3). Mild cases are treated medically with antibiotics and gut rest, but severe cases may lead to surgery, long-term developmental impairments or death in 20-30% of patients (2). Despite the urgency and devastating consequences of the disease, there have been minimal research advancements in the prevention of NEC (2).

The type of enteral nutrition fed to preterm and critically ill infants has been studied as a therapy to help protect against NEC. Previous studies suggest that bovine milk-based nutrition products may lead to more NEC cases than human milk (4), but reasons for this hypothesis are unclear and an exclusive human milk diet is limited by supply and cost. There are multiple considerations to weigh when deciding on an appropriate diet for an infant in the neonatal intensive care unit (NICU). A brief description of enteral nutrition sources investigated in this thesis, and the advantages and disadvantages for each follows.

Sources of enteral nutrition for infants in the neonatal intensive care unit

Mother's own milk (MOM) is typically the first choice of nutrition for infants in the NICU, including those at risk of NEC, because of its many associated benefits (5). Among the numerous

valuable components of MOM, it contains protein, growth factors, immunological agents, and pro- and prebiotics to promote appropriate growth, enhance immunoprotection, and develop a healthy microbiota (6). Unfortunately, the supply of MOM for some mothers may be difficult to establish, leading to insufficient volumes of milk available in the first several days or weeks. Pasteurized donor human milk (DHM) purchased from registered milk banks is an alternative source of human milk prioritized for preterm infants when MOM is unavailable. DHM contains many of the immunological and digestive benefits as MOM. A limitation of DHM is the added cost and, often, its deficiency in protein and other nutrients required by preterm infants. If both MOM and DHM are unavailable, preterm or term bovine-milk based formula is used. Bovine milk-based formulas are useful because they contain consistent amounts of nutrients, may be concentrated to meet an infant's growth requirements and are cost effective. Compared to human milk, however, formulas may not be tolerated as well, and do not include the previously mentioned beneficial components of human milk. These differences may impact an infant's defenses against NEC.

Another nutrition consideration for preterm infants is fortification of MOM and DHM with human milk fortifier (HMF). Although the proportion of protein, carbohydrate and fats in MOM and DHM is appropriate for preterm infants, the amount of calories, protein, vitamins and minerals is insufficient for very and extremely low birth weight infants. Fortification is necessary for infants born <1500 g in order to meet the infant's energy, protein and micronutrient needs during the preterm period when there is a rapid rate of growth (7, 8). The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) and North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) also recommend the use of fortified human milk for infants with a birth weight <1800 g to facilitate appropriate growth (9). The most commonly used HMF is a powder made from bovine milk, but recently, a new HMF has been marketed as a better option for preterm infants. In order to provide an exclusive

human milk diet, Prolacta® Bioscience (Prolacta) concentrated DHM to create a liquid DHM-based fortifier (10) to be added to MOM or DHM. Prolacta has reported lower incidence of NEC with the use of its product. These results seem promising, but it is a costly option. The average cost of Prolacta HMF for an infant born at 28 weeks gestation and 750-1000 grams is approximately $12,000 CAD for a 5-6 week course of treatment (personal communication, Mike Hamilton, Prolacta Business Director, Canada, 22 Apr 2016).

Another type of enteral nutrition is protein-hydrosylated ("hydrolyzed") formulas or fortifiers. These products have proteins broken down to individual amino acids or small peptides, as well as carbohydrates and fats that are easy to absorb. The main indication for these formulas is digestive and absorptive issues, such as short bowel syndrome. An innovative use of hydrolyzed products, though, is to assist infants with immature or immunocompromised digestive systems absorb nutrients. Efficient nutrient absorption may promote weight gain, development of the gut wall barrier (11) and avoid pro-inflammatory digestive processes (12), which may reduce the risk of NEC.

Study justification and objectives

Over the past few decades, nutrition products, research and recommendations for the best source of enteral nutrition in the NICU have evolved. The type of enteral nutrition fed to infants is being recognized as a potential approach to protect against NEC. Previous studies and systematic reviews have compared human milk to bovine milk-based products with respect to NEC and have favored an exclusive human milk diet, but several uncertainties remain.

Systematic reviews on this topic focus on RCTs, indicating that the totality of evidence on all types of quantitative study designs (e.g. cohort studies, case control studies), neonatal characteristics and diet comparisons have not yet been synthesized. A compendium of quantitative studies on enteral diets and NEC, using scoping review methodology, may offer

useful information for clinicians and researchers, and identify directions for future research. Additionally, as far as we are aware, hydrolyzed nutrition products have not been systematically reviewed as an intervention for NEC. It is unknown how hydrolyzed products compared to bovine milk-based products with intact-protein or human milk affect NEC incidence, and the quality of this evidence.

Lastly, in order to develop meaningful research going forward, protocol decisions should be based on existing evidence and input from key stakeholders. Patient and community engagement is being increasingly integrated into research to ensure that results are important to patients and their families as the end users of health research and services. To our knowledge, parent priorities for research outcomes related to infant health and nutrition in the NICU has not been explored. Findings from this work may help researchers in the field of nutrition and NEC generate future studies that measure outcomes and answer problems most important to families.

Research questions

This thesis reviews the evidence on different enteral diets and NEC, systematically reviews hydrolyzed nutrition products, and investigates parent priorities for research outcomes related to nutrition and NEC. The research questions addressed in this thesis are:

1. How is the type of enteral nutrition fed to infants in a NICU associated with NEC?
2. What evidence is available associating NEC with different enteral diets?
3. What is the effect of hydrolyzed formulas or fortifiers compared to intact-protein formula or fortifiers, or human milk on NEC?
4. What are parent priorities for infant health and nutrition outcomes regarding quantitative research in the NICU?

5. How do parent priorities compare to commonly reported primary outcomes in the NEC and enteral nutrition literature?

Thesis outline

This thesis includes three studies. Chapter 2 is a narrative review that describes NEC and its risk factors, nutrition considerations for preterm infants and how different sources of nutrition may impact an infant's risk of NEC. Chapter 3 is a scoping review that maps the primary quantitative literature on different enteral diets (e.g. MOM, DHM and DHM-based fortifier, bovine milk-based formula and fortifier, and hydrolyzed formulas and fortifiers) and NEC, and a systematic review of hydrolyzed nutrition products and the effect on NEC. The review will highlight study characteristics, categorize diet comparisons and identify gaps in the literature. Lastly, Chapter 4 is a parent survey on outcome prioritization to determine outcomes most important to parents. Parent priorities will also be compared to commonly reported primary outcomes in the literature. Overall, this work will provide direction for the development and design of future studies on NEC and nutrition.

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Chapter 2[1]

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Abstract

Preterm infants are extremely vulnerable to a range of morbidities and mortality. Underdeveloped cardiac, respiratory, gastrointestinal, and immune systems in the preterm period increase the risk of necrotizing enterocolitis (NEC), a serious disease of the gut. NEC affects 5–12% of very–low birthweight infants, leads to surgery in 20–40% of cases, and is fatal in 25–50% of cases. There are multiple factors that may contribute to NEC, but the exact cause is not yet fully understood. Severe cases can result in intestinal resection or death, and the health care costs average >$300,000/infant when surgical management is required. Different types of nutrition may affect the onset or progression of NEC. Several studies have indicated that bovine milk–based infant formulas lead to a higher incidence of NEC in preterm infants than does human milk (HM). However, it is not clear why HM is linked to a lower incidence of NEC or why some infants fed an exclusively HM diet still develop NEC. An area that has not been thoroughly explored is the use of semielemental or elemental formulas. These specialty formulas are easy to digest and absorb in the gut and may be an effective nutritional intervention for reducing the risk of NEC. This review summarizes what is known about the factors that contribute to the onset and progression of NEC, discusses its health care cost implications, and explores the impact that different formulas and HM have on this disease.

Introduction

Preterm infants: Prematurity and risk of mortality and morbidity

Prematurity in infants is defined as birth at <37 wk gestation (1). The preterm birth rate was 7.7% in Canada in 2010 (2), 9.57% in the United States in 2014 (3), and 11.1% worldwide in 2010 (4). Of the premature infants in Canada and the United States, 6% and 8% were low birth weight (LBW) (<2500 g), respectively (1, 3). Medical advancements have improved the rate of survival for preterm infants; however, survival in many cases has been coupled with health and developmental complications later in life (1). Preterm birth is a major cause of LBW (1), which is a preliminary indicator for health status. Although LBW is not a direct cause of mortality, the literature indicates that it is associated with adverse outcomes (e.g., respiratory distress syndrome, cardiovascular disorders, compromised immune system, limited ability to mitigate inflammation and infections, neurological impairments) that may lead to mortality (1, 5). A recent Japanese study found that the odds of mortality increase as the SD for birth weight decreases for growth-restricted extremely preterm infants (6). LBW (1500–2499 g), very low birth weight (VLBW) (1000–1499 g), and extremely low birth weight (<1000 g) have been linked to several morbidities, including chronic lung disease, retinopathy of prematurity, sepsis, and necrotizing enterocolitis (NEC) (1, 6-8). Preventing and ameliorating the effects of these morbidities is an ongoing challenge in neonatology.

NEC

Description and incidence of disease

NEC is a serious intestinal inflammatory disease in neonates first described in 1965 by Mizrahi et al. (9). The disease is characterized by inflammation and injury of the gut wall barrier that may advance to necrosis and, potentially, perforation of the gut (10, 11). The diagnosis of NEC

is commonly determined with the use of Bell's modified staging criteria (12). Mild cases of NEC may be effectively treated by withholding enteral feeds, decompressing the stomach with a nasogastric tube, and starting broad-spectrum antibiotics. Advanced cases, however, may lead to surgery, extensive intestinal necrosis (NEC totalis), and death (10).

In Canada, 5.1% of infants aged <33 wk are affected by NEC (10). The incidence of NEC across developed countries is ~5–12% for VLBW infants (13-18), depending on certain risk factors. Three major risk factors for NEC are <32 wk gestational age, <1500 g at birth, and cardiac complications (10). NEC is more prevalent in preterm infants (19), with ~85% of cases occurring in infants born <35 wk gestation, whereas only 7–15% of cases occur in late-preterm (35–36 wk gestation) or term infants (37–42 wk gestation) (20-22). The incidence of NEC also drastically increases from 0.7% for infants with a birth weight >1500 g to 6.6% for infants <1500 g (10). NEC is less common in infants with a birth weight >1500 g, but the expected prognosis of larger infants is worse than smaller infants (23).

NEC has been studied for decades. Although some evidence has been found to elucidate the potential causes and progression of the disease, minimal advancements have been made in this field because of its complex nature. Clinical and theoretic knowledge of the disease mechanisms and interventions to protect an infant from NEC, including nutritional approaches, require further research.

Multifactorial causes of NEC

Prematurity is a risk factor for poor health outcomes, largely because of the underdevelopment of cardiac, respiratory, gastrointestinal, and immune systems. Immaturity of the lungs, a problem especially affecting infants born <32 wk gestation, results in impaired gas exchange and insufficient oxygenation of tissues (24). Cardiac complications during the preterm period, such as a large patent ductus arteriosus, limit the availability of oxygen and nutrients to other

tissues and organs (25). Immaturity of the gut is also a concern. The preterm gut is characterized by reduced peristalsis, a thin mucous layer, reduced tight junctions, increased enterocyte apoptosis, and impaired enterocyte regeneration (26, 27). These deficiencies may result in a “leaky” gut barrier, thereby facilitating the penetration of bacteria from the lumen (26, 27). Decreased structural integrity and functionality of the gut result in poor digestion and absorption of energy, protein, and other nutrients necessary for growth, the development of organs, and immunoprotection (26). Last, there are distinct differences between term and preterm infants in regard to the expression of immune cells and signaling pathways. A preterm immune system cannot readily detect pathogens and protect against infections due to multiple associated factors such as 1) the decreased production of IgA, IgM, IgG, and defensins; 2) changes in the expression of toll-like receptors (TLRs), especially TLR4 and TLR9, which are involved in pathogen recognition and the activation of the innate immune system (14, 28, 29); and 3) upregulation of proinflammatory TLRs (26) and/or proinflammatory cytokines such as TNF-a, IL-6, IL-8, and IL-1b (26, 27). The culmination of these factors increases a preterm infant’s vulnerability to infections and disease, particularly NEC.

Prematurity is a predominant risk factor for NEC, but several other medical risk factors have been identified. Infants with high clinical acuity or severe comorbidities may be at a greater risk for NEC. Low Apgar scores at birth, cardiac lesions, bowel obstruction, the use of ≥1 inotropes, and compromised respiratory function are a few indicators of clinical severity (26). Medical events or pharmaceuticals that reduce perfusion to the gut or oxygen saturation of the blood have also been linked to NEC (10, 26). Hypoperfusion or hypoxic conditions in the intestine occur when the metabolic requirements of epithelial cells are not met by the mesenteric blood supply. Incomplete reduction of oxygen in the mitochondria during hypoxic conditions produces reactive oxygen species, which in turn activate adenosine monophosphate-activated protein kinase through calcium-dependent channels (30). Adenosine monophosphate-activated protein

kinase downregulates energy-consuming anabolic mechanisms such as Na^+/K^+-ATPase activity and favors catalytic processes in an effort to spare energy (30). These ensuing intracellular responses may set the stage for NEC. Over time, this catabolic, oxidative system may fail to maintain digestive and absorptive functionality and cellular integrity and increase the gut's susceptibility to uncontrolled inflammation and necrosis.

For preterm infants, hypoxia-ischemia and respiratory complications such as bronchopulmonary dysplasia limit nutrient and oxygen delivery to the gut (10). Vasoconstrictive medications such as cyclooxygenase inhibitors (e.g., indomethacin), which are used in the treatment of patent ductus arteriosus in preterm infants, can also impair gut perfusion (10, 31). In a hypoxic gut environment, the introduction of enteral nutrients may cause oxygen to be preferentially used for digestion at the expense of maintaining the physical gut wall barrier (32). At the tissue level, hypoxic conditions or vasoconstrictive medications may lead to an inadequate supply of nutrients and oxygen needed to generate energy, produce immune cells, build membrane proteins to protect the integrity of the gut wall, and perform digestive and absorptive processes. Therefore, an inability to maintain the structure and function of the gut wall because of hypoperfusion may be an underlying catalyst for NEC.

With very few exceptions, NEC occurs after infants have been enterally fed (26). This may be related to the gut microbiome. Preterm infants have a lower diversity of microbiota (10, 26) and higher proportion of potentially harmful species such as Proteobacteria (10, 33) than term infants. Disruptions of the microbiota have been attributed to the prophylactic use of antibiotics at birth, contact with harmful bacteria on the mother's skin during a cesarean delivery, or the inability to transfer beneficial bacteria and prebiotics through breast milk shortly after birth. Ineffective digestion and absorption of enteral feeds in the lumen allows the microbiota to use these nutrients for their own growth and proliferation (27, 34). Bacterial overgrowth combined with an underdeveloped immune system and gut structure can facilitate bacterial adherence to

the gut wall and increased mucosal permeability. Intestinal bacterial overgrowth, diagnosed by clinical symptoms (e.g., vomiting, diarrhea, gas, abdominal pain, etc.), breath tests measuring hydrogen and methane gas, or the aspiration and culture of intestinal fluids, is typically treated with antibiotics (35). Eradicating existing bacterial colonies by antibiotics combined with an underdeveloped immune system and gut structure can facilitate the adherence of successive bacterial colonies to the gut wall and mucosal permeability. The translocation of bacteria may, in turn, initiate the inflammatory processes involved in NEC.

Proton pump inhibitors or H2 blockers have been linked to NEC because of changes to the intestinal microbiota (27, 33). The mechanism of action is not clear, but researchers suspect that H2 blockers increase the intestinal pH, consequently promoting the growth of Proteobacteria and overgrowth of the microbiota. The interaction between these microbiota and the intestinal epithelium has been associated with increased leukocytes and calprotectin, indicating mucosal inflammation (36). This inflammation may predicate NEC.

Health consequences

NEC is associated with widespread effects. The length of hospital stay (LOS) is considerably longer for NEC patients than infants without NEC. One study that evaluated 291 VLBW infants found that LOS was much longer for infants with NEC than without (85 ± 36 d compared with 70 ± 33 d, respectively) (13). Another study reported similar differences in LOS, in which infants with NEC had a mean incremental LOS of 11.7 d (95% CI: 6.9, 16.5) compared with infants without NEC (16). Prolonged hospital stay is often used as a proxy for illness severity, but it may also be a risk factor for nosocomial infections and further complications.

Severe forms of NEC lead to surgery in ~20–40% of cases (37, 38). Surgery involves laparotomy (often with intestinal resection) and ostomy creation, with potential long-term health effects and a mortality rate of ≤50% (27, 37, 39). Surgical NEC survivors may be affected by

short bowel syndrome or intestinal failure, with attendant failure to thrive and postoperative complications such as intestinal strictures, bowel obstruction, enterocutaneous fistulas, intraabdominal abscess, wound dehiscence, central line sepsis, or poor neurodevelopmental outcomes (14, 19, 37, 40-42).

Long-term outcomes for NEC survivors are also concerning. Ganapathy et al. (17) found that surgical NEC survivors were much more likely to have feeding difficulties and gastrointestinal ostomies from chronological ages 6–36 mo than matched controls with no diagnosis of NEC during birth hospitalization. Medical NEC infants (those treated with nonsurgical approaches) were more likely to have a higher risk of failure to thrive, feeding difficulties, neurodevelopmental delay, and open gastrointestinal ostomies between 6 and 12 mo than matched controls with various chronic conditions (17).

Health care costs

The health care costs associated with NEC are substantial. Data from the United States in 2011 and 2012 indicate that the cost of NEC is $180,000 to $198,000/infant (13, 16) and nearly doubles to $313,000/infant for surgically treated NEC (13). By comparison, the mean neonatal intensive care unit (NICU) hospitalization cost for infants without NEC is ~$134,500/infant (13). In the first 3 y of life, NEC survivors also accrue substantially higher outpatient costs. Ganapathy et al. (17) determined that between 6 and 36 mo of age, the cost difference between surgical NEC survivors and matched controls (no diagnosis of NEC) was ~$97,000/infant. Medical NEC survivors incurred a mean $5000 more in health care costs than controls from 6 to 12 mo (17).

The type of enteral nutrition product used for preterm infants affects health care costs. Human milk (HM) may be supplied by a baby's mother [mother's own milk (MOM)] or a human donor (DHM). An exclusive HM diet for preterm infants weighing typically <1800 g and with a

gestational age <32 wk at birth also includes an HM-based human milk liquid fortifier such as Prolact+ H^2MF, which is manufactured by Prolacta® Bioscience (Prolacta) (43). One study estimated that an exclusive HM diet resulted in net hospital cost savings (excluding physician fees and posttreatment care costs) of $8167/extremely premature infant (95% CI: 4405, 11,930; $p < 0.0001$) and 3.9 fewer days in the NICU (95% CI: 3.25, 4.58; $p < 0.0001$) (16) (note: this study was funded by Prolacta, but Prolacta had no editorial control over any part of the publication). However, an exclusive HM diet is substantially more expensive than a diet containing bovine milk–based products. The mean cost of 0.8 kcal enteral feed/mL that uses bovine milk–based products is $0.03/mL for preterm formula or ~$0.05/mL MOM with human milk fortifier (16, 44) (**Table 1**). Alternatively, MOM with Prolact+ H^2MF costs ~$1.25/mL, and DHM with Prolact+ H^2MF costs $1.33/mL for 0.8 kcal feed/mL (16, 43) (**Table 1**). Another retrospective study calculated hospital and physician costs for preterm infants ≤28 wk gestation and/or VLBW fed 4 different diets (45). The authors estimated that total hospital charges per infant were much lower for the exclusive HM diet ($237,647) than diets consisting of MOM with a bovine milk–based fortifier ($265,035), formula only ($266,825), and a combination of MOM, bovine-based fortifier, and formula ($344,615). A caveat to this study is that selection bias may have been a concern given the single-center design and small sample size (n = 293). In addition, the study commenced in March 2009, the exclusive human milk diet was introduced in March 2012, and the study ended in March 2014. Confounding factors such as changes to clinical practices other than infant diets over the 5-y period may have affected the results. Nonetheless, these results suggest that nutritional interventions have an impact on service utilization and health care expenses. It is unknown how these costs compare to other nutritional products such as semielemental or elemental formulas because, to our knowledge, this topic has not yet been studied.

Clearly, NEC is a multifactorial disease with substantial health consequences and costs. There are many research avenues available on this topic, but the focus of this review is on different types of enteral nutrition for the prevention of NEC.

Feeding protocols for preterm infants

Typical feeding progression

Several challenges exist for preterm nutritional support. Many preterm infants, especially those born <1500 g and/or <34 wk gestation, are not able to breastfeed or start enteral feeds shortly after birth. The suck-swallow-breathe rhythm of oral feeding may not be possible for preterm infants because of coordination issues and/or low body stores of energy (27). Intense respiratory or cardiac support can limit or preclude an infant from oral or enteral feeds. The use of high-dose or multiple medications that compromise gut perfusion, cardiac lesions, substantial bladder pressure, acute abdominal issues, 48-h posthypoxic-ischemic encephalopathy or cardiopulmonary resuscitation, or persistent feeding intolerance are also contraindications for enteral feeds. Aggressive enteral feeding in the presence of ≥1 of these contraindications may potentiate NEC (46, 47). For these reasons, intravenous delivery of nutrients [parenteral nutrition (PN)] is often initiated for preterm infants after birth. PN is initiated slowly, individually prescribed to ensure tolerance and safety, and advanced to meet the infant's nutritional and fluid needs. There are several risks associated with PN, such as line infections, liver damage, or gut atrophy (48, 49). A clinician's aim is to wean PN and start enteral feeds as soon as possible while maintaining adequate energy and protein intake to promote appropriate growth velocity.

Nutritional practices of feeding initiation and advancement vary among neonatal practitioners, but enteral feeds typically follow a standard progression (50). Trophic feeding, also known as minimal enteral feeding or gut priming, of 10–24 mL kg^{-1} d^{-1} HM is started for 1–4 d when appropriate to stimulate gastrointestinal functioning and promote endocrine and metabolic

maturity (50-52). If tolerated, feeds are advanced by 20–30 mL kg^{-1} d^{-1} for VLBW infants and 15–25 mL kg^{-1} d^{-1} for extremely-low-birth-weight infants (50) or more slowly (10 mL kg^{-1} d^{-1}) for infants with gastrointestinal or cardiac issues (11). Advancements continue until goal feeds are achieved. Enteral feeding goals are monitored daily and adjusted based on estimated energy requirements, fluid restrictions, medications, and clinical stability. PN is weaned as enteral intake increases to ensure nutritional goals are met. In general, caloric and protein goals for normal preterm development are 110–135 kcal kg^{-1} d^{-1} and 3–4.5 g protein kg^{-1} d^{-1} (8, 50, 53). Most preterm infants cannot meet these high needs through enteral intake of breast milk or standard formula alone (53-55). Therefore, fortification is required. Bovine milk–based and HM-based HM fortifiers (HMFs) contain additional energy, protein, fat, vitamins, and minerals (56) to ensure adequate growth, neurodevelopment, and bone mineralization (53). HMFs are typically added once enteral intake reaches 100 mL kg^{-1} d^{-1} to ensure the gut can tolerate more concentrated feeds. Some clinicians prefer to start the fortifier at 80 mL kg^{-1} d^{-1} or earlier to meet protein and energy goals sooner. HMFs are discontinued generally when the infant is 32–34 wk corrected gestational age and meeting growth expectations, but this practice may vary between centres and clinicians. Preterm infants are constantly monitored for feeding intolerance, including excessive gastric residuals, vomiting, diarrhea, distended abdomen, or bloody stools. If signs of feeding intolerance are observed, enteral feeds are either reduced or discontinued (50) to prevent exacerbating a problem that may trigger NEC.

Growth and development goals

A tool used to monitor and evaluate health and nutritional status for preterm infants is the Fenton preterm growth charts for boys and girls (57). Expected postnatal growth velocities of preterm infants are based on an intrauterine growth of ~15 g kg^{-1} d^{-1} (8, 54). Although this approach may not be precise given the differences between intra- and extrauterine environments, to our knowledge there are currently no alternative standards (57).

Nutritional goals for clinically stable infants are set to help them reach their genetic growth potentials and track on corrected gestational age- and sex-specific Fenton growth chart centiles for weight, length, and head circumference. After birth, it is expected that infants lose ≤10% of their birth weight (mean: 5.7–6.6%), but this weight is normally regained within 2 wk (58). Preterm infants often require enteral or parenteral nutritional support to help them achieve their growth potential. Many clinical experts agree that there is no need, however, to accelerate weight gain beyond the centile that the infant is tracking provided that growth is meeting patient-specific expectations. Doing so may lead to further harm from overfeeding. The key message is that although many preterm infants have considerably lower birth weights than their term counterparts and LBW is a risk factor for morbidities and mortality, preterm infants can still grow and develop at a rate that tracks the preterm growth chart and is appropriate for each infant's genetic and physiologic potential. The difficulty of nutritional support is balancing adequate growth while avoiding complications and comorbidities that may predispose an infant to diseases such as NEC.

Sources of nutrition

MOM

HM includes breast milk from an infant's mother (MOM) or DHM. There are many benefits of HM, including improved gastrointestinal functioning, protection against respiratory illnesses and infections (e.g., sepsis, urinary tract infections), improved bonding between the mother and baby, faster achievement of full enteral feeds, shorter LOS, and improved cognitive and visual development (54, 59). Breast milk is a functional food that contains the appropriate proportion of macronutrients for the optimal growth and development of infants (60-62) and bioactive agents to help them grow and mount immunologic defenses against diseases such as NEC (26, 63). For instance, lactoferrin is a glycoprotein in breast milk that is believed to aid in iron transport,

but it also has antimicrobial properties. Lactoferrin has been found to mitigate the release of proinflammatory cytokines from monocytic cells in the presence of lipopolysaccharides (11). Breast milk also contains a host of immune cells such as mucosal-protective IgA; growth factors to promote enterocyte development; a phospholipid mediator, platelet-activating factor acetylhydrolase, which may be protective against NEC; Igs, cytokines, chemokines, prostaglandins, neuropeptides, and nucleotides; an appropriate pH and osmolarity for a newborn's naïve gut; microbiota to colonize the gut and establish a healthy mucosal layer; and probiotic human milk oligosaccharides to facilitate the colonization of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (7, 64, 65). Together, these active breast milk components promote the proliferation of beneficial microbiota relative to enterobacteria and influence immune system responses to favor an anti-inflammatory environment that is suspected to be protective against NEC and other diseases (26, 33).

MOM has been recognized as the best source of nutrition for term and preterm infants (**Figure 1**) (7, 11, 54, 64, 66). The composition of breast milk changes over time to support an infant's nutritional needs at different developmental stages. Notably, protein content in the preterm period is higher in preterm breast milk than in term breast milk, especially in the first week of life (**Table 2**) (67). Colostrum contains the highest protein content in both term and preterm breast milk, with preterm colostrum having the highest concentration at 2.7 g compared with 2 g/100 mL in term colostrum (67). Higher protein intake is especially important to preterm infants given the accelerated rate of growth, anabolism, and brain development during the preterm period (55). Despite the benefits of preterm MOM, its macro- and micronutrient content alone is not sufficient to meet a preterm infant's estimated high needs. As discussed previously, to meet the caloric, protein, and micronutrient requirements for most preterm infants, MOM must be fortified with HMF (68, 69). Another consideration for preterm nutrition in the NICU is the availability of MOM. Delayed milk letdown, illness, psychologic stress, lack of understanding or social support

(70), inability to put the baby to breast to stimulate milk production, or drug use may limit a mother's supply. In these cases, alternative sources of nutrition are necessary.

DHM

Pasteurized DHM is considered to be the next best source of nutrition for preterm infants if MOM is unavailable (**Figure 1**) (55, 66, 71). Compared with bovine milk–based formula, HM is efficiently digested and absorbed and contains immunologic cells and bioactive factors for infant growth and development (72). Although DHM shares some of the benefits of preterm MOM, its nutritional profile is different (**Table 2**). In accordance with the Human Milk Banking Association of North America, DHM is batched at human milk banks from 3 to 5 donors to maintain similar composition and quality of the milk across batches (73). Most donors have older infants or term infants and have been lactating for weeks or months (74). Because protein content decreases over time, the mean protein content of batched DHM is lower than preterm breast milk (67, 75). Adequate protein intake is essential for preterm infants given their rapid rate of weight gain and anabolism, so the limited protein content in DHM is a concern. As with preterm MOM, protein and energy deficits are corrected with fortification; however, more fortification may be required for DHM to compensate for its low mean protein content (76). Furthermore, the heating process of pasteurization can denature proteins and immunologic agents in DHM (77), possibly reducing the effectiveness of DHM in developing a preterm infant's gut and immune systems. Despite these shortcomings, DHM is recommended as an alternative form of nutrition for preterm infants (66) because it is well-tolerated and still contains many potentially beneficial bioactive components.

Standard infant formula

Bovine milk–based preterm formulas are another feeding option. The advantages of formula are that it provides a consistent amount of calories and macronutrients for adequate growth (78)

and is less expensive than DHM (16). Several studies, however, have indicated that bovine milk–based products may increase the risk of NEC (8, 13, 18, 79). The mechanism of action is unclear. A possible explanation is that formula or bovine milk–based HMF does not contain oligosaccharides such as HM and that this deficiency may select for potentially pathogenic microbiota such as enterobacteria (26). The overgrowth of pathogenic microbiota and proinflammatory immune responses to the microbiota may contribute to the initiation of NEC (26).

Some researchers have suggested that casein rather than whey protein in formula may be responsible for the gut lesions and proinflammatory immune responses that precede NEC (80). However, evidence regarding this hypothesis is conflicting. Thymann et al. (81) compared preterm piglets fed formula containing 100% whey to 40% whey and 60% casein for 30 h. Both formulas were isocaloric and equivalent with respect to the total amount of protein, maltodextrin, lactose, and fat. The piglets were killed after 30 h of feeding to determine NEC development and gut function. No significant difference was found with respect to the incidence and severity score of NEC, diversity of anaerobic and aerobic bacteria, glucose absorption, and lactase activity between the groups. The authors concluded that factors other than casein should be investigated in relation to NEC.

The processing of formula also leads to the removal of the milk fat globule membrane (82). One study found that the supplementation of the bovine milk fat globule membrane in infant formula for term infants led to several beneficial outcomes, including the decreased incidence of acute otitis media, decreased use of antipyretic medications, and increased production of serum IgG in response to the pneumococci vaccine (82). Effects from plant-based lipids such as soy oil added to infant formulas may also be problematic for the developing immune system of preterm infants. Higher ratios of ω-6 to ω-3 FAs and a higher proportion of arachidonic acid in soy oils are associated with proinflammatory responses (83, 84). The synthesis of leukotrienes and

prostaglandins from arachidonic acid may propagate inflammation in response to cellular injury or infection (84), as seen with NEC. Therefore, the current composition or structure of bovine milk–based or artificial formulas may be unfavorable for certain infants, but again, this mechanism is not completely understood.

Hydrolyzed formula

The other types of enteral formula for preterm infants are semielemental or elemental formulas. These formulas are primarily made from broken-down proteins (semielemental or protein-hydrosylated formula) or amino acids (elemental formula), medium-chain TGs, and a carbohydrate source (e.g., corn syrup solids) (**Figure 2**) (85, 86). The purpose of semielemental or elemental formulas is to facilitate nutrient digestion and absorption because there is minimal reliance on the gut to produce the enzymes, bile salts, and gastric juices needed to digest complex nutrients (87). Amino acids or small peptides, easily absorbed fats (e.g., medium-chain TG oil), and simple sugars (e.g., glucose) are especially beneficial for patients that are severely ill, have a feeding intolerance, or are at risk of gastrointestinal complications (87). In relation to NEC, semielemental or elemental formulas may protect against cytotoxicity of enterocytes and the propagation of proinflammatory processes. A cell-based in-vitro study by Penn et al. (88) designed to test the cytotoxicity of enzymatically digested breast milk and infant formulas on intestinal epithelial cells offers a potential mechanism of action for the effectiveness of hydrolyzed formulas. The authors hypothesized that unbound free FAs (FFAs) produced by lipase digestion of standard formula would be cytotoxic to rat intestinal cells but that the digestion of fresh breast milk would not. Cytotoxicity was defined as the death of >5% of rat intestinal epithelial cells or >15% of neutrophil death. In total, 9 different infant formulas were tested, and all 9 resulted in significantly greater cytotoxicity after digestion with lipase or lipase plus proteases ($p < 0.007$; $p < 0.025$ was considered significant), as determined by greater epithelial cell death. Fresh breast milk digestion did not result in cytotoxicity. Interestingly, the

addition of orlistat (a lipase inhibitor) ($p < 0.0023$; $p < 0.017$ was considered significant), bovine serum albumin (BSA) ($p < 0.00008$; $p < 0.05$ was considered significant) or proteases ($p < 0.008$; $p < 0.025$ was considered significant) reduced cytotoxicity significantly. The authors hypothesized that the inhibition of lipase reduced the production of unbound FFAs and BSA bound and neutralized the unbound FFAs, thereby minimizing cell death. Similarly, proteases may help deactivate unbound FFAs by opening intact proteins, exposing their hydrophobic core, and increasing the ability of proteins to bind unbound FFAs. Fresh breast milk was hypothesized to resist cytotoxicity, potentially through the deactivation of pancreatic lipases and its lipid profile, which consists of fats that are less susceptible to lipase digestion (88).

Studies in piglets that compared elemental diets with bovine milk–based diets have also uncovered potential benefits of elemental diets. Piglets are the best nonprimate model for studying neonatal nutrition because the gastrointestinal anatomy, physiology, and nutrient requirements of pigs are the most similar to humans (89). In one such study, Connor et al. (90) compared polymeric and elemental formulas for 3 surgically created short-bowel syndrome groups: 1) midintestinal resection with a jejunoileal anastomosis (equal amount of jejunum and ileum remaining) ($n = 16$); 2) distal intestinal resection, including the ileum, cecum, and 5 cm of the spiral colon, with a jejunocolic anastomosis ($n = 17$); and 3) sham surgery ($n = 15$). After surgical treatment, enteral nutrition was initiated with either a polymeric or isocaloric and isonitrogenous elemental formula on postoperative day 2. The polymeric formula contained nonfat milk and whey protein concentrate, lactose and glucose polymers, and high-fat oleic sunflower or safflower, soy, and coconut oils. The authors did not specify whether the polymeric formula was bovine milk–based, but this was likely the case. The outcomes of interest were functional and structural adaptations of the intestine, as well as glucagon-like peptide 2 (GLP-2), a gut-specific hormone that improves nutritional absorption and intestinal barrier function (90). No difference was found in structural measures such as intestinal lengthening, villus height,

crypt depth, and colon weight between the diet groups. The concentration of plasma GLP-2 was higher at the end of the trial for the jejunocolic anastomosis piglets fed the polymeric formula than those on the elemental formula. The authors reasoned that higher GLP-2 concentrations for the polymeric diet may have resulted from undigested polymeric nutrients being used by bacteria in the colon that produced short-chain FAs and, in turn, GLP-2. However, in the same jejunocolic anastomosis group, the elemental diet led to significantly fewer days of diarrhea (9.9 ± 0.8 d on the elemental diet compared with 12.3 ± 0.4 d on the polymeric diet; $p = 0.023$) and PN support (12.7 ± 0.6 d on the elemental diet compared with 14.1 ± 0.1 d on the polymeric diet; $p = 0.047$). These improved functional measures with the elemental diet were considered by the authors to be highly beneficial for an animal model with a surgically removed ileum.

With the use of a healthy piglet model, Stoll et al. (91) investigated the effects of bovine milk–based formula and an elemental formula fed over 6 d in piglets aged 3 wk. The elemental diet consisted of crystalline amino acids, glucose, and a lipid emulsion, and the polymeric diet was a bovine milk–based formula. Piglets on the elemental diet were fed intragastrically at a continuous rate, whereas piglets on the polymeric formula were fed orally 3 times/d. The elemental diet provided less calories and protein than the polymeric diet (165 kcal kg^{-1} d^{-1} and 10.6 g amino acids kg^{-1} d^{-1} compared with 195 kcal kg^{-1} d^{-1} and 12.5 g protein kg^{-1} d^{-1}, respectively). The main purpose was to compare small intestinal growth and function between the 2 diet groups. There was no difference in total body weight or intestinal cell morphology (crypt depth, villus height, and muscle thickness) at the end of the 6-d trial. In contrast to the aforementioned piglet study (89), Stoll et al. (91) found that cell proliferation and protein synthesis, measured by the percentage of labeled crypt cells in the S-phase and ornithine decarboxylase activity, were considerably higher in the proximal jejunum and ileum of the piglets fed an elemental diet. Furthermore, concentrations of gut hormones GLP-2 and glucose-dependent insulinotropic polypeptide, but not peptide YY, were considerably higher in the

elemental diet group. The authors concluded that an elemental diet matches a polymeric diet with respect to intestinal growth and cell morphology, with an added benefit of stimulating gut hormone production, cell proliferation, and protein synthesis. These conclusions should be interpreted with caution because of the difference in feeding protocols between the diet groups and short study duration (i.e., observations over several weeks would provide more robust results regarding cell morphology and gut function).

Overall, these cell and animal studies highlight the possible benefits of hydrolyzed formula in terms of intestinal structure, function, and absorption and provide insight for future clinical studies.

Current Status of Knowledge

In this section, we evaluate several randomized controlled trials (RCTs) and a Cochrane systematic review that compared the effect of different types of nutritional products (MOM, DHM, bovine milk–based formula, and an elemental fortifier) on the incidence of NEC in preterm infants.

MOM compared with preterm formula

Sullivan et al. (18) conducted a multicenter RCT to evaluate the health effects of an exclusive HM diet compared with a diet containing both HM and bovine milk–based products. This study analyzed 207 preterm infants. Eligibility criteria included a birth weight between 500 and 1250 g, mothers' intention to provide breast milk, enteral feedings started within 21 d of life, and PN started within 48 h of life. Infants with major congenital malformations were excluded from enrollment. The authors did not mention whether gastrointestinal comorbidities were considered a part of the eligibility criteria. Randomization to 3 groups occurred in blocks of 4 that were stratified by birth-weight categories (500–750, 751–1000, and 1001–1250 g) and whether the

infants were appropriate or small for gestational age. Comparison groups were based on the type of enteral feeds and when fortifier was added. These groups were defined as follows: 1) HM100: HM-based fortifier was added once enteral feeds of MOM reached 100 mL kg^{-1} d^{-1}, and DHM was used if MOM was unavailable; 2) HM40: same intervention as the HM100 group, except the fortifier was started once enteral feeds reached 40 mL kg^{-1} d^{-1}; and 3) BOV: after enteral feeds of MOM were started, bovine milk–based fortifier was added once feeds reached 100 mL kg^{-1} d^{-1}, and bovine milk-based preterm formula was used if MOM was unavailable. Standard feeding protocols were maintained for all infants. Outcomes were measured until the earlier of 91 d of life, hospital discharge, or 50% of oral feed goals were achieved.

No significant differences were found for days of PN, LOS, late-onset sepsis, or growth, although a subsequent analysis found that the probability of needing PN was significantly reduced by 11–14% for an exclusive HM diet (92). There were no differences between the HM100 and HM40 for any of the outcomes. After adjusting for confounding factors with the use of multivariate logistic regression, the OR for NEC was 0.23 (95% CI: 0.08, 0.66), or a 77% reduction in the odds of developing NEC, in favor of an exclusive HM diet.

A criticism of the study is that the method of randomization was not clear. The randomized block number (blocks of 4) was not divisible by the 3 comparison groups or 3 birth weight strata. This approach may have led to imbalances between the groups for known and unknown factors. A more transparent method would have been to create random blocks of a number divisible by 3 (93). Another important note is that 3 infants (4.5%) in the HM100 group and 5 (7.0%) in the HM40 group developed NEC (**Table 3**). Of these cases, NEC led to mortality for 1 infant in each of the HM groups, although the authors reported that both of these infants were protocol violators who had received some amount of bovine milk–based formula or fortifier during the study. Nonetheless, this finding reinforces that NEC is a multifactorial disease, and an exclusive HM diet may not fully protect infants from NEC.

DHM compared with formula

Cristofalo et al. (79) performed an RCT that paralleled Sullivan et al. (18) in objectives and methodology. The difference in Cristofalo et al. (79) was that MOM was not used—only DHM. In this multicenter blinded trial, 53 preterm infants weighing between 500 and 1250 g at birth were randomly assigned to 2 groups: DHM with HM-based fortifier (concentration not reported) (n = 29) or preterm formula concentrated to 0.8 kcal/mL (n = 24).

Unlike Sullivan et al. (18), Cristofalo et al. (79) found a significant reduction in the days of PN (27 compared with 36; p = 0.04) in favor of the HM group. Surgical NEC was significantly lower in the HM group (0 compared with 4 cases; p = 0.036), but the incidence of NEC (1 compared with 5 cases; p = 0.08) (**Table 3**) and NEC and/or death (1 compared with 5 cases; p = 0.08) were not significant. The findings were affirmed even after controlling for race, antenatal steroids, Apgar score, and age at the first enteral feed. Note that because the study was powered on the duration of PN as the primary outcome, it may not have been adequately powered to detect differences between the groups on NEC outcomes. The authors acknowledged that a potential issue with the study was that eligibility included no intention to provide MOM. The unavailability of MOM may have been caused by exposure to medications or medical problems, mother's absence, or illicit drug use. These variables may have been confounders for NEC.

On the whole, the study found no significant difference between the DHM and preterm formula on the incidence of NEC (possibly because of the smaller sample size), but the incidence of surgical NEC supported the previous study. Both Sullivan et al. (18) and Cristofalo et al. (79) recommended an exclusive HM diet as a strategy for improving clinical outcomes, namely to reduce the incidence of NEC.

In 2014, a Cochrane systematic review compared bovine milk–based formula with DHM for feeding preterm or LBW infants (78). Nine RCTs, including the RCT conducted by Cristofalo et al. (79), involving 1070 infants were analyzed. The included RCTs compared formula with DHM in preterm or LBW infants in regard to short- and long-term (6 mo post-term) growth and neurodevelopmental outcomes. Secondary outcomes were all-cause mortality, NEC, days to full enteral feeds, feeding intolerance, and invasive infections. Most studies analyzed included patients who were stable, aged <2 wk, and weighed <1800 g at birth. Four trials compared term formula with DHM, and 5 trials compared preterm formula with DHM. One trial used unpasteurized DHM.

A meta-analysis that included 5 studies (n = 802 patients) on preterm formula and 1 study (n = 67 patients) on term formula determined that formula had a 2.77 greater risk of NEC than DHM (95% CI: 1.4, 5.46; $I^2 = 0$) (78). There was a slightly lower risk for preterm formula-only compared with DHM (RR: 2.61; 95% CI: 1.27, 5.35; $I^2 = 0$). A subgroup analysis of 360 patients further examined the effect of preterm formula as a sole source of nutrition or supplemental nutrition. Preterm formula as a sole source of nutrition was associated with a significantly higher risk of NEC (RR: 4.62; 95% CI: 1.47, 14.56). The CI around the risk ratio was wide, suggesting either a small sample size or considerable heterogeneity within the sample with respect to the treatment effect. As supplemental nutrition, there was no significant difference between DHM and preterm formula for the incidence of NEC (RR: 1.96; 95% CI: 0.82, 4.67); however, there were twice as many NEC cases in the formula group (n = 15) than there were in the DHM group (n = 7), a non-significant difference (**Table 3**).

A limitation of this evidence is the unclear or high selection bias for nearly half of the included studies and unclear performance and detection bias for most. Unclear allocation concealment and lack of blinding may have influenced the results; therefore, the findings should be interpreted with caution. The authors also noted that several included studies were conducted

>20 y ago, but formula, DHM technologies, and clinical practice have evolved since that time. Outdated evidence poses even more questions for clinical practice. This limitation emphasizes the need for more trials to accurately assess the harms and benefits of current nutritional products.

Hydrolyzed nutrition products

Kim et al. (53) conducted a nonblinded, multicenter, noninferiority RCT that involved protein-hydrosylated HMF. The trial compared liquid HMF with extensively hydrolyzed proteins (LE-HMF) to powdered HMF with intact proteins (PI-HMF) for enterally fed preterm infants. All infants were born <33 wk gestation, had a birth weight between 700 and 1500 g, and were fed MOM. DHM was not used during the study unless indicated by the clinician or principal investigator. HMF was added once feeds reached 100 mL kg^{-1} d^{-1}. The HMFs were similar in caloric density, fat, carbohydrate, phosphorus, and vitamin D content. However, LE-HMF had more protein (3.6 compared with 3 g/100 kcal), twice the amount of docosahexaenoic acid, less calcium (153 compared with 175 mg/100 kcal), higher osmolality (450 compared with 385 mOsm water/kg), and added lutein (23 mg/100 kcal). Infants were followed for 29 d after HMF was started or until hospital discharge. The primary outcome was weight gain per day.

There were 63 and 66 infants included in the intention-to-treat analysis for the PI-HMF and LE-HMF groups, respectively. Noninferiority was achieved for the primary outcome, weight gain, because there was no significant difference between the study groups when the intention-to-treat analysis was used. However, the analysis that compared only the strict protocol followers found a substantially higher weight for the infants fed LE-HMF in the last 14 d of the study. Both HMFs were well-tolerated. There were no significant differences between the groups for length and head circumference gain, stool characteristics, and energy intake. The LE-HMF contained more protein than the PI-HMF and, as expected, infants in the LE-HMF group had higher protein

intake (3.9 compared with 3.3 g kg^{-1} d^{-1}; $p < 0.0001$), blood urea nitrogen (9.31 ± 0.53 compared with 5.81 ± 0.38 mg/dL), and prealbumin concentrations (10.01 ± 0.35 compared with 9.08 ± 0.35 mg/dL). All biochemistries were within normal limits. NEC incidence was low in the LE-HMF and PI-HMF groups (1.5% and 3.2% of infants, respectively). The incidence of sepsis was also low in both groups (4.5% of infants fed LE-HMF and 3.2% of infants fed PIHMF). Of note, significantly fewer infants discontinued HMF because of feeding intolerance in the LE-HMF (2% of infants) than the PI-HMF (10%of infants) group ($p = 0.048$).

The authors concluded that the use of both HMFs achieved weight gain goals. Feeding intolerance and morbidities were minimal in the 2 groups; therefore, both HMFs were deemed safe. LE-HMF may have the potential to optimize growth without increasing the risk of morbidities, as evidenced by the significantly higher mean weight of infants by the study endpoint and low incidence of NEC and sepsis. A larger equivalence trial or one powered to detect a significant difference for the incidence of NEC is needed to support this hypothesis.

Conclusions

In summary, HM has been acknowledged as the best source of nutrition for preterm infants and those at risk for NEC (8, 13, 16, 18, 26, 78, 79, 97-99). Two RCTs on preterm infants weighing between 500 and 1250 g at birth compared the effect of bovine milk–based preterm infant formula to MOM or DHM on the incidence of NEC (18, 79). Both trials found that an exclusive HM diet results in a lower incidence of NEC. A Cochrane systematic review that evaluated the effect of DHM or bovine milk–based formula on health outcomes for preterm infants also determined that formula significantly increases the risk of NEC (78). The review authors cautioned, however, that potential sources of bias, particularly the lack of blinding and unclear allocation concealment, may have influenced the results. These pivotal studies have prompted the ongoing research and development of HM-based products such as pasteurized DHM and

Prolacta fortifiers. Indeed, several questions remain. Based on previous trials and a Cochrane systematic review, ~1–3% of infants fed an exclusively human milk diet develop NEC (18, 78, 79). These studies have not explained why HM is superior or why some infants, albeit a small percentage, fed an exclusively HM diet still develop NEC.

Semielemental or elemental formulas may be an effective nutritional intervention to reduce the risk of NEC in preterm infants. The nutrients in semielemental or elemental formulas are easy to absorb, which is expected to reduce stress on the gut and potentially avoid the proinflammatory processes that lead to NEC. Although semielemental or elemental formulas do not contain immunologic factors such as MOM, the benefit of readily absorbed nutrients may outweigh this deficit. Limited research on semielemental or elemental formulas and NEC was found for this review; hence, more research evaluating the effect of these specialty formulas on the incidence of NEC is warranted. This is an area of study our group is pursuing.

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Abbreviations used: BSA, bovine serum albumin; DHM, donor human milk; FFA, free FA; GLP-2, glucagon-like peptide 2; HM, human milk; HMF, HM fortifier; HM40, exclusive HM diet fortifier added when feeds reached 40 mL/kg; HM100, exclusive HM diet fortifier added when feeds reached 100 mL/kg; LBW, low birth weight; LE-HMF, liquid HMF with extensively hydrolyzed proteins; LOS, length of hospital stay; MOM, mother's own milk; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; PI-HMF, powdered HMF with intact proteins; PN, parenteral nutrition; RCT, randomized control trial; TLR, toll-like receptor; VLBW, very low birth weight.

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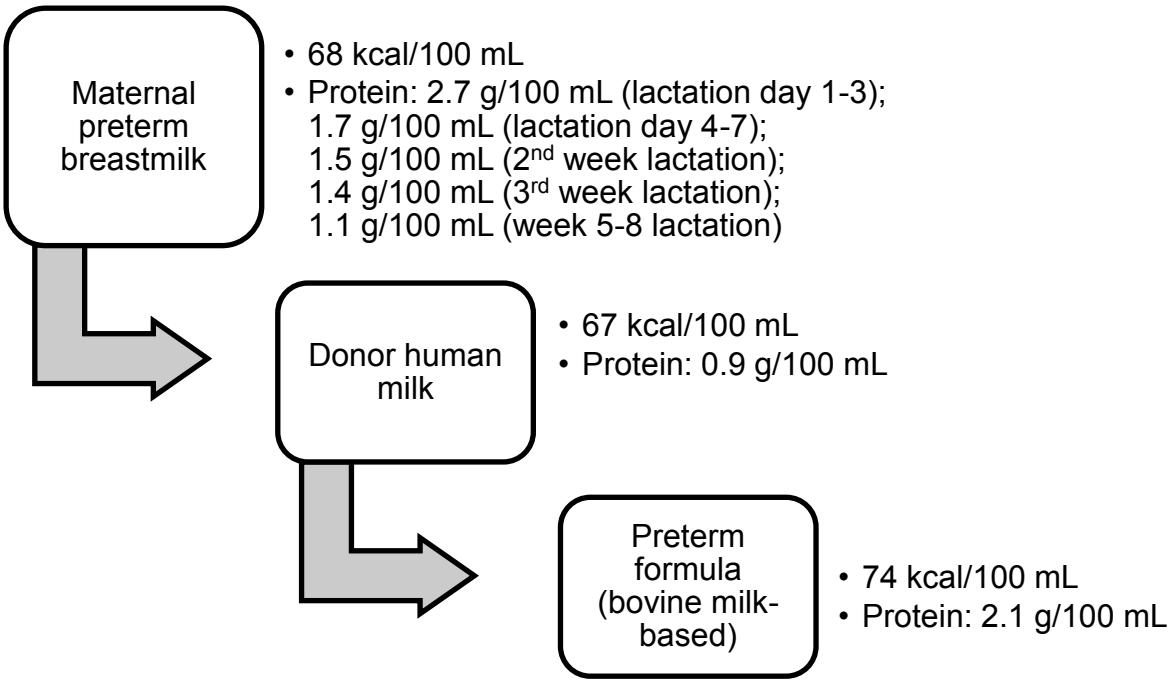

FIGURE 1 Preference for type of preterm nutrition. Mother's own milk is the first choice of nutrition for preterm infants, followed by pasteurized donor human milk (66) and then bovine milk–based preterm formula. The composition of each nutrition source is different.

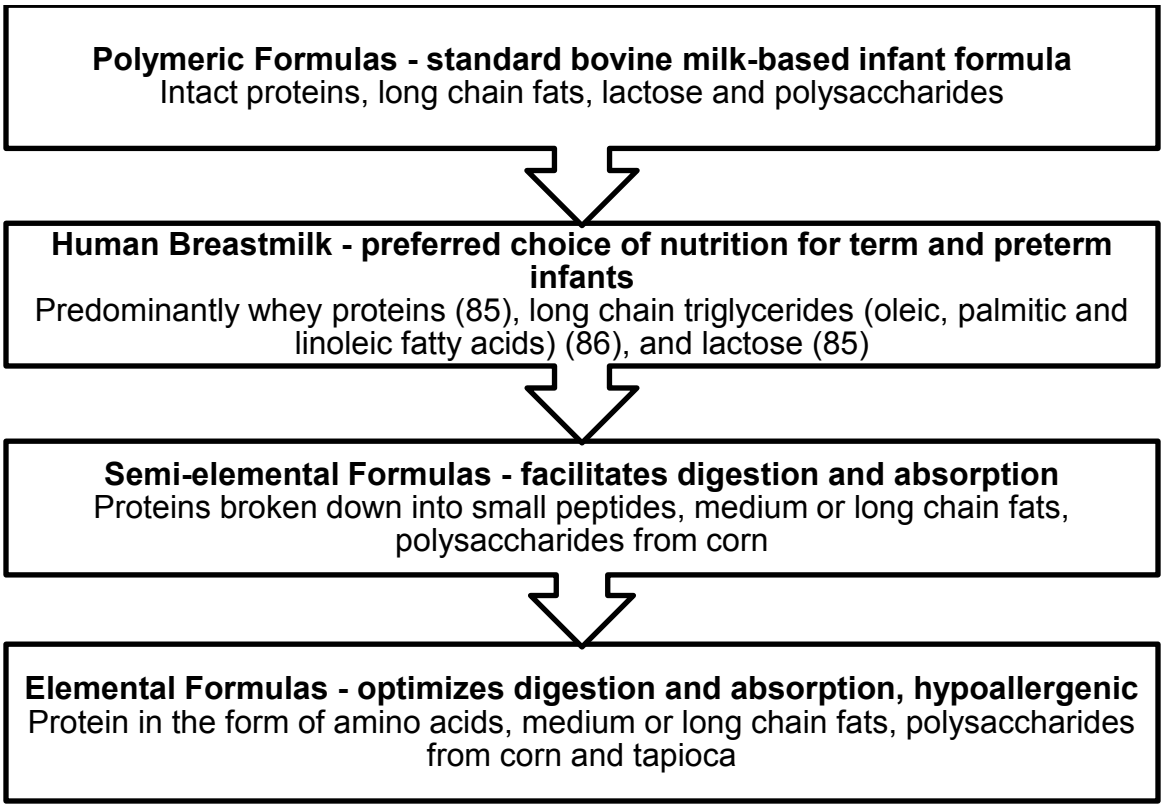

FIGURE 2 Breakdown of macronutrient composition in infant nutrition sources and corresponding uses (85, 86).

Table 1. Cost comparison of Enfamil® Human Milk Fortifier (HMF) and Prolacta+ H²MF® to prepare enteral feeds of 0.8 kcal/mL for a preterm infant.

	Enfamil® HMF (powder)	**Prolacta+4 H²MF® (liquid)**
Cost of fortifier	$1.20/sachet[1] × 4 sachets = $4.80	20 mL Prolact+4 H2MF × $6.25/mL[2] = $125
Volume to prepare 0.8 kcal/mL	(0.71g sachet[1] × 4 sachets × 0.84 mL/g[3] displacement) + 100 mL breastmilk = 102.4 mL	20 mL Prolacta + 80 mL breastmilk[4] = 100 mL
If mixed with maternal breastmilk	$4.80/102.4 mL = $0.05/mL	$125/(100 mL) = $1.25/mL
If mixed with donor human milk	[$4.80 fortifier + ($0.10/mL[2])(100 mL)]/102.4 mL = $0.14/mL	[$125 + ($0.10/mL[2])(80 mL)]/100 mL = $1.33/mL

[1] Drugstore.com (44).
[2] Ganapathy et al. (16).
[3] Mead Johnson Nutrition Product Information Database, accessed 25 April 2016.
[4] Prolacta® Bioscence (43).

Table 2. Nutrition comparison of maternal preterm breastmilk, donor human milk and preterm formula[1].

	Maternal preterm breastmilk	**Donor human milk**	**Preterm formula (bovine milk-based)**
Calories (kcal/100 mL) (67) Protein (g/100 mL) (67)	68 2.7 ± 1.5 (Day 1-3) 1.7 ± 0.5 (Day 4-7) 1.5 ± 0.4 (Week 2) 1.4 ± 0.4 (Week 3-4)	67 0.9	74 2.1
Fortification	Fortification needed	More fortification needed than preterm breastmilk given the low protein content	Concentrate as needed (patient-specific)
Bioactive components (e.g. immune cells, growth factors, prebiotics)	Present	Present, but reduced by processing and pasteurization	Absent

[1] MOM, mother's own milk.

Table 3. Summary of recent studies evaluating the effect of infant diets on the incidence of NEC[1].

Author, year	Study Design	Population	Duration of intervention	Comparison Groups (n)	NEC, n (%)
Sullivan et al., 2010 (18)[2]	RCT	500-1250 g	≤91 days old, hospital discharge, or 50% oral feeds (4 complete feeds/day) achieved	HM100, HM40, and BOV	HM100: 3 (4.5%); HM40: 5 (7.0%); and BOV: 11 (15.9%)*
Cristofalo et al., 2013 (79)	RCT	500-1250 g	≤91 days old, hospital discharge, or 50% oral feeds (4 complete feeds/day) achieved	HM and BOV	HM: 1 (3%); BOV: 5 (21%)
Quigley et al., 2014 (78)	SR – 4 RCTs of relevance (79, 94-96)	500-1250, <1600, <1850, and <1500 g	≤91 days old, hospital discharge, or 50% oral feeds (76); until weight reached 1800 g (94); until discharge/transfer or 2000 g (95); 10th day of life until 2000 g or illness requiring intravenous nutrition (96)	DHM and BOV	DHM: 3 (1.6%)**; BOV: 13 (7.6%)
Kim et al., 2015 (53)	RCT	<33 weeks GA and 700-1500 g	Until 29 days after fortification or hospital discharge	LE-HMF and PI-HMF	LE-HMF: 1 (1.5%); PI-HMF: 2 (3.2%)

[1] *HM100 compared with BOV, p = 0.04; HM100 + HM40 compared with BOV, p = 0.02; and HM40 compared with BOV, p = 0.09. **DHM compared with BOV, p = 0.009. BOV, bovine milk–based preterm formula provided if MOM unavailable or bovine milk–based fortifier added when breast milk intake reached 100 mL/kg; DHM, donor human milk as sole diet; GA, gestational age; HM, pasteurized donor human milk plus human milk–based human milk fortifier; HM40, exclusive human milk diet, fortifier added when feeds reached 40 mL/kg; HM100, exclusive human milk diet, fortifier added when feeds reached 100 mL/kg; LE-HMF, liquid human milk fortifier with extensively hydrolyzed proteins; MOM, mother's own milk; NEC, necrotizing enterocolitis; PI-HMF, powdered human milk fortifier with intact proteins; RCT, randomized controlled trial; SR, systematic review.

[2] In both the HM100 and HM40 groups, 1 NEC case was a protocol violator that had received some amount of bovine milk–based formula or fortifier.

Chapter 3

Shulhan J, Larsen BMK, Kumar M, Jones CA, Shave K, and Hartling L. A scoping review of enteral nutrition and necrotizing enterocolitis and a systematic review of hydrolyzed formulas.

Abstract

Background. Necrotizing enterocolitis (NEC) is a complex and devastating disease of the intestine affecting preterm and critically ill neonates. The type of enteral nutrition used to feed infants may influence their risk of NEC. Systematic reviews have evaluated subsets of enteral diets, with a focus on bovine milk-based nutrition products compared to human milk (HM); however, a comprehensive review describing all types of evidence on this topic does not yet exist. Further, the effect of protein-hydrosylated products has not been thoroughly explored. The objectives of this review are to (1) map the literature on NEC and different enteral diets, and (2) systematically review the evidence on hydrolyzed formulas compared to other sources of enteral nutrition and the effect on NEC in neonates.

Methods. Five databases and grey literature were searched. Eligible studies were primary, quantitative research published in English from 1990-2017 that compared different diets and reported on NEC events in neonates enterally fed before day of life 30. Two reviewers independently completed study selection. Data were extracted by one reviewer, verified by a second reviewer, and analyzed using summary statistics. Homogenous data regarding protein-hydrosylated nutrition products were pooled in a meta-analysis.

Results. Seventy-six studies were included: 44 cohort, 18 case control, 13 randomized control trials (RCT) and 1 before-and-after studies. The majority of studies (56.6%) were conducted in the United States and involved very or extremely low birth weight infants born <34 weeks of gestation. The median sample size was 213 infants (interquartile range: 101-396). The most common comparison was an exclusive or predominantly HM diet versus intact-protein formula or combination diets containing some amount of bovine milk-based products. One of 5 RCTs, 8/21 cohort studies and 11/16 case control studies showed that an exclusive or predominantly HM diet significantly reduced NEC compared to a diet containing more bovine milk-based

products. Protein-hydrosylated formulas were evaluated by 9 studies, including 2 RCTs and 2 non-concurrent cohort studies that compared hydrolyzed fortifiers to a standard bovine milk-based fortifier with intact proteins. Meta-analysis of the 2 RCTs (n=140) indicated no significant difference between the hydrolyzed and standard fortifiers (RR 1.44, 95% CI 0.18-11.31, I^2 39%). The overall risk of bias for the 2 RCTs was unclear (Figure 5).

Conclusions. There are a large number of studies with variable designs comparing HM to bovine milk-based products. Data from RCT and cohort studies suggest that an exclusive or predominantly HM diet may be protective against NEC. Additional RCTs, adequately powered to the outcome of NEC, are required to determine the effect of hydrolyzed fortifiers on the incidence of NEC.

Introduction

Premature and critically ill neonates with poor gut and immune functions are at an increased risk of necrotizing enterocolitis (NEC), a serious inflammatory disease of the gut. NEC is defined as transmural inflammation and damage to the gut wall that may progress to intestinal perforation and necrosis (1). In Canada, 5.1% of babies born <33 weeks of gestation develop NEC (2). Consequences of the disease can be devastating in that severe cases may lead to laparotomy, surgical resection of damaged intestinal tissue or, in 20-30% of cases, death (3).

NEC is a multifactorial disease that may stem from various risk factors, including prematurity, colonization and proliferation of pathogenic microbiota, and poor gut perfusion (3, 4). Although the exact etiology is not well-understood, the type of enteral (through the gut) nutrition administered is a modifiable factor that may influence an infant's susceptibility to NEC. Bovine milk-based infant formulas may cause more infants to develop NEC compared to infants only fed human breast milk (5, 6). The presence of immune and growth factors, probiotic human milk oligosaccharides, and the high bioavailability of iron, fat and other nutrients are thought to contribute to this hypothesis (4). An exclusive human milk (HM) diet involving use of donor human milk (DHM)-based fortifiers may be better than standard infant formula, but it is an expensive approach (7, 8) and does not completely prevent NEC (5).

Modern nutrition products that have not been thoroughly explored are hydrolyzed formulas. These formulas contain partially or extensively broken down proteins, and easily digested fats and carbohydrates (9). Hydrolyzed products are beneficial because broken down nutrients facilitate digestion and absorption, which is expected to reduce stress and pro-inflammatory processes in the gut (10). The disadvantages of hydrolyzed formulas are the reduced pH in liquid solutions and higher osmolarity compared to formulas with intact proteins. Hyperosmolarity and acidity may impede complete digestion in an immature gut.

Several studies have evaluated the type of enteral feeds and NEC, but knowledge syntheses on this topic only focus on randomized controlled trials or one specific diet comparison (e.g. formula versus mother's own milk, or formula versus donor human milk). An inclusive review describing all forms of quantitative literature has not yet been done. In addition, the effect of protein-hydrosylated products on NEC compared to formulas/fortifiers with intact proteins or HM is unknown. The goals of this review are 1) to comprehensively identify and organize the evidence regarding enteral diets and NEC, and 2) systematically assess hydrolyzed products. Findings from this study are intended to inform clinical decisions regarding the type of nutrition used for infants at risk of NEC, and guide future research.

Objectives

The objectives of this review were:

(1) To map the literature on NEC and enteral diets (including hydrolyzed formulas, bovine milk-based products, mother's own milk and donor human milk products) in infants enterally fed before day of life 30.
(2) To systematically review the evidence on hydrolyzed formulas compared to other sources of enteral nutrition regarding NEC events in neonates.

Methods

The scoping review on enteral nutrition and NEC was completed concurrently with the systematic review of hydrolyzed formulas; therefore, literature searching and study selection for these 2 components were performed simultaneously. The review protocol was registered on the PROSPERO register, CRD42016046805 (registration 27 September 2016, last updated 30 January 2017).

Search strategy

Five databases were searched in July 2016, in collaboration with a health sciences librarian: MEDLINE, EMBASE, Cochrane Library, CINAHL and ProQuest. Two proceedings were searched: the Pediatric Academic Society, and European Society for Paediatric Gastroenterology Hepatology and Nutrition. Grey literature, including the Food and Drug Administration, Canadian Drug Products Database, European Medicines Agency – EU Clinical Trials Register, and clinicaltrials.gov were also searched. In order to focus on modern nutrition product formulations and manufacturing technologies, publication year was restricted to 1990 – 2016. Language was restricted to English. The database searches were updated in February 2017 to capture references added to the databases between July 2016 and February 2017. See **Appendix 1** for the MEDLINE search strategy.

Inclusion/Exclusion criteria

Primary quantitative studies that compared different types of feeds and reported on NEC events in neonates enterally fed before day of life 30 were included. Only studies on enteral feeds containing protein, carbohydrate and fat in proportions intended to promote growth, development and infant disease prevention were considered. Interventions or exposures to nutrition supplements such as individual amino acids, polyunsaturated fatty acids or probiotics were not included.

Study selection

Two reviewers (JS and KS) independently screened the titles and abstracts of each study to assess for eligibility. Studies identified as “include” or “unclear” by at least one reviewer at the title/abstract level were retrieved and the full text was independently assessed for eligibility. Discrepancies were resolved by consensus or third party adjudication.

Relevant abstracts were identified but not included in the analysis to avoid reliance on incomplete or preliminary data and/or insufficient details about the methods and results.

Data extraction

Data were extracted using a standardized form in Microsoft Excel (Microsoft, Redmond, WA) by one reviewer and verified by a second review. Discrepancies were resolved through consensus. Study characteristics, including design, sample size, population and intervention characteristics, and outcomes were extracted. Study design classifications were based on a taxonomy developed for this purpose and presented in the Agency for Healthcare Research and Quality report 11-EHC-007 (11).

Data extracted for the systematic review of hydrolyzed formulas included the number, stage and type of NEC cases; medical events (e.g. gastrointestinal problems, in-hospital mortality); growth and nutrition outcomes; and health care cost data. Quality assessments were completed using the Cochrane Risk of Bias tool (12) for randomized controlled trials and the Newcastle Ottawa Scale (13) for cohort or case control studies. One reviewer (JS) independently completed quality assessments and a second reviewer (DD) verified decisions. Discrepancies were resolved through consensus.

Data synthesis

Data for the scoping review was analyzed descriptively and quantitatively. Study characteristics were summarized using proportions, mean and median values using Microsoft Excel. Weighted means were calculated for birth weight, gestational age, sex, and morbidities. The weighted value was divided by the total population of the studies reporting each variable. For example, respiratory distress syndrome was reported by 11 studies (n=9822) and occurred in 4118 infants. The weighted mean proportion, as determined by Microsoft Excel, was 42.2%.

Included studies were categorized and described regarding types of interventions and primary outcomes. Homogenous data, based on clinical and methodological considerations, from the systematic review of hydrolyzed formulas was pooled in a meta-analysis using Cochrane's Review Manager 5.3 (14).

The quality of evidence was assessed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach (15) and the GRADEpro GDT software (16). GRADE provides an overall rating of the body of evidence by outcome; ratings can be very low, low, moderate and strong. These ratings indicate how confident we are in the estimates of effect based on the available evidence. Two reviewers (JS and AW) independently assessed the following GRADE domains: study design, risk of bias, inconsistency, indirectness, imprecision and other considerations. Discrepancies were resolved by consensus or third party adjudication.

Results

The vast majority of records (5202/5237) were retrieved from library databases (**Figure 1**). Two-hundred and thirty studies were assessed for eligibility and 76 studies were included (**Figure 1** and **Appendix 2**). No records were identified from the food and drug regulatory agencies. Five relevant abstracts were identified but not included in the analysis (**Appendix 3**). Studies excluded at full-text review are provided in **Appendix 4** along with reasons for exclusion.

General study characteristics

Characteristics of the included studies are outlined in **Table 1**. The majority of studies originated from the United States (n=43, 56.6%), were published with a median year of mid-2012 (interquartile range [IQR]: 2008-2015.25), and included a median of 213.5 (IQR: 101.5-396) infants. The most common types of study design were case control and non-concurrent cohort

studies (n=18, 23.7% each). Thirteen RCTs were identified, representing 17.1% of included studies.

Across the 76 included studies, the median recruitment duration was 3 years. The majority (51.3%) of studies recruited or collected data from birth or hospital admission to discharge or death, and the average neonatal intensive care unit (NICU) stay reported by 20 studies was 68.6 ± 37.0 days (9.8 ± 5.3 weeks).

Population characteristics

The majority of studies included very (<1500 g) or extremely (<1000 g) low birth weight infants born <34 weeks gestational age (**Figures 2** and **3**). Of the 52 studies that reported mean birth weight, the weighted mean was within the very low birth weight category at 1199 ± 413 g (**Table 1**). Small-for-gestational-age (SGA), typically defined as birth weight <10th percentile for age, was reported by 26 studies (n=9452 infants). The weighted mean proportion of infants born SGA in those 26 studies was 18.8%. Note that some authors defined SGA differently, e.g. birth weight <3rd percentile or standard deviation <-2. Intrauterine growth restriction (IUGR) was reported by 6 studies (n=2970 infants), and affected an average 7.0% of infants in those studies.

Forty-eight studies reported morbidities other than NEC at baseline or as outcomes. Three of the 48 studies enrolled specific populations (infants with cardiac conditions or gastroschisis). The most frequently reported morbidities were patent ductus arteriosus and sepsis, reported by 22 and 21 studies, respectively. However, the morbidity with the highest weighted proportion of infants was respiratory distress syndrome, which affected 42.2% of 9822 infants in 11 studies (**Table 1**). Bronchopulmonary dysplasia was the second most common morbidity, affecting 25.8% of 7837 infants in 16 studies.

Intervention

The most frequent diet comparison with respect to NEC was exclusive or predominantly HM compared to exclusive formula or combination diets (e.g. bovine milk-based formula, HM and bovine milk-based human milk fortifier [HMF]) (**Table 2**). Five RCTs, 21 cohort studies and 16 case control studies compared HM to formula or combination diets; 3/5 RCTs (of those, 2/5 compared DHM-only diets with no MOM to formula) and 4/21 cohort studies evaluated an exclusive HM diet containing Prolacta+ H^{2}MF®, a DHM-based HMF. One RCT, 8 cohort studies and 11 case control studies found that an exclusive or predominantly HM diet significantly reduces NEC compared to a diet containing bovine milk-based products; the remaining studies found no significant difference. Only 1 prospective and 1 retrospective cohort study reported that NEC rates were higher in infants fed a predominantly or exclusively HM compared to a formula diet, but these findings were not significant.

Six cohort studies compared MOM to DHM or pasteurized MOM. Fortification of DHM and MOM with bovine milk-based HMF was only specified by 3/6 studies, but may have been used by at least 5/6 studies because the study populations included infants with a mean birth weight <1250 g. Only one retrospective cohort study (Dickey 2017) found that the number of NEC events for infants fed pasteurized MOM was significantly lower than infants fed fresh MOM. The other 5 cohorts found no significant difference between MOM and DHM on NEC events.

Quality improvement initiatives (n=6), standardized nutrition protocols (n=4), or pre- and post-introduction of DHM in the NICU (n=4) were the focus of 14 cohort studies. The quality improvement and standardized protocol studies involved several components in the intervention, including strategies to prioritize MOM or DHM as the first choice for enteral feeds. For example, Alshaikh 2015 studied a quality improvement project to promote breastfeeding by training nurses to help mothers pump and express breast milk early, prioritize colostrum as the

first feeds and understand the protective effects of breast milk. Although this type of study contributes to the overall body of literature on enteral diets and NEC, it is difficult to disentangle the effect of a specific diet on NEC in a multicomponent intervention.

Primary outcomes

The most frequently reported type of primary outcome across the 76 studies pertained to nutritional intake or nutrition status. This category included outcomes such as days of parenteral nutrition, days to initiate enteral feeds, human milk feeding at discharge, and episodes of feeding intolerance (**Table 4**). Mortality or morbidities, NEC events, risk factors for NEC, and growth measures were also frequently used as primary outcomes.

Parent-focused outcomes

Only two studies (Schanler 2005 and Schanler 1999) reported parent-focused measures as secondary outcomes, including: median parent visits and visits >50% of hospital stay, episodes of skin-to-skin contact for mother and father, mean episodes of maternal skin-to-skin contact, mean duration of maternal skin-to-skin contact, and median duration of infant holding. Therefore, outcomes centred on parents' perceptions of care, or interactions with their infants were seldom captured in this quantitative body of literature.

Systematic review of protein-hydrosylated products

Study characteristics

Four RCTs (Kim 2015, Mihatsch 2002, Mihatsch 2001 and Moya 2012), 3 non-concurrent cohort studies (Cibulskis 2015, del Castillo 2010 and Thoene 2016) and 2 case control studies (Jayanthi 1998 and Iannucci 2013) investigated protein-hydrosylated formulas or fortifiers. One study (Erasmus 2002) compared Lactaid-treated to placebo-treated formula or liquid fortifier.

Erasmus 2002 is related to the other 9 studies because the intervention breaks down carbohydrate molecules (hydrolysis of lactose to produce galactose and glucose); however, it was not included in the analysis because the focus of this review was on protein-hydrosylated products.

Of the 9 studies on hydrolyzed products, 6 were conducted in the United States, 2 in Germany and 1 in the United Kingdom. The median publication year was 2012 (IQR: 2002 – 2015). The median recruitment duration was 2.9 (IQR: 1.7 – 4.5) years and the median sample size was 100 (IQR: 98 – 129) infants. Only 4/9 studies (Cibulskis 2015, Jayanthi 1998, Kim 2015 and Moya 2012) including 429 infants provided mean and standard deviation data for birth weight and gestational age. The weighted mean was 1258 ± 180 g and 29.4 ± 1.4 weeks at birth.

General findings

Two RCTs with different participants by a similar German research team, published in 2001 and 2002, tested hydrolyzed products for very and extremely low birth weight infants (Mihatsch 2001 and Mihatsch 2002). The first trial compared high lactose formula containing hydrolyzed protein (whey 60:casein 40) to low lactose formula containing hydrolyzed whey, and starch and maltodextrin in place of lactose. There were 5 cases of NEC (10.2%) in 49 infants fed low lactose formula with hydrolyzed whey versus no NEC in 50 infants fed the high lactose formula, p=0.027 (Fisher's exact test). The RCT published one year later compared a formula with hydrolyzed whey and casein (Aptamil Prematil HA) to standard preterm formula (Aptamil Prematil). One NEC event in 66 infants (1.5%) fed the hydrolyzed formula was reported compared to 2 NEC events in 63 infants (3.2%) fed the standard formula, p=0.61 (Fisher's exact test).

Hydrolyzed formulas were evaluated in 3 studies targeting specific patient populations: hypoplastic left heart syndrome (del Castillo 2010, non-concurrent cohort study), congenital

heart disease (Iannucci 2013, case control study) and gastroschisis (Jayanthi 1998, case control study). Although hydrolyzed formulas were suspected to help reduce NEC in cardiac populations, del Castillo 2010 and Iannucci 2013 did not provide complete data on enteral feed sources for all patients. Further, hydrolyzed formula (Pregestimil) was studied by del Castillo 2010 as part of a multi-component intervention. The authors reported that Pregestimil as a stand-alone variable was not significantly associated with NEC. In a gastroschisis population, Jayanthi 1998 found that NEC was significantly higher in infants fed extensively hydrolyzed formulas (Pregestimil or Pepti-Junior, 7 NEC events in 23 patients) compared to MOM (0 NEC events in 12 patients), $p<0.02$.

Meta-analysis of hydrolyzed fortifiers

Two RCTs and 2 non-concurrent cohort studies compared powdered intact-protein HMF to a liquid hydrolyzed HMF. The main differences between these fortifiers were (1) intact versus extensively hydrolyzed proteins, (2) powder versus liquid form, in which powder displaces less human milk volume than a liquid HMF, (3) osmolarity, in which the hydrolyzed fortifier has a higher osmolarity, and (4) some differences in nutritional composition (e.g. Moya 2012 liquid hydrolyzed HMF contained 20% more protein than the powdered HMF; Kim 2015 liquid hydrolyzed HMF contained 23 µg lutein versus 0 µg per 100 kcal in the powdered HMF). Characteristics of these studies are summarized in **Table 5**.

Meta-analysis of the RCTs found no significant difference in NEC between the intact-protein and hydrolyzed-protein HMFs (**Figure 4**: RR 1.44, 95% CI 0.18-11.31, I^2 39%). The cohort studies found that the hydrolyzed HMF was associated with a non-significantly higher risk of NEC; however, the small sample size gave rise to a large confidence interval that crossed the line of no effect.

The overall risk of bias assessment for both RCTs was unclear (**Figure 5**). Moya 2012 did not provide sufficient details regarding random sequence generation and allocation concealment to accurately assess selection bias. Performance bias was unclear because no description was provided by Moya 2012 and Kim 2015 did not blind participants or personnel (although lack of blinding was deemed to probably not influence the results because NEC diagnosis is an objective outcome). Finally, attrition bias was unclear in both studies due to moderately high drop-out rates (25-28%) with an intention-to-treat analysis plan.

The non-concurrent cohort studies, Thoene 2016 and Cibulskis 2015, each scored 7/8 stars on the Newcastle-Ottawa Scale, indicating good quality based on this tool (**Table 6**). The studies appropriately addressed the selection and outcome domains of the scale. Comparability between the cohorts in both studies was unclear because the cohorts were not matched in the design, and confounders (e.g. birth weight or gestational age) were not adjusted for in the analyses. It is important to note that a cohort design, especially one that fails to address comparability between the cohorts, does not provide the strongest level of primary evidence regarding comparative effectiveness. Well-designed RCTs provide more robust evidence, given that randomization balances known and unknown factors between comparison groups. Another warning regarding the Thoene 2016 study was that the acidified liquid HMF was discontinued early due to the higher incidence of metabolic acidosis, suboptimal growth, and significantly higher events of NEC (13% in the acidified liquid HMF cohort versus 0% in the powdered intact-protein HMF cohort, p=0.03) (17). This led to half the number of participants receiving the acidified liquid HMF (n=23) compared to the powdered intact-protein HMF (n=46) and failure to achieve the study's projected sample size. In the same non-concurrent cohort study, the authors later studied a third cohort using non-acidified liquid HMF (n=51), which did not produce the adverse events experienced by the acidified liquid cohorts. Only the acidified liquid and

powdered intact-protein HMFs comparison groups from Thoene 2016 were included in the meta-analysis, as this comparison was consistent with the other 3 studies in the meta-analysis.

GRADE was also used to rate the quality of evidence in the meta-analysis (**Table 7**). Due to the small sample sizes and imprecision of the results, the RCTs were rated as low quality evidence. The cohort studies provide low quality evidence given the observational study design and were downgraded twice for imprecision of the effect estimate, resulting in a very low rating for quality of evidence.

In conclusion, there was insufficient evidence to determine the effectiveness of hydrolyzed fortifiers compared to intact-protein fortifiers on the risk of NEC. Based on the GRADE approach, confidence in the effect estimate of the RCTs is limited and the true effect may be substantially different from the estimate of effect. Additional RCTs powered to detect a significant difference in NEC incidence between comparison groups are needed.

Discussion

This review identified and described primary quantitative studies published since 1990 that compared different enteral diets with respect to NEC. The majority of evidence was identified from database searches. No records were identified from a search of food and drug regulatory agency websites; this may be due to unsophisticated search functions or lack of indexing. The majority of studies included very or extremely low birth weight infants, which is reflective of the population most vulnerable to NEC (2, 18). Most of the identified literature evaluated HM and bovine-milk based formula with intact proteins. There was mounting evidence on the protective effect of HM on NEC. The majority of RCTs and cohort studies did not use NEC as a primary outcome; consequently, sample sizes were often too small to have confidence in the results (i.e.

it is unclear whether no significant difference is due to effects of the interventions or lack of power). Despite this, 1/5 RCTs and 6/21 cohort studies that compared HM to formula diets found an exclusively or predominantly HM diet significantly reduced NEC. This evidence was consistent with a Cochrane systematic review updated in 2014 that compared formula to donor breast milk for preterm or low birth weight infants (5). The meta-analysis included 4 RCTs and showed that exclusive preterm formula significantly increased the risk of NEC compared to exclusive DHM (RR 4.62, 95 CI 1.47-14.56, I^2 0%). A limitation of the Cochrane review was that 3 of the 4 RCTs pooled in the meta-analysis were published in 1983 and 1984. Outdated data calls into question the relevance of the findings, given that product formulations, technologies, and clinical practices have changed in the past 30 years (10). Nonetheless, most studies in this review that examined predominantly/exclusively HM and partial/exclusive formula found a trend or significant difference in favor of HM diets with respect to NEC protection.

Other systematic reviews have addressed different types of enteral feeds and NEC (**Appendix 3**). Our review was unique in 3 ways. First, all forms of enteral feeds, including partial or exclusive formula, partial or exclusive maternal or donor breast milk, bovine or human milk-based HMFs, and specialty formulas were included in this review. Second, all quantitative study designs were included to capture the totality of evidence on this topic. Third, to our knowledge, this was the first systematic review to evaluate the effect of protein-hydrosylated products compared to other types of enteral diet on NEC.

The use of hydrolyzed formulas for infants at risk of NEC is a relatively novel approach to nutrition therapy in the NICU (19). A limited number of clinically diverse studies have been found on this topic. Two German RCTs demonstrated that hydrolyzed formulas are being considered as a nutritional therapy for preterm infants who may be at risk for NEC. However, the first study compared two formulas that each contained some form of hydrolyzed proteins, and the second study did not have sufficient power to detect a difference in NEC events between the groups. A

case control and non-concurrent cohort study on infants with congenital heart disease and hypoplastic left heart syndrome compared an extensively hydrolyzed formula to intact-protein formulas or breast milk. There was incomplete data from these studies on feeding type and NEC to adequately assess this relationship. Finally, one case control study on infants after gastroschisis repair or matched controls compared extensively hydrolyzed formula to mother's own milk alone or in addition to hydrolyzed formula. There were significantly more NEC events in infants fed the hydrolyzed formula compared to mother's own milk; however, the data dates back to 1990-1996. As the nutritional profile of hydrolyzed products evolves, current evidence on hydrolyzed formulas and NEC in gastroschisis patients is needed. Based on this incomplete or outdated evidence, the effect of hydrolyzed formulas on NEC in specialized cardiac and gastrointestinal populations is inconclusive. More evidence is needed to accurately compare extensively hydrolyzed formulas to formulas with intact proteins in the general neonatal population and specialty areas (e.g. cardiac or gastrointestinal populations).

Only 2 RCTs testing hydrolyzed fortifiers could be pooled in a meta-analysis. The small sample size and imprecision of the effect estimate resulted in low quality of evidence. Two non-concurrent cohort studies also compared intact-protein fortifiers to hydrolyzed fortifiers and found non-significantly higher NEC events for infants receiving the hydrolyzed fortifier. As such, more data is required to increase our confidence in the effect estimate. These findings will be followed by another systematic review with similar objectives. Since our original literature search, a Cochrane systematic review protocol has been published, aiming to compare standard formula to protein-hydrosylated formula (20). The Cochrane review will only include RCTs and will assess feeding intolerance, NEC, morbidities and mortality outcomes. Current status of the Cochrane review is unknown. In the interim, the key recommendation of our review is that a larger RCT evaluating hydrolyzed products, bovine milk-based products and/or an

exclusive HM diet is needed. A high-quality, sufficiently powered trial is necessary to guide evidenced-based nutrition practices for neonates at risk of NEC going forward.

We acknowledge that, although RCTs provide the highest level of primary evidence, there are several challenges to overcome in order to conduct a trial on this topic. A recurrent problem with the existing literature is that most trials have not been powered on NEC as a primary outcome. Because the control event rate for NEC is approximately 5% for preterm infants <33 weeks gestation, thousands of participants would be needed for a well-powered clinical trial, depending on the design (superiority versus non-inferiority) and minimal clinically important difference considered. Unfortunately, personnel and coordination costs of a large trial is prohibitive for individual research teams.

The cost of nutrition products is another issue. An exclusive HM diet for very or extremely low birth weight infants often requires pasteurized DHM from milk banks and HM-based HMF such as Prolacta+ H^{2}MF®. Combined, Prolacta and DHM would cost an estimated $1.33/mL, nearly 10 times the price of DHM fortified with a bovine milk-based HMF (10). Hydrolyzed products are also expensive, with powdered formula for term infants costing $0.14/g CAD (21) compared to $0.06/g CAD for bovine milk-based term formula (22). We are not familiar with the cost of a hydrolyzed HMF but, presumably, it is more expensive that bovine milk-based HMF. Clearly, the main barriers for high-level evidence are the resources and costs involved. Collaboration across multiple centres and pooling of resources is the best approach to further our understanding of the role nutrition may play to prevent NEC.

A possible limitation of this review was the English language restriction, which may have led to underrepresentation of non-English studies. Justification for the language restriction was based on a 2014 Cochrane review (5) determining the effect of formula compared to DHM on growth and development for premature and low birth weight infants. The Cochrane reviewers searched

literature in all languages and the included studies were all published in English. Given that the Cochrane review studied a comparable population, intervention and outcome as this review, and did not find relevant non-English studies, our results likely would not have changed if non-English studies were included in the search. A recent methodological study also evaluated the impact of non-English reports on the results of meta-analyses in a sample of 129 child health systematic reviews (23). The authors found that non-English studies contributed a small proportion of included studies to the reviews and rarely impacted the conclusions. Therefore, the English language restriction of the literature search in this review likely did not affect the conclusions.

A number of secondary outcomes were extracted for the studies on hydrolyzed nutrition products, including patient-oriented (e.g. type and severity of NEC, growth) and parent-oriented (e.g. parent-child bonding, satisfaction with care) outcomes. These secondary outcomes were not reported here as the focus of this review was on NEC events.

Conclusions

Overall, several studies have compared diets containing varying proportions of human milk and bovine milk-based products on the effect of NEC. The majority of studies have found a non-significantly lower occurrence of NEC with predominantly human milk-based diets. While preliminary evidence suggests there might be higher risk of NEC with hydrolyzed fortifiers, additional data is required to determine the effect of these fortifiers on NEC with more confidence.

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Abbreviations

DHM – donor human milk
HM – human milk
HMF – human milk fortifier
IQR – interquartile range
MOM – mother's own milk
NEC – necrotizing enterocolitis
NICU – neonatal intensive care unit
RCT – randomized controlled trial
SGA – small-for-gestational-age

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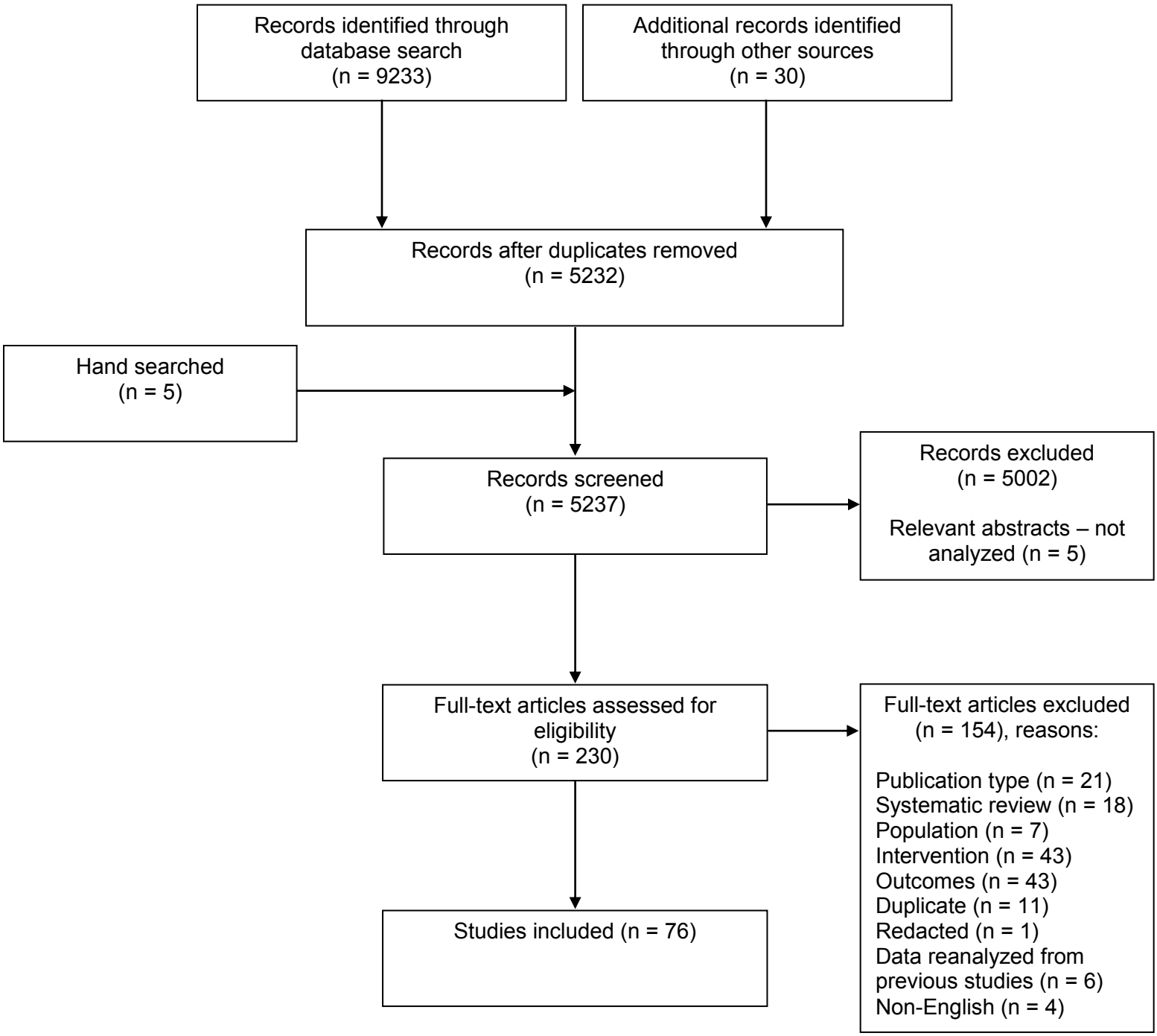

Figure 1. Flow diagram of study selection.

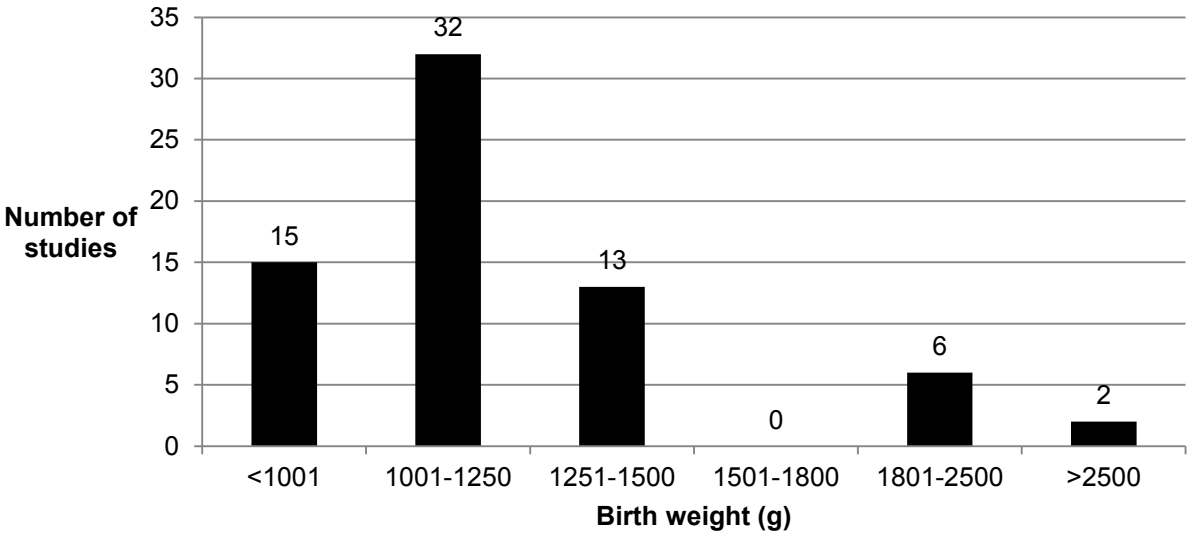

Figure 2. Birth weight classes studied based on overall mean, median or range reported by 68 included studies. Summary statistics were unclear or not reported by 8 studies.

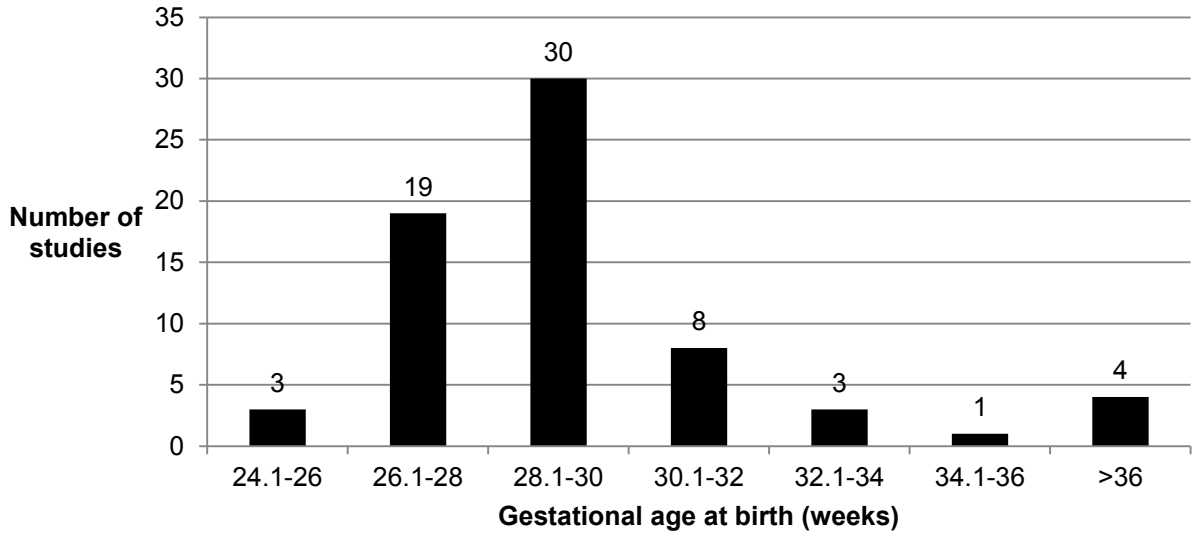

Figure 3. Gestational age at birth categories studied based on overall mean, median or range reported by 68 included studies. Summary statistics were unclear or not reported by 8 studies.

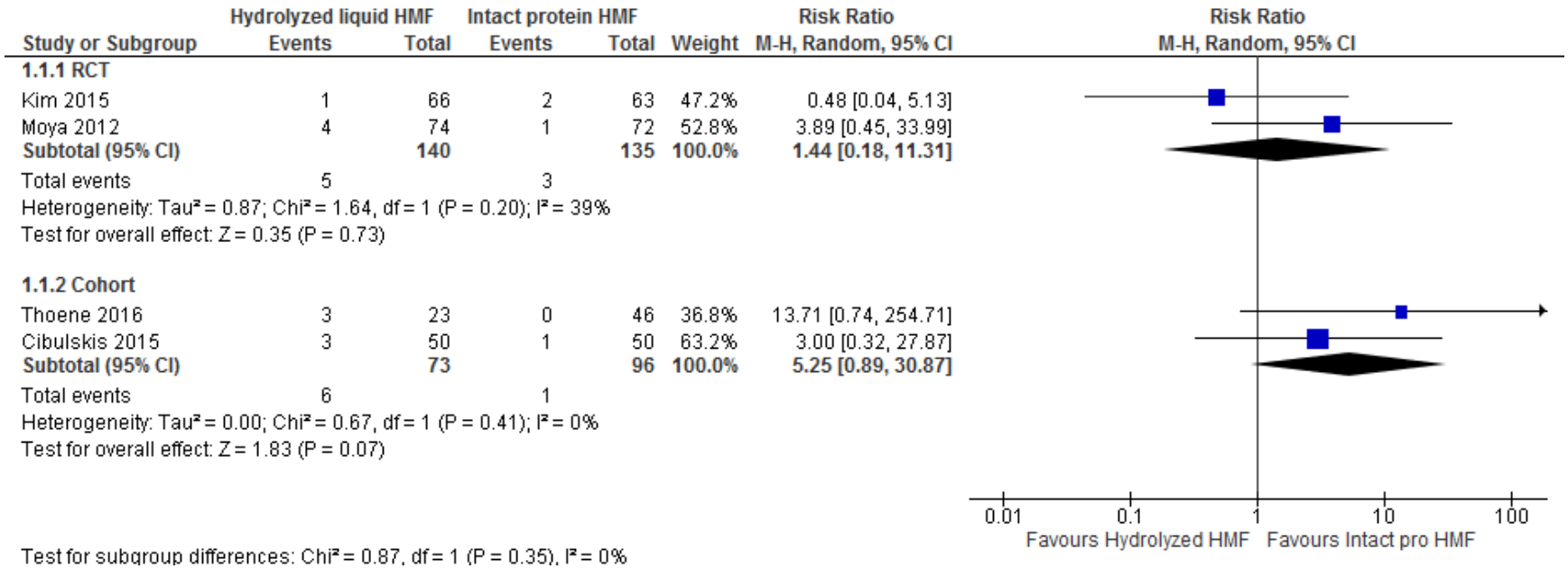

Figure 4. Hydrolyzed liquid human milk fortifier versus powdered intact-protein human milk fortifier and necrotizing enterocolitis events.

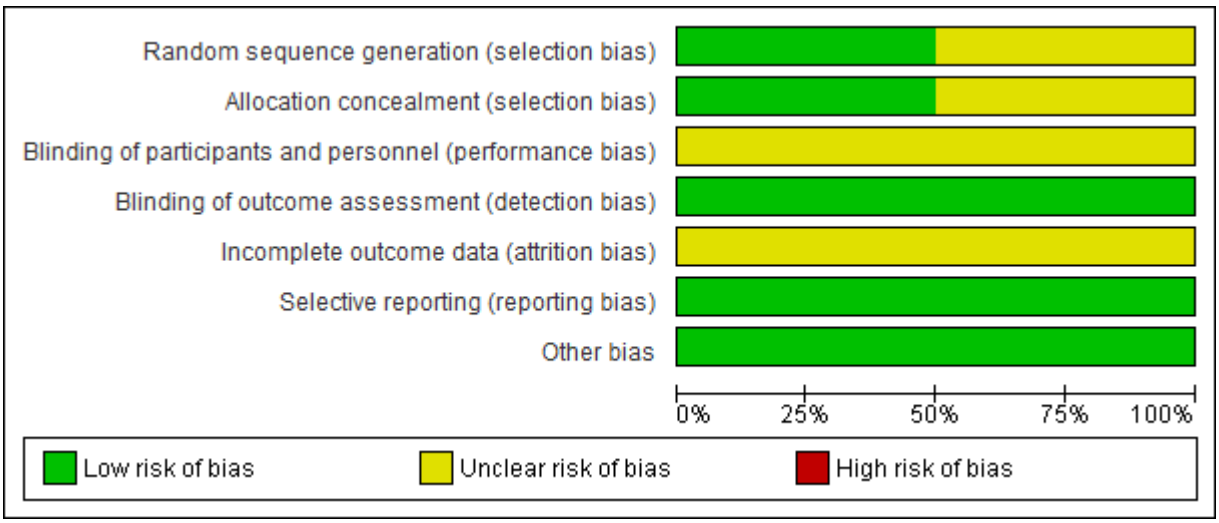

Figure 5. Risk of bias graph for Kim 2015 and Moya 2012: review authors' judgments about each risk of bias item presented as percentages across all included studies. Both random sequence generation and allocation concealment were unclear for Moya 2012, and low for Kim 2015.

Table 1. Description of included studies

Variable	n studies	
N	**76**	
Country of corresponding author		
United States	43	56.6%
Canada, United Kingdom	4 each	5.3% each
Germany	3	3.9%
Australia, Brazil, France, Greece, Italy, The Netherlands	2 each	2.6% each
Austria, Finland, India, Israel, Oman, Romania, South Africa, Spain, Thailand, Turkey	1 each	1.3% each
Study design		
Case control study	18	23.7%
Non-concurrent cohort study	18	23.7%
Retrospective cohort study	14	18.4%
Randomized controlled trial	13	17.1%
Prospective cohort study	12	15.8%
Before-and-after study	1	1.3%
Year of publication		
Median		2012.5
Interquartile Range		2008-2015.25
Sample size of individual studies		
Median N		213.5
Interquartile Range		101.5 – 396
Range		33 – 422,877
Population characteristics		
Birth weight, weighted mean ± SD (grams)	52[a]	1199 ± 413
Gestational age at birth, weighted mean ± SD (weeks)	51[b]	28.7 ± 3.3
Male sex, proportion	58[c]	55.2%
Common morbidities[d], weighted proportion		% population
Respiratory distress syndrome	11	42.2%
Bronchopulmonary dysplasia	16	25.8%
Patent ductus arteriosus	22	23.5%
Retinopathy of prematurity	14	17.4%
Sepsis	21	13.6%
Other chronic lung diseases	5	12.0%
Intraventricular hemorrhage	15	11.7%
Recruitment duration	70[e]	
Median		3 years
Interquartile Range		1.92 – 5.5 years
Range		0.5 – 17 years
Duration of interventions/exposures		
Admission to discharge or death	39	51.3%
1-4 weeks or (some studies) the earlier of discharge or death	12	15.8%
Until 32-36 weeks CGA or (some studies) the earlier of discharge, transfer, death or NEC	8	10.5%
Until NEC diagnosis	5	6.6%
Until weight goal (1400 g, 2000 g or 2500 g)	4	5.3%
Until 90-91 days old or (some studies) discharge or	3	3.9%

Variable	n studies	
N	**76**	
50% enteral feeds achieved		
3-7 days before and/or after NEC diagnosis	3	3.9%
Post-operation to discharge or death	2	2.6%
Length of stay, weighted mean ± SD (days)	20[f]	68.6 ± 37.0
Type of enteral feeds evaluated		
Exclusive HM diet	28	36.8%
HM with bovine milk-based fortifier	27	35.5%
Exclusive formula	25	32.9%
Mostly HM (≥50% of intake) with some formula[g]	24	31.6%
HM, bovine milk-based fortifier and formula[g]	22	28.9%
Mostly formula[g] (≥50% of intake) with some HM	15	19.7%
Multicomponent intervention	12	15.8%
Hydrolyzed formula or fortifier	9	11.8%

Abbreviations: CGA = corrected gestational age; HM = human milk; NEC = necrotizing enterocolitis; SD = standard deviation.

[a] One study (Lambert 2007) excluded as an outlier. 17 studies reported median values or ranges, and 6 studies did not report birth weight.

[b] One study (Lambert 2007) excluded as an outlier. 19 studies reported median values or ranges, and 5 studies did not report gestational age at birth.

[c] 18 studies did not report subtotals for males and females.

[d] 18 studies did not report morbidities; 3 studies (del Castillo 2010; Iannucci 2013; Jayanthi 1998) were excluded because of targeted study populations (e.g. gastroschisis or cardiac disease only).

[e] 6 studies did not report recruitment dates or dates of study period.

[f] 2 studies (Ellsbury 2016; Gane 2014) were excluded as outliers; 15 studies reported median or range values; 38 did not report length of stay.

[g] Intact-protein bovine milk-based formula

Table 2. Diet or primary comparisons evaluated by the 76 included studies.

Diet or primary intervention evaluated	RCTs	Cohort studies	Case control studies
Exclusive or Predominantly HM Diet vs. Formula or Combination Diets, n=26	1. Corpeleijn 2016 2. Cristofalo 2013 **[EHM]** 3. Lucas 1990 **[EHM]** 4. Schanler 2005 5. Sullivan 2010 **[EHM]***	1. Assad 2016 **[EHM]*** 2. Beattie 2014 3. Calabro 2012 4. Colaizy 2016 5. Colaizy 2012 6. Corpeleijn 2012 7. Furman 2003 8. Hair 2016 **[EHM]*** 9. Herrmann 2014 **[EHM]** 10. Huston 2014 **[EHM]*** 11. Maayan-Metzger 2012***** 12. Manea 2016 13. Meinzen-Derr 2009 **[EHM]** 14. O'Connor 2003 15. Oncel 2014 **[EHM]** 16. Parker 2012 17. Schanler 1999***** 18. Serrao 2016 19. Sisk 2016***** 20. Sisk 2007***** 21. Spiegler 2016*****	
Case control studies determining risk factors for NEC, n=16			1. Beeby 1992 2. Cordero 2010 3. Gane 2014* 4. Gephart 2017* 5. Gregory 2008* 6. Gregory 2015 7. Hallstrom 2003* 8. Henderson 2009* 9. Kimak 2015* 10. Lambert 2007* 11. Martin 2013 12. Miner 2013* 13. Sdona 2016* 14. Sullivan 2016* 15. Thompson 2011* 16. Vinocur 1990
Hydrolyzed formulas,	1. Kim 2015	1. Cibulskis 2015	1. Jayanthi 1998

Diet or primary intervention evaluated	RCTs	Cohort studies	Case control studies
n=9	2. Mihatsch 2002 3. Mihatsch 2001 4. Moya 2012	2. del Castillo 2010 3. Thoene 2016	2. Iannucci 2013
Lactase-treated feeds	1. Erasmus 2002		
MOM vs. DHM or pasteurized MOM, n=6		1. Aprile 2010 2. Dicky 2017 3. Dritsakou 2016 4. Giuliani 2012 5. Montjaux-Regis 2012 6. Stock 2015	
Bovine milk-based fortifier vs. no fortifier or placebo, n=4	1. Bhat 2001 2. Khorana 2014 3. Lucas 1996	1. Moody 2000	
NICU quality improvement initiatives which include prioritizing HM, n=6		1. Alshaikh 2015 2. Ellsbury 2016 3. Johnson 2015 4. Lee 2012 5. Montgomery 2008 6. Patel 2014	
Pre- and post-introduction of DHM, n=4		1. Bishop 2010 2. Chowning 2016 3. Coutsoudis 2011 4. Kantorowska 2016	
Standardized nutrition protocol (multicomponent intervention) which includes prioritizing HM, n=4		1. Bulter 2013 2. Kamitsuka 2000 3. Lapointe 2016 4. Stefanescu 2016	

Abbreviations: DHM = donor human milk, HM = human milk, MOM = mother's own milk, NEC = necrotizing enterocolitis, NICU = neonatal intensive care unit.
[EHM] = Study evaluated an exclusive human milk diet including Prolacta® fortifier.
*Exclusive or predominantly human milk diet found to significantly reduce necrotizing enterocolitis compared to exclusive formula or diet containing bovine milk-based products.

Table 3. Randomized controlled trials and cohort studies comparing human milk versus bovine milk-based diets and the effect on necrotizing enterocolitis in neonates.

Study design	Author, year	Intervention, n	Control, n	NEC events
Exclusive human milk diet vs. Exclusive formula or Combination diets				
RCT	Cristofalo 2013	DHM + Prolacta, n=29	PTF, n=24	HM = 1 (3%) PTF = 5 (21%)
RCT	Lucas 1990	Exclusive DHM, n=86	Exclusive PTF, n=76	HM = 1 (1.2%) PTF = 4 (5.3%), OR = 4.7 (95% CI 0.5-43)
		DHM with or without MOM, n=243	Preterm formula with or without MOM, n=249	HM = 3 (1.2%) PTF/MOM = 9 (3.6%), OR = 3.1 (95% CI 0.8-11.7, p=0.07)
RCT	Sullivan 2010	(1) MOM + Prolacta + DHM if MOM unavailable, start fortifier when feeds at 100 mL/kg/d (HM100), n=67; (2) MOM + Prolacta + DHM if MOM unavailable, start fortifier when feeds at 40 mL/kg/d (HM40), n=71; (3) HM(100+40), n=138	(4) MOM + bovHMF + PTF if MOM unavailable (BOV), n=69	(1) HM100 = 3 (4.5%)* (2) HM40 = 5 (7%) (3) HM(100+40) = 8 (5.7%)* (4) BOV = 11 (15.9%) (1) vs. (4), p=0.04; (2) vs. (4), p=0.09; (3) vs. (4), p=0.02
Non-concurrent cohort	Assad 2016	(1) MOM + DHM + Prolacta, n=87	(2) MOM + bovHMF (BOV), n=127; (3) MOM + bovHMF + PTF if MOM unavailable (Mixed), n=49	(1) HM = 1 (1.1%) (2 and 3) BOV and Mixed = 18 (10%), p<0.011
Non-concurrent cohort	Hair 2016	MOM or DHM + Prolacta, n=819	MOM + bovHMF and/or PTF, n=768	HM = 57 (6.9%) PTF = 128 (16.7%), p<0.00001
Non-concurrent cohort	Herrmann 2014	Until 33 weeks gestation: MOM + Prolacta + DHM if MOM unavailable; after 33 weeks gestation: MOM + PTF and/or bovHMF, n=199	Not specified. Likely did not include DHM or Prolacta, n=443	HM = 7 (3.5%) Control = 17 (3.8%)
Retrospective cohort	Huston 2014	(1) MOM or DHM + Prolacta (EHM), n=44	(2) Exclusive PTF or MOM + bovHMF + PTF (FORM), n=93	(1) EHM = 1 (2.3%) (2) FORM = 8 (8.6%)*

Study design	Author, year	Intervention, n	Control, n	NEC events
			(3) MOM or DHM + bovHMF (HM+HMF), n=224	(3) HM+HMF = 11 (4.9%) *EHM vs. FORM, OR=0.06 (0.003-0.445, p=0.019)
Retrospective cohort	Meinzen-Derr 2009	MOM only or Any MOM in the first 14 days of life for NEC/death patients (n=173 of which 98 had NEC) and	MOM only or Any MOM in the first 14 days of life for Survivors (no NEC, n=1099)	MOM only in first 14 days of life: NEC/death = 49 (28%), Survivors = 353 (32%); Any MOM in first 14 days of life: NEC/death = 116 (67%), Survivors = 811 (74%)
Prospective cohort	Oncel 2014	(1) MOM, n=17	(2) MOM + bovHMF, n=94; (3) PTF, n=51	(1) HM = 0 (0%) (2) HM+HMF = 5 (5.3%) (3) PTF = 5 (9.8%)
Predominantly human milk diet vs. Diet containing more bovine-milk based products (formula and/or fortifier)				
RCT	Corpeleijn 2016	MOM or DHM if MOM unavailable, bovHMF added after day of life 10, n=183	MOM (bovHMF added after day of life 10) or PTF if MOM unavailable, n=190	HM+HMF = 17 (9.3%) MOM/PTF+HMF = 17 (8.9%) Adjusted HR = 0.98 (95% CI 0.49-1.93, p=0.95)
RCT	Schanler 2005	(1) MOM + bovHMF added when feeds reached 100 mL/kg/day, n=70	(2) MOM + supplemental DHM + bovHMF added when feeds reached 100 mL/kg/day, n=81 (3) MOM + supplemental PTF, n=92	NEC for infants that received ≥50 mL/kg of assigned feeds: (1) MOM + HMF = 4 NEC of 70 infants (5.7%) (2) MOM + DHM + HMF = 5 NEC of 78 infants (6.4%) (3) MOM + PTF = 10 NEC of 88 infants (11.4%) (2) vs. (3), p=0.27 (1) vs. (2 and 3), p=0.39

Study design	Author, year	Intervention, n	Control, n	NEC events
Prospective cohort	Beattie 2014	(1) MOM, n=12 (bovHMF added to n=2) (2) MOM + DHM, n=5	(3) MOM + DHM + PTF, n=10 (bovHMF added to n=3) (4) MOM + PTF, n=27 (HMF added to n=3) (5) DHM + PTF, n=1 (6) PTF, n=1	(1) MOM = 8 (67%) (2) MOM + DHM = 5 (100%) (3) MOM + DHM + PTF = 7 (70%) (4) MOM + PTF = 11 (40%) (5) DHM + PTF = 0 (0%) (6) PTF = 0 (0%)
Prospective cohort	Calabro 2012	(1) MOM or DHM with or without bovHMF, n=41	(2) MOM and/or DHM + PTF, n=6 (3) Exclusive PTF, n=7	(1) MOM/DHM, bovHMF = 2 (4.9%) (2) MOM/DHM + PTF = 6 (8.3%) (3) PTF =2 (28.6%)
Retrospective cohort	Colaizy 2016	(1) ≥98% MOM + bovHMF, n = 77	(2) <98% MOM (no DHM), n = 573 (3) Exclusive PTF, n = 198	(1) ≥98% MOM = 1 (1.3%) (2) <98% MOM = 47 (8.2%) (3) PTF = 22 (11.1%)
Retrospective cohort	Colaizy 2012	(1) >75% volume HM (MOM or DHM), n = 88	(2) 50-75% HM, n = 36 (3) 25-50% HM, n = 30 (4) <25% HM, n = 17	(1) >75% HM, NEC = 2 (2.3%) (2) 50-75% HM = 1 (2.8%) (3) 25-50% HM = 0 (0%) (4) <25% HM cases = 0 (0%) p-value (ANOVA) = 1
Retrospective cohort	Corpeleijn 2012	(1) >50.01% MOM from DOL 1-10, n = (100% MOM, n=7)	(2) 0.01-50% MOM from DOL 1-10, n = (3) Exclusive PTF, n = 42	Overall rate of NEC was 6.3% during the study but authors did not delineate based on feeding groups.
Prospective cohort	Furman 2013	(1) HM ≥50 mL/kg, n = 32	(2) HM 25-49 mL/kg, n = 18 (3) HM 1-24 mL/kg, n = 29 (4) Exclusive PTF, n = 40	(1) HM ≥50 mL/kg = 0 (0%) (2) HM 25-49 mL/kg = 2 (11%)

Study design	Author, year	Intervention, n	Control, n	NEC events
				(3) HM 1-24 mL/kg = 2 (7%) (4) PTF = 3 (8%) (1) vs. (4), OR = 0 (95% CI 0-3.56) (2) vs. (4), OR = 1.99 (95% CI 0.14-21.03) (3) vs. (4), OR = 1.15 (95% CI 0.8-12.13)
Retrospective cohort	Maayan-Metzger 2012	(1) HM (≥7 of 8 meals were HM only) + bovHMF, n = 54	(2) Mainly HM, n = 134 (3) Equal amount HM and PTF feedings, n = 40 (4) Mainly PTF (5-6 of 8 meals were PTF only), n = 122 (5) PTF (≥7 of 8 meals were PTF only), n = 50	(1 and 2) HM and Mainly HM = 0 (0%) (4 and 5) Formula and Mainly Formula = 5 (2.9%) p=0.044
Prospective cohort	Manea 2016	Breast milk (unknown if DHM available) + bovHMF, n = 18	Formula (unknown if term or preterm formula), n = 16	Breast milk = 0 (0%) Formula = 2 (12.5%)
Retrospective cohort	O'Connor 2003	(1) Predominantly HM (PHM-T, consumed <100 mL/kg birth weight of formula or fortifier for the total duration of their initial hospital stay and >80% of all feedings provided as HM at term corrected age; unknown if DHM used), n = 43	(2) ≥50% energy from HM before hospital discharge, n = 98 (3) <50% of energy from HM before hospital discharge, n = 203 (4) Predominantly formula fed (PFF-T, consumed <100 mL/kg birth weight of HM for the total duration of their initial hospital stay and >80% of all feedings provided as formula until term corrected age), n = 119	(1) PHM-T = 0 (0%) (2) ≥50% HM = 5 (5%) (3) <50% HM = 2 (1%) (4) PFF-T = 8 (7%)
Retrospective cohort	Parker 2012	>49% breast milk (unknown if DHM used), n =40	Exclusive formula (unknown if preterm or term formula), n = 40	>49% HM = 1 (2.5%) Exclusive formula = 3 (7.5%), p=0.59
Retrospective	Schanler	Mean >50 mL/kg/day MOM +	Exclusive PTF, n = 46	MOM + HMF = 1 (1.6%)

Study design	Author, year	Intervention, n	Control, n	NEC events
cohort	1999	bovHMF, n = 62		Exclusive PTF = 6 (13%), p≤0.01
Prospective cohort	Serrao 2016	MOM or DHM if MOM unavailable ≥50 mL/kg/day until 32 weeks post-menstrual age, n=22	MOM or DHM if MOM unavailable ≥50 mL/kg/day until 32 weeks post-menstrual age, n = 30	≥50 mL/kg/day HM = 0 (0%) <50 mL/kg/day HM = 0 (0%)
Retrospective cohort	Sisk 2016	(1) ≥50% MOM + bovHMF + Beneprotein; PTF used if MOM unavailable for first half of study; DHM used if MOM unavailable for second half of study; n = 299	(2) ≥50% Pasteurized DHM + bovHMF + Beneprotein, n = 139 (3) ≥50% Preterm formula, n = 113	(1) ≥50% MOM = 16 (5.3%) (2) ≥50% DHM = 6 (4.3%) (3) ≥50% PTF = 13 (11.5%), p=0.038 (significant based on Bonferroni correction)
Prospective cohort	Sisk 2007	≥50% MOM or DHM if MOM unavailable for first 2 weeks of life, n = 156	<50% MOM or DHM if MOM unavailable, n = 46	≥50% HM = 5 (3.2%) <50% HM = 5 (10.9%), p<0.05
Prospective cohort	Spiegler 2016	(1) MOM and/or DHM + bovHMF and/or protein supplements, n = 223	(2) Exclusive PTF, n = 239 (3) PTF + HM + bovHMF and/or protein supplements, n = 971	(1) HM + bovHMF = 2 (0.9%) (2) PTF = 14 (6.1%) (3) PTF + HM + bovHMF = 26 (2.7%) (1) vs. (2), p=0.004
Bovine milk-based fortifier versus no fortifier or placebo				
RCT	Bhat 2001	MOM only, n = 50 (3 infants received supplemental formula over 4 days)	MOM + bovHMF, n = 50 (3 infants received supplemental formula over 3 days)	MOM only = 4 (8%) MOM + bovHMF = 3 (6%), p > 0.05
RCT	Khorana 2014	MOM + bovHMF, PTF used if MOM unavailable (excluded infants if PTF >20% intake), n = 18	MOM + post-discharge formula, n = 15	MOM + bovHMF = 2 (11.1%) MOM + post-discharge formula = 0 (0%), p = 0.489
RCT	Lucas 1996	MOM + bovHMF, PTF used if MOM unavailable, n = 137	MOM + Phosphate and vitamins supplement, PTF used if MOM	MOM + bovHMF = 8 (5.8%)

Study design	Author, year	Intervention, n	Control, n	NEC events
			unavailable, n = 138	MOM + control supplement = 3 (2.2%), p = 0.12
Before-after	Moody 2000	5 days after introduction of bovHMF to MOM (once feeds reached 100 mL/kg/day), n = 76	5 days before introduction of bovHMF to MOM (after feeds reached 100 mL/kg/day), n = 76	5 days before bovHMF = 0 (0%) 5 days after bovHMF = 0 (0%)
Mother's own milk versus Donor human milk or pasteurized mother's own milk				
Prospective cohort	Aprile 2010	Raw or pasteurized MOM (bovHMF not specified but likely used), n = 10	DHM (bovHMF not specified but likely used), n = 30	MOM = 0 (0%) DHM = 3 (10%), p=0.5597
Retrospective cohort	Dicky 2017	Fresh MOM (bovHMF not specified but likely used), n = 636	Pasteurized MOM (bovHMF not specified by likely used), n = 290	Fresh MOM = 28 (4.4%) Pasteurized MOM = 5 (1.7%), p = 0.05
Retrospective cohort	Dritsakou 2016	Raw MOM (~70%) + DHM (~30%) if MOM unavailable + bovHMF when volume reached 70 mL/kg/day, n = 192	DHM only until 3rd week of life then PTF until discharge, DHM fortified with bovHMF when volume reached 70 mL/kg/day, n = 192	Raw HM = 12 (6.3%), DHM + PTF = 16 (8.3%) OR 0.67 (95% CI: 0.27-1.63), p=0.374
Retrospective cohort	Giuliani 2012	>80% MOM + bovHMF when volumes reached 100 mL/kg/day until 1.8 kg or discharge, n = 46	>80% DHM + bovHMF when volumes reached 100 mL/kg/day until 1.8 kg or discharge; DHM switched to PTF at 1.8 kg or discharge; n =46	>80% MOM = 4 (8.7%) >80% DHM = 1 (2.2%), p = 0.36
Prospective cohort	Montjaux-Regis 2012	(1) ≥80% MOM and some DHM + bovHMF when volumes reached 100 mL/kg/day, n = 17	(2) DHM and 20-<80% MOM + bovHMF at 100 mL/kg/day,, n = 11 (3) DHM and <20% MOM + bovHMF at 100 mL/kg/day, n = 20 (14 infants received no MOM)	(1) ≥80% MOM = 0 (0%) (2) 20-<80% MOM = 0 (0%) (3) <20% MOM = 3 (15%) p=0.23 (assuming ANOVA used)
Non-concurrent cohort	Stock 2015	Unpasteurized breast milk (unclear if DHM was used; bovHMF not specified but likely used), n = 164	Pasteurized breast milk (unclear if DHM was used or if MOM was pasteurized; bovHMF not specified but likely used), n = 159	Unpasteurized HM = 4 (2.4%) Pasteurized HM = 7 (4.4%), p=0.254

Study design	Author, year	Intervention, n	Control, n	NEC events
Pre- and post-introduction of donor human milk				
Non-concurrent cohort	Bishop 2010	Post-pasteurization DHM era (Apr 2003 - Dec 2004), MOM provided 54% of enteral feeds, bovHMF added to MOM and DHM, n = 150	Pre-pasteurized DHM era (Jan 2001 - Mar 2003), MOM provided 51% of enteral feeds, bovHMF added to MOM and DHM, n = 175	Pre-pasteurized DHM era = 21 (12%) Post-pasteurized DHM era = 15 (10%) Binary logistic regression of feeding practices and overall NEC: Mostly maternal milk (>50%), OR=1.06 (CI 0.51-2.2), p=0.95; Mostly donor milk (>50%), OR=0.46 (CI 0.11-1.87), p=0.28; Mostly formula (>50%), OR = 1.47 (CI 0.5-4.29), p=0.48; bovHMF, OR = 1.03 (CI 0.81-1.32), p=0.81.
Retrospective cohort	Chowning 2016	(1) ≥90% MOM or DHM + liquid bovHMF, n = 71 (2) ≥50% MOM or DHM + liquid bovHMF, n = 290	(2) <50% MOM or DHM + liquid bovHMF, n = 260 (3) 0% MOM or DHM (exclusive PTF), n = 76	(1) ≥90% HM = 0 (0%) (2) ≥50% HM = 10 (3.4%) (3) <50% HM = 35 (13.5%) (4) 0% HM = 8 (10.5%) (1) vs. (4), p=0.005 (2) vs. (3), p<0.001
Prospective cohort	Coutsoudis 2011	(1) MOM and DHM if MOM unavailable (bovHMF not specified but likely used), n = 18	(2) MOM (likely added bovHMF) + PTF, n = 66 (3) DHM (likely added bovHMF) + PTF, n = 22	(1) MOM + DHM = 2 (11%); (2) MOM + PTF = 10 (15%); (3) DHM + PTF = 5 (23%)

Study design	Author, year	Intervention, n	Control, n	NEC events
Non-concurrent cohort	Kantorowska 2016	Post-DHM availability	Pre-DHM availability	Post-DHM era resulted in mean difference of 2.6% decrease in NEC rate (95% CI -3.9 to -1.5%, p = 0.0006))

Abbreviations: bovHMF=bovine milk-based human milk fortifier; CI=confidence interval; DHM=donor human milk; HM=human milk group; MOM=mother's own milk; OR=odds ratio; Prolacta=donor human milk-based human milk fortifier, Prolact+ $H^2MF^®$; PTF=preterm formula (bovine milk-based) group; RCT=randomized controlled trial

Table 4. Categories of primary outcomes

Primary outcomes[a]	**All studies (N=76), n (%)**	**Excluding case control studies (N=58), n (%)**
Nutritional intake or status (e.g. days of parenteral nutrition, time to full enteral feeds, exclusive or partial human milk feeding at discharge)	19 (25.0%)	18 (31.0%)
Mortality, morbidities or complications	15 (19.7%)	15 (25.9%)
NEC	15 (19.7%)	14 (24.1%)
Risk factors for NEC	15 (19.7%)	
Growth (e.g. weight gain, length, head circumference)	13 (17.1%)	13 (22.4%)
Length of stay	3 (3.9%)	3 (5.2%)
Biochemical outcome (serum folate, serum IGF-1[b])	2 (2.6%)	2 (3.4%)
Health care costs	2 (2.6%)	2 (3.4%)
Other		
Neurodevelopment	1 (1.3%)	1 (1.7%)
Metabolic acidosis	1 (1.3%)	1 (1.7%)
Fecal and breast milk analysis	1 (1.3%)	1 (1.7%)
Abdominal signs before NEC	1 (1.3%)	

[a] Some studies reported more than one primary outcome.

[b] IGF-1 = insulin-like growth factor-1.

Table 5. Characteristics of studies evaluating hydrolyzed fortifiers

	Kim 2015	Moya 2012	Thoene 2016	Cibulskis 2015
Methods	Randomized controlled trial	Randomized controlled trial	Non-concurrent cohort	Non-concurrent cohort
Participants				
N	129	146	69[a]	100
Mean birth weight, grams	1175 ± 201	1001 ± 163	Median[b]: Group 1 = 1305 Group 2 = 1481 Group 3 = 1340	1161 (standard deviation not reported)
Mean gestational age at birth, weeks	28.8 ± 1.6	27.8 ± 1.7	Median[b]: Group 1 = 29.15 Group 2 = 31.00 Group 3 = 29.60	28.8 (standard deviation not reported)
Exclusions	No enteral feeds before DOL 21, severe congenital anomalies, expected facility transfer, 5-minute Apgar <5, severe IVH, mechanical ventilation, major abdominal surgery, severe asphyxia, NEC at baseline	Congenital malformations affecting growth, 5-minute Apgar ≤4, major surgery requiring anesthesia, severe IVH, glucocorticoids for 3 consecutive days, >3 feedings of fortified breast milk , feeding intolerance to HM, fluid restriction <120 mL/kg/day, creatinine >2 mg/dL, probiotics received, >40% FiO2 via mechanical ventilation or CPAP at baseline	Congenital malformations affecting growth	Congenital anomalies affecting feeding
Multicentre	14 sites across the US	14 sites	No	No
Recruitment years	Not reported	October 2008 – July 2010	Group 1 = Oct 2009 – Jul 2011 Group 2 = Apr – Jul 2011 Group 3 = Aug 2012 – Jul 2014	Powdered intact-protein HMF: Apr - Jun 2010, Acidified liquid hydrolyzed HMF: Dec 2011 - Apr 2012

	Kim 2015	Moya 2012	Thoene 2016	Cibulskis 2015
Duration of intervention	The earlier of 29 study days or hospital discharge	The earlier of 28 study days, hospital discharge, or discontinuation of fortified breast milk feedings	Growth data collected until 36 weeks CGA, CO2 data collected until DOL 30; or birth until discharge	Powdered intact protein HMF: median 22.8 (IQR: 19.1-26.5) days; Acidified liquid hydrolyzed HMF: median 16 (IQR: 11.7-20.2) days, p=0.02
Intervention	MOM or DHM fortified with liquid HMF containing extensively hydrolyzed protein (n=66) versus powdered intact protein HMF (n=63)	MOM or DHM fortified with liquid HMF containing hydrolyzed whey protein (n=74) versus powdered intact protein HMF (n=72)	MOM or DHM fortified with acidified liquid HMF containing whey protein isolate hydrolysate (Group 2, n=23) versus powdered intact protein HMF (Group 1, n=46) versus non-acidified liquid HMF (Group 3, n=51)	MOM or DHM fortified with liquid HMF containing hydrolyzed whey protein (n=50) versus powdered intact protein HMF (n=50)
Outcomes				
Primary	Growth: weight, length, head circumference	Rate of weight gain	Growth: weight, length, head circumference, percentile rankings, weight gain	Metabolic acidosis and feeding intolerance
Secondary	Tolerance (stool characteristics, withheld feeds, *nil per os* periods), serum biochemistries (e.g. electrolytes and prealbumin), intake of HM and supplements, morbidities including NEC	Rate of length gain, achieved growth (weight, length, head circumference), ponderal index, enteral and parenteral intake	Nutrition outcome (e.g. days to start enteral feeds), morbidities including NEC, serum biochemistries (e.g. carbon dioxide minimum on DOL 14 and 30)	NEC, late-onset infection, death, length of stay, switched off of HMF

	Kim 2015	Moya 2012	Thoene 2016	Cibulskis 2015
Notes			Acidified liquid HMF contained higher in protein and iron than the powdered intact protein HMF	(1) Olive oil was added to twice as many infants in the intact-protein cohort (n=10) than the hydrolyzed cohort (n=5); (2) Protein modulator was added to n=12 in the intact-protein cohort and n=0 in the hydrolyzed cohort; (3) polycose powder was added to n=1 in the intact-protein and n=0 in the hydrolyzed cohort.

Abbreviations: CGA=corrected gestational age; CPAP=continuous positive airway pressure; DHM=donor human milk; DOL=day of life; FiO2=fraction of inspired supplemental oxygen; HM=human milk; HMF=human milk fortifier; IQR=interquartile range; IVH=intraventricular hemorrhage; MOM=mother's own milk.

[a]Thoene 2016 compared 3 treatments: powdered fortifier with intact-proteins (n=46), acidified liquid hydrolyzed fortifier (n=23), and non-acidified liquid hydrolyzed fortifier (n=51). Data from the third cohort was not included in the meta-analysis to maintain consistency with the randomized controlled trials.

[b]Interquartile range not reported.

Table 6. Newcastle-Ottawa quality assessment scale of the cohort studies

Domain	**Thoene 2016**	**Cibulskis 2015**
Selection		
1. Representativeness of exposed cohort	*	*
2. Selection of non-exposed cohort	*	*
3. Ascertainment of exposure	*	*
4. Outcome not present at start of study	*	*
Comparability		
1. Comparability of cohorts on the basis of design or analysis		
Outcome		
1. Assessment of outcome	*	*
2. Follow-up long enough for outcomes to occur	*	*
3. Adequacy of follow-up of cohorts	*	*
Total Stars	**7/8**	**7/8**

Table 7. GRADE evidence table – Hydrolyzed fortifiers compared to intact-protein fortifiers for the prevention of necrotizing enterocolitis (NEC)

Quality assessment							№ of patients		Effect		Quality	Import-ance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other**	**hydrolyzed fortifiers**	**intact-protein fortifiers**	**Relative (95% CI)**	**Absolute (95% CI)**		
NEC events - RCT (assessed with: Modified Bell's Staging Criteria, >/=stage 2)												
2	randomized trials	not serious[a]	not serious[b]	not serious	very serious[c]	none	5/140 (3.6%)	3/135 (2.2%)	**RR 1.44** (0.18 to 11.31)	**10 more per 1,000** (from 18 fewer to 229 more)	⊕⊕◯◯ LOW	CRITICAL
NEC events - Cohort (assessed with: Modified Bell's Staging Criteria, >/=stage 2)												
2	observational studies	not serious	not serious	not serious	very serious[d]	none	6/73 (8.2%)	1/96 (1.0%)	**RR 5.25** (0.89 to 30.87)	**44 more per 1,000** (from 1 fewer to 311 more)	⊕◯◯◯ VERY LOW	CRITICAL

CI: Confidence interval; **RR:** Risk ratio

Explanations

a. Moderately high drop-out rates (25-28%); however, drop-outs were balanced between groups. Risk of bias was not a serious quality concern.
b. Infants in Kim 2015 were born larger (birth weight) and later (gestational age) than Moya 2012. This may explain heterogeneity in the meta-analysis, but inconsistency was not downgraded because all included infants were very or extremely low birth weight and preterm, and the I-squared statistic was moderate (39%).
c. The optimal information size (~2000-4000 infants) was not met, given the low sample sizes and very few observed NEC events.
d. Optimal information size (~2000-4000 infants) was not reached.

Appendix 1. MEDLINE search strategy – July 11, 2016

Database: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present

1. Enterocolitis, Necrotizing/
2. exp Intestinal Diseases/
3. (gastro* or gut).jw.
4. gut.tw,kf.
5. intestin*.tw,kf.
6. necroti?ing enterocolitis.tw,kf.
7. NEC.tw,kf.
8. or/1-7
9. Enteral Nutrition/
10. Food, Formulated/
11. Infant Formula/
12. Infant Nutritional Physiological Phenomena/
13. Milk Banks/
14. Milk, Human/
15. Parenteral Nutrition/
16. artificial formula*.tw,kf.
17. ((bovine or breast or donor or human or maternal or mother*) adj3 milk).tw,kf.
18. ((enrich* or fortif*) adj3 (formula* or HM or milk)).tw,kf.
19. ((enteral or infant or parenteral) adj (feed* or nutrit*)).tw,kf.
20. (formula* adj (diet* or milk)).tw,kf.
21. ((hydrosyl* or hydroly* or intact) adj protein*).tw,kf.
22. ((pre term or preterm or prem* or term) adj2 (formula* or milk)).tw,kf.
23. or/9-22
24. Infant, Extremely Low Birth Weight/
25. Infant, Extremely Premature/
26. Infant, Low Birth Weight/
27. Infant, Newborn/
28. exp Infant, Newborn, Diseases/
29. Infant, Premature/
30. exp Infant, Premature, Diseases/
31. Infant, Small for Gestational Age/
32. Infant, Very Low Birth Weight/
33. Intensive Care Units, Neonatal/
34. Neonatology/
35. Perinatal Care/
36. Perinatology/
37. Postnatal Care/
38. Premature Birth/
39. ELBW*.tw,kf.
40. low birth weight*.tw,kf.
41. (neonat* or perinat* or postnat*).tw,kf,jw.
42. NICU*.tw,kf.
43. (post matur* or postmatur* or pre matur* or prematur* or pre term* or preterm*).tw,kf.
44. (small* adj2 gestational age).tw,kf.
45. VLBW*.tw,kf.

46. or/24-45
47. and/8,23,46
48. (comment or editorial or letter).pt.
49. 47 not 48
50. limit 49 to yr="1990-current"
51. limit 50 to english language
52. remove duplicates from 51

Appendix 2. List of included studies

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Appendix 3. Relevant abstracts not included in analysis

	Abstract Reference
1.	Cheah FC, Tiew WT, Raja Lope RJ, Ismail J. A randomized controlled trial comparing the effects of individualized and standardized fortification of expressed breast milk on the growth of preterm infants in the NICU. J Perinat Med. 2015. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/646/CN-01134646/frame.html.
2.	Maxwell A, Abraham A, Valdes-Greene R, Aboudi D, Brumberg H, LaGamma E, Parvez B. The impact of exclusive breastmilk diet on growth and NEC. Pediatric Academic Societies Abstract Archive. 2015. Available from: http://www.abstracts2view.com/pasall/view.php?nu=PAS15L1_400.
3.	Narogan M, Ryumina I, Grosheva E. Feeding of very preterm infants: the results application of modern standardized approaches in the practices. J Pediatr Gastroenterol Nutr. 2016;62(Suppl 1):859-860.
4.	Pauls J, Bauer K, Versmold H. Randomized controlled trial of formulas with hydrolyzed versus non-hydrolized protein for starting enteral feedings in preterm infants <1500g body weight. J Pediatr Gastroenterol Nutr. 1996; (4):450. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/594/CN-00227594/frame.html.
5.	Wicks JS, Esquerra-Zwiers AL, Rogers LM, Scala CM, Chen S, Silvestri JM, Patel AL. Reduced formula intake with donor milk (DM) impacts necrotizing enterocolitis (NEC) in very low birth weight (VLBW) infants. American Academy of Pediatrics National Conference. 2015 Oct. Available from: https://aap.confex.com/aap/2015/webprogrampress/Paper29414.html.

Appendix 4. Excluded full-text records with reasons for exclusion

	Excluded Reference	Reason for Exclusion
1.	Abdelhamid AE, Chuang S-L, Hayes P, Fell JME. In vitro cow's milk protein-specific inflammatory and regulatory cytokine responses in preterm infants with necrotizing enterocolitis and sepsis. Pediatr Res. 2011;69(2):165-9. doi: http://dx.doi.org/10.1203/PDR.0b013e31820263e7. PubMed PMID: 20975616.	Wrong intervention
2.	Abrams SA, Schanler RJ, Lee ML, Rechtman DJ. Greater mortality and morbidity in extremely preterm infants fed a diet containing cow milk protein products. Breastfeed Med. 2014;9(6):281-5. doi: http://dx.doi.org/10.1089/bfm.2014.0024. PubMed PMID: 24867268; PMCID: PMCPMC4074755.	Reanalyzed data from another study
3.	Akintorin SM, Kamat M, Pildes RS, Kling P, Hill J, Pyati S. A prospective study comparing feeding methods in VLBW infants. Pediatr Res. 1996; (4):190a. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/594/CN-00271594/frame.html.	Wrong intervention
4.	Amin HJ, Zamora SA, McMillan DD, Fick GH, Butzner JD, Parsons HG, Scott RB. Arginine supplementation prevents necrotizing enterocolitis in the premature infant. J Pediatr. 2002;140(4):425-31. PubMed PMID: 12006956.	Wrong intervention
5.	Andorsky DJ, Lund DP, Lillehei CW, Jaksic T, Dicanzio J, Richardson DS, Collier SB, Lo C, Duggan C. Nutritional and other postoperative management of neonates with short bowel syndrome correlates with clinical outcomes. J Pediatr. 2001;139(1):27-33. PubMed PMID: 11445790.	Wrong population
6.	Anonymous. Poster-Surgery. Journal of Gastroenterology and Hepatology (Australia). 2015;30:408-25. doi: http://dx.doi.org/10.1111/jgh.13190. PubMed PMID: 2015533770.	Wrong population
7.	Arco A, Gitto E, Sacco F, Barberi I, Mondello I, Nicolo A, D'Asero G, Lombardo F, Colombo A, Polizzi B, et al. Growth parameters in very-low-birth-weight infants feeding human milk fortified and formulas with different protein concentration Multicentric study. Journal of Pediatric Gastroenterology and Nutrition. 2002; (4):488. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/374/CN-00419374/frame.html.	Wrong outcomes
8.	Armand M, Hamosh M, Mehta NR, Angelus PA, Philpott JR, Henderson TR, Dwyer NK, Lairon D, Hamosh P. Effect of human milk or formula on gastric function and fat digestion in the premature infant. Pediatr Res. 1996; (3):[429-37 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/733/CN-00131733/frame.html.	Wrong population
9.	Aynsley-Green A, Lucas A, Lawson GR, Bloom SR. Gut hormones and regulatory peptides in relation to enteral feeding, gastroenteritis, and necrotizing enterocolitis in infancy. J Pediatr. 1990;117(1 Pt 2):S24-32. PubMed PMID: 2163441.	Wrong study design
10.	Bartick MC, Jegier BJ, Green BD, Reinhold AG, Schaefer AJ, Bogen DL, Schwarz EB, Stuebe AM, Jobe AH, Oh W, et al. Impact of optimized	Duplicate

	Excluded Reference	Reason for Exclusion
	breastfeeding on the costs of necrotizing enterocolitis in extremely low birthweight infants. J Pediatr. 2016;175:100-5.e2. doi: http://dx.doi.org/10.1016/j.jpeds.2016.03.040. PubMed PMID: 610178769.	
11.	Battersby CW, Santhakumaran S, Modi N. Feeding practices in babies born less than 33 weeks gestation: A population-based study using operational clinical data. J Neonatal Perinatal Med. 2013; (2):190-1. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/562/CN-01006562/frame.html.	Wrong outcomes
12.	Bellagamba MP, Carmenati E, D'Ascenzo R, Malatesta M, Spagnoli C, Biagetti C, Burattini I, Carnielli VP. One extra gram of protein to preterm infants from birth to 1800 g: a single-blinded randomized clinical trial. J Pediatr Gastroenterol Nutr. 2016;62(6):879-84. doi: http://dx.doi.org/10.1097/MPG.0000000000000989. PubMed PMID: 26418211.	Wrong intervention
13.	Berseth CL, Van Aerde JE, Gross S, Stolz SI, Harris CL, Hansen JW. Growth, efficacy, and safety of feeding an iron-fortified human milk fortifier. Pediatrics. 2004;114(6):e699-706. PubMed PMID: 15545616.	Wrong intervention
14.	Berseth CL, Walsh K, Moore N, Harris C, Mitmesser SH. A new liquid human milk fortifier improves linear growth in preterm infants. Journal of Pediatric Gastroenterology and Nutrition. 2011:[E13-e4 pp.]. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/152/CN-01035152/frame.html.	Wrong intervention
15.	Bhat BA, Gupta B. Effects of human milk fortification on morbidity factors in very low birth weight infants. Ann Saudi Med. 2001; (5-6):292-5.	Duplicate
16.	Bhat BA, Gupta B. Effects of human milk fortification on morbidity factors in very low birth weight infants. Ann Saudi Med. 2003;23(1-2):28-31. PubMed PMID: 17146218.	Duplicate
17.	Bigger HR, Fogg LJ, Patel A, Johnson T, Engstrom JL, Meier PP. Quality indicators for human milk use in very low-birthweight infants: are we measuring what we should be measuring? J Perinatol. 2014;34(4):287-91. Doi: http://dx.doi.org/10.1038/jp.2014.5. PubMed PMID: 24526005.	Wrong outcomes
18.	Bilenko N, Ghosh R, Levy A, Deckelbaum RJ, Fraser D. Partial breastfeeding protects Bedouin infants from infection and morbidity: prospective cohort study. Asia Pac J Clin Nutr. 2008;17(2):243-9. PubMed PMID: 18586643.	Wrong outcomes
19.	Boehm G, Senger H, Friedrich M, Muller DM, Beyreiss K. Protein supplementation of human milk for the nutrition of VLBW-infants: human milk protein vs. meat protein hydrolysate. Klin Padiatr. 1990;202(5):316-20. PubMed PMID: 2214590.	Wrong outcomes
20.	Boyd CA, Quigley MA, Brocklehurst P. Donor breast milk versus infant formula for preterm infants: systematic review and meta-analysis Archives of disease in childhood Fetal and neonatal edition. 2007; (3):f169-f75.	Systematic review
21.	Brown JVE, Embleton ND, Harding JE, McGuire W. Multi-nutrient fortification of human milk for preterm infants. Cochrane Database Syst Rev. 2016(5):CD000343. doi: http://dx.doi.org/10.1002/14651858.CD000343.pub3. PubMed PMID: 27155888.	Systematic review

	Excluded Reference	Reason for Exclusion
22.	Brown Jennifer VE, Embleton Nicholas D, Harding Jane E, McGuire W. Multi-nutrient fortification of human milk for preterm infants. Cochrane Database Syst Rev. 2016; (5). Available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD000343.pub3/abstract.	Duplicate
23.	Brunton JA, Saigal S, Atkinson SA. Growth and body composition in infants with bronchopulmonary dysplasia up to 3 months corrected age: a randomized trial of a high-energy nutrient-enriched formula fed after hospital discharge. J Pediatr. 1998;133(3):340-5. PubMed PMID: 9738713.	Wrong intervention
24.	Cacho NT, Parker LA, Neu J. Necrotizing enterocolitis and human milk feeding: a systematic review. Clinics in Perinatology. 2017;44(1):49-67. doi: https://dx.doi.org/10.1016/j.clp.2016.11.009. PubMed PMID: 28159209.	Systematic review
25.	Carroll K, Herrmann KR. The cost of using donor human milk in the NICU to achieve exclusively human milk feeding through 32 weeks postmenstrual age. Breastfeed Med. 2013;8(3):286-90. doi: http://dx.doi.org/10.1089/bfm.2012.0068. PubMed PMID: 23323965; PubMed Central PMCID: PMCPMC3663453.	Wrong outcomes
26.	Catassi C, Bonucci A, Coppa GV, Carlucci A, Giorgi PL. Intestinal permeability changes during the first month: effect of natural versus artificial feeding. J Pediatr Gastroenterol Nutr. 1995;21(4):383-6. PubMed PMID: 8583288.	Wrong outcomes
27.	Chierici R, Sawatzki G, Thurl S, Tovar K, Vigi V. Experimental milk formulae with reduced protein content and desialylated milk proteins: influence on the faecal flora and the growth of term newborn infants. Acta Paediatr. 1997;86(6):557-63. PubMed PMID: 9202787.	Wrong outcomes
28.	Civardi E, Garofoli F, Longo S, Mongini ME, Grenci B, Mazzucchelli I, Angelini M, Castellazzi A, Fasano F, Grinzato A, et al. Safety, growth, and support to healthy gut microbiota by an infant formula enriched with functional compounds. Clin Nutr. 2015;27:27. doi: http://dx.doi.org/10.1016/j.clnu.2015.11.006. PubMed PMID: 26718667.	Wrong intervention
29.	Collaborative Group for the Multicenter Study on Human Milk Fortification. [Multicenter study on the effects of human milk fortification in premature infants]. Chinese J Pediatr. 2012 May;50(5):336-342. PUBMED 22883034; EMBASE 22883034	Non-English
30.	Cordova J, Sriram S, Patton T, Jericho H, Gokhale R, Weinstein D, Sentongo T. Manifestations of Cow's-Milk Protein Intolerance in Preterm Infants. J Pediatr Gastroenterol Nutr. 2016;62(1):140-4. doi: http://dx.doi.org/10.1097/MPG.0000000000000933. PubMed PMID: 26252918.	Wrong intervention
31.	Corpeleijn WE, de Waard M, Christmann V, van Goudoever JB, Jansen-van der Weide MC, Kooi EMW, Koper JF, Kouwenhoven SMP, Lafeber HN, Mank E, et al. Effect of donor milk on severe infections and mortality in very low-birth-weight infants. JAMA Pediatr. 2016;170(7):654-61. doi: 10.1001/jamapediatrics.2016.0183. PubMed PMID: 116677891.	Duplicate
32.	Cossey V, Vanhole C, Verhaegen J, Schuermans A. Intestinal colonization patterns of staphylococci in preterm infants in relation to type of enteral feeding and bacteremia. Breastfeed Med. 2014;9(2):79-85. doi: http://dx.doi.org/10.1089/bfm.2012.0116. PubMed PMID: 23786310.	Wrong outcomes

	Excluded Reference	Reason for Exclusion
33.	Dempsey E, Miletin J. Banked preterm versus banked term human milk to promote growth and development in very low birth weight infants. Cochrane Database Syst Rev. 2009;(1) (CD007644). doi: http://dx.doi.org/10.1002/14651858.CD007644. PubMed PMID: 2009485684.	Systematic review
34.	De Nisi G, Berti M, De Nisi M, Bertino E. Early enteral feeding with human milk for VLBW infants. J Biol Regul Homeost Agents. 2012;26(3 Suppl):69-73. PubMed PMID: 23158518.	Wrong study design
35.	De Schepper J, Cools F, Vandenplas Y, Louis O. Whole body bone mineral content is similar at discharge from the hospital in premature infants receiving fortified breast milk or preterm formula. J Pediatr Gastroenterol Nutr. 2005;41(2):230-4. PubMed PMID: 16056105.	Wrong outcomes
36.	Diamanti A. Enteral formulas in children: which is the best choice? Nutritional Therapy & Metabolism. 2010;28(1):40-5 6p. PubMed PMID: 105045600.	Wrong study design
37.	Di Lorenzo C, St James-Roberts I. Summary and conclusions. Journal of Pediatric Gastroenterology and Nutrition. 2013;57(SUPPL.1):S42-S5. doi: http://dx.doi.org/10.1097/01.mpg.0000441935.99845.c4. PubMed PMID: 2014018766.	Wrong study design
38.	Downard CD, Renaud E, St Peter SD, Abdullah F, Islam S, Saito JM, Blakely ML, Huang EY, Arca MJ, Cassidy L, et al. Treatment of necrotizing enterocolitis: an American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review. J Pediatr Surg. 2012;47(11):2111-22. doi: http://dx.doi.org/10.1016/j.jpedsurg.2012.08.011. PubMed PMID: 23164007.	Systematic review
39.	Ecevit A, Abbasoglu A, Tugcu U, Silahli M, Laleli Y, Tarcan A. Neonatal outcomes of very low birth weight infants who received enteral nutrition with and without olive oil support: Randomised controlled pilot study. Arch Dis Child. 2014:A444. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/438/CN-01023438/frame.html.	Wrong intervention
40.	Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL, Katsikiotis V, Tyson JE, Oh W, Shankaran S, et al. Longitudinal growth of hospitalized very low birth weight infants. Pediatrics. 1999;104(2 Pt 1):280-9. PubMed PMID: 10429008.	Wrong intervention
41.	el-Kholy MS, Halim HY, Marzouk AH. Beta-glucuronidase and hyperbilirubinemia in breast-fed versus formula-fed babies. J Egypt Public Health Assoc. 1992;67(3-4):237-48. PubMed PMID: 1296961.	Wrong outcomes
42.	Fairey AK, Butte NF, Mehta N, Thotathuchery M, Schanler RJ, Heird WC. Nutrient accretion in preterm infants fed formula with different protein:energy ratios. J Pediatr Gastroenterol Nutr. 1997;25(1):37-45. PubMed PMID: 9226525.	Wrong intervention
43.	Favre A, Szylit O, Popot F, Catala I, Rondeau C, Maurage C, Gold F, Borderon JC, Butel MJ. Diet, length of gestation, and fecal short chain fatty acids in healthy premature neonates. J Parenter Enteral Nutr. 2002;26(1):51-6. PubMed PMID: 11833751.	Wrong outcomes
44.	Fenton TR, Premji SS, Al-Wassia H, Sauve RS. Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane Database	Systematic review

	Excluded Reference	Reason for Exclusion
	Syst Rev. 2014;4:CD003959. doi: http://dx.doi.org/10.1002/14651858.CD003959.pub3. PubMed PMID: 24752987.	
45.	Fenton Tanis R, Premji Shahirose S, Al-Wassia H, Sauve Reg S. Higher versus lower protein intake in formula-fed low birth weight infants. Cochrane Database Syst Rev. 2014; (4). Available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD003959.pub3/abstract.	Duplicate
46.	Fewtrell MS, Morley R, Abbott RA, Singhal A, Isaacs EB, Stephenson T, MacFadyen U, Lucas A. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. Pediatrics. 2002;110(1 Pt 1):73-82. PubMed PMID: 12093949.	Wrong intervention
47.	Fomon SJ, Ziegler EE, Nelson SE, Rogers RR, Frantz JA. Infant formula with protein-energy ratio of 1.7 g/100 kcal is adequate but may not be safe. J Pediatr Gastroenterol Nutr. 1999;28(5):495-501. PubMed PMID: 10328124.	Wrong outcomes
48.	Freeman V, van't Hof M, Haschke F. Patterns of milk and food intake in infants from birth to age 36 months: the Euro-growth study. J Pediatr Gastroenterol Nutr. 2000;31 Suppl 1:S76-85. PubMed PMID: 10896092.	Wrong outcomes
49.	Ganapathy V, Hay JW, Kim J. Analysis of necrotizing enterocolitis costs among extremely preterm infants FED exclusively human-milk based diet vs. human-milk fortified with bovine-milk based supplements. Value in health. 2010; (3):A183-4. Available from: http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/698/CN-01008698/frame.html.	Reanalyzed data from another study
50.	Ganapathy V, Hay JW, Kim JH. Costs of necrotizing enterocolitis and cost-effectiveness of exclusively human milk-based products in feeding extremely premature infants. Breastfeed Med. 2012;7(1):29-37. doi: http://dx.doi.org/10.1089/bfm.2011.0002. PubMed PMID: 21718117.	Reanalyzed data from another study
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146.	Unknown. ISRHML abstracts: Breastfeeding and the nutrition transition. 2010;26(4):419-445. doi: 10.1177/0890334410386918	Wrong intervention
147.	Unknown. Online articles. Pediatr. 2015 May;135(5):932-944.	Wrong intervention
148.	Unknown. Pediatr electronic pages. 2005;116(6):1529-1541.	Wrong intervention
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151.	Vandenplas Y, Alarcon P, Fleischer D, Hernell O, Kolacek S, Laignelet H, Lonnerdal B, Raman R, Rigo J, Salvatore S, et al. Should partial hydrolysates be used as starter infant formula? A working group consensus. J Pediatr Gastroenterol Nutr. 2016;62(1):22-35. doi: http://dx.doi.org/10.1097/MPG.0000000000001014. PubMed PMID:	Wrong study design

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152.	Wahlen E, Strandvik B. Effects of different formula feeds on the developmental pattern of urinary bile acid excretion in infants. J Pediatr Gastroenterol Nutr. 1994;18(1):9-19. PubMed PMID: 8126625.	Wrong outcomes
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Chapter 4

Shulhan J, Larsen BMK, Kumar M, Jones CA, and Hartling L. Parent prioritization of infant health and nutrition outcomes in the neonatal intensive care unit.

Abstract

Background: Patient and community engagement is an important step for prioritizing outcomes in health research. There is limited literature on parent priorities for infant health and nutrition outcomes for research in neonatal intensive care units (NICUs), especially with respect to critical diseases like necrotizing enterocolitis (NEC).

Objectives: (1) To determine parent priorities of infant health and nutrition outcomes, including NEC outcomes, for quantitative research in the NICU, and (2) to compare parent priorities with outcomes commonly reported in the NEC and enteral nutrition literature.

Methods: Parent participants with at least 6 weeks of experience in the NICU completed an online survey about the relative importance of health and nutrition outcomes (No NEC group). Additional NEC-specific outcomes were presented to parent participants whose child had NEC (NEC group). Participants identified, rated and ranked the outcomes important to them. Data were analyzed descriptively and using summary statistics. Outcomes identified as most important to participants were compared to commonly reported primary outcomes in the NEC and enteral nutrition literature, as described in a recent scoping review.

Results: Fourteen participants in the No NEC group and one participant in the NEC group completed the survey. Infant death was ranked as the most important outcome by the No NEC and NEC groups. Adverse events (e.g. bloody stools, trouble breathing), gut problems (e.g. feeding intolerance, vomiting), and quality of life were ranked as next most important by the No NEC group. Development, severity and type of NEC were all rated by one participant (NEC group) as very important and ranked second, third and fourth overall. Participants' priorities for mortality and NEC aligned with the relatively frequent use of these outcomes in the literature. Outcomes related to the timing, type or duration of specific feeds, e.g. days on parenteral

nutrition, were the most commonly used primary outcomes in the literature but ranked as lower-priority outcomes by participants.

Conclusions: Participants' priority of mortality as a research outcome was also frequently used as a primary outcome in the existing quantitative literature. However, participant prioritization of nutrition-related outcomes like duration of parenteral nutrition, and outcomes involving parents like quality of life did not align with the frequency of those outcomes reported in the literature. NEC-specific outcomes were identified as very important to one participant whose child had NEC, but more feedback is required to determine if these outcomes are priorities to parents. Collaborating with parents on outcome prioritization or study development for future research in neonatal nutrition and NEC is recommended to ensure that results are relevant to families.

Introduction

Parent engagement in the development of health and social services and research in the neonatal intensive care unit (NICU) is being recognized as an important driver for high-quality care. Several studies have sought to improve the delivery of health services in the NICU by understanding sources of parental stress (1, 2), perceptions of safety (3) and satisfaction with care (4). Higher levels of parent participation in research have also been used to design and implement family-centered programs (5) or health services (6). However, parent engagement in the planning and development of NICU quantitative studies, especially clinical trials to test new products or procedures, is not a mainstream approach.

Parent engagement may occur at any point during the research process, including input on study design features (e.g., the development of study eligibility criteria); raising awareness for study participation; peer-reviewing protocols, funding applications and manuscripts; and developing or coordinating knowledge translation initiatives (7-10). Outcome prioritization is another important step in the research process where parents may contribute valuable insights. Several agencies have provided a framework for or described public participation in priority setting and outcome definition (7, 8, 11-13). Engaging parents in outcome prioritization adds value to research by ensuring transparency and accountability to end users with respect to outcomes reported, and relevance to families (14).

There are two prominent organizations involved in outcome definition and prioritization: the James Lind Alliance (JLA) and Core Outcome Measures in Effectiveness Trials (COMET) Initiative. Both agencies have identified necrotizing enterocolitis (NEC), a serious inflammatory disease of the gut, as a priority for neonatal and preterm infant research. Complications from NEC may lead to surgery, death or long-term neurodevelopmental impairments (15); however, due to the complexity of the disease, there have been minimal advancements regarding

effective prevention and treatment practices (15). Of the “Top 10 uncertainties” list developed by the JLA Preterm Birth Priority Setting Partnership, the third highest priority was research to determine “which interventions are most effective to prevent necrotising enterocolitis in premature babies” (16). Similarly, the COMET Initiative identified a need to establish an “evidence-based case-definition” of NEC in order to include this definition in a core outcomes set (a list of outcomes that should be reported in all trials on a specific topic) (17). Clearly, there is a need for patient-oriented research on NEC, and one of the key steps involved in addressing this gap is outcome prioritization.

To understand the breadth of literature on enteral nutrition and NEC, we recently completed a scoping review on this topic (Chapter 3 of this thesis). The scoping review included 76 quantitative studies comparing different enteral diets that reported on NEC events. In addition to NEC outcomes, we extracted primary outcomes from each included study. Primary outcomes were organized into categories and tallied. This information was used to assess the type of primary outcomes typically used for sample size calculations and reporting. We were interested in the similarities and differences between parent priorities for research outcomes and outcomes commonly reported in this body of literature.

Therefore, the goals of this study were to (1) understand which research outcomes for infants in the NICU are most important to parents, and (2) determine if there are discrepancies between parent priorities and primary outcomes in the existing literature. The findings may be used to guide future clinical trials on NEC and preterm nutrition to generate evidence most relevant to parents.

Objectives

The objectives of this cross-sectional survey were to:

1. determine parent priorities of infant health and nutrition outcomes, including NEC outcomes, for quantitative research in the NICU; and
2. compare parent priorities with outcomes commonly reported in the literature reporting on clinical research relevant to NEC and enteral nutrition.

Methods

Based on the Canadian Task Force on Preventive Health Care patient preferences protocol (18), 20 participants were needed to complete an outcome prioritization survey: 10 parents of children who were diagnosed with NEC Stage ≥2 during the NICU stay (NEC group), and 10 parents of children who did not develop NEC (No NEC group). Eligible participants were parents or caregivers who had at least 6 weeks of experience in a NICU immediately after the birth of their child in the past 10 years. A minimum NICU stay of 6 weeks was agreed upon by the study investigators to obtain insight from parents who have likely experienced challenges or setbacks with feeding due to prematurity, disease states or clinical status. To ensure consistency with current clinical practices and nutrition formulary, participation was restricted to parents with NICU experience in the past 10 years. Finally, this study targeted nutrition and health outcomes during the neonatal period, including the first enteral (through the gut) feedings, so only families admitted or transferred to a NICU immediately after birth were included.

A survey was developed in consultation with clinical experts, and pilot tested with 3 non-participating parents and 5 health sciences researchers. There were 5 sections of the survey. Participants were asked to: (1) indicate if their child was diagnosed with NEC during the NICU admission, (2) review a list of potential research outcomes (based on a preliminary review of the data collected in Chapter 3 of this thesis and pilot testing feedback) and identify outcomes important to them which were not already on the list, (3) use a 10-point Likert scale to rate all outcomes, (4) rank all outcomes from most (#1) to least (#15 for No NEC group; #18 for NEC

group; or more if parent added outcomes to the pre-defined list) important, and (5) complete 11 demographic questions.

There were 2 separate pathways of the survey: one pathway listed 15 general clinical and nutrition outcomes for the No NEC group; and the other pathway included 3 additional outcomes specific to NEC for the NEC group. NEC-related outcomes were considered irrelevant to participants with children who did not develop NEC. These parents may not have been familiar with the disease, and did not have experience dealing with the disease treatment or consequences. As such, the first question of the survey routed participants to the appropriate pathway by asking if they have a child who was diagnosed with NEC.

See **Appendix 1** for the complete survey tool. The online survey was hosted by Nooro, a secure data management platform (19). The survey was open from February 8, 2016 until March 31, 2017.

Recruitment occurred via Facebook and e-mail invitations. Notifications of the study were posted on private Facebook groups for parents of preterm infants. These groups were created by the Canadian Premature Babies Foundation or individual parents, and included members residing within the local Edmonton area or across Canada. Interested parents were asked to contact the first author (JS) for screening and enrolment. Eligible parents were e-mailed a unique link to the online survey. Parents who did not complete the survey were sent one reminder email, but were not asked to provide a reason for withdrawal to maintain privacy.

Ethics approval was received from the University of Alberta Research Ethics Board – Health Panel (No. 00068447). De-identified data was summarized descriptively and analyzed using mean and median values in Microsoft Excel (Microsoft, Redmond, WA). NEC-specific outcomes (development of NEC, severity and type of NEC) presented to the NEC group were reported separately from the No NEC group. Weighted means were used when ratings and rankings from

the NEC and No NEC groups were combined. Outcomes rated and ranked as highly important to parents were compared to commonly reported primary outcomes in the NEC and enteral nutrition literature, as described in Chapter 3 of this thesis. In addition to analyzing individually rated and ranked outcomes, 6 categories of the pre-specified outcomes were created by the first author (JS) and verified by the co-authors to analyze general areas or themes. The categories were:

1. NEC (only applicable to NEC survey questions) – development of NEC, NEC type and NEC severity;
2. Medical outcomes: infant deaths, gut problems (e.g. feeding intolerance, colitis, bloody stools, ileus, pneumoperitoneum, intestinal perforation, pneumatosis intestinalis), days on a breathing tube, and adverse events (e.g. bloody stools, trouble breathing, blood infection);
3. Hospital stay: days in the neonatal or pediatric intensive care unit, and total hospital length of stay (in NICU/PICU and other inpatient wards);
4. Nutrition outcomes: Number of days food is given into the blood (parenteral nutrition or TPN), number of days until first feeds are given by mouth or feeding tube, number of days to reach full feeds by mouth or feeding tube, and growth (e.g. weight, length, head circumference, rate of weight gain);
5. Health care system: health care costs (including doctors' feeds, inpatient and outpatient costs, formula costs, etc.), and satisfaction with care;
6. Outcomes involving parents: quality of life, daily function, and bonding or parent's relationship with infant.

Mean ratings and rankings for the outcome categories were determined by calculating the weighted average of the outcomes in each category from the No NEC and NEC group. For example, the mean rating for the category "hospital stay" was the weighted mean of the ratings

for the “NICU/PICU length and stay” and “total hospital stay” outcomes from the No NEC and NEC groups.

Results

Sixteen participants were enrolled in the study and 15 participants (14 females, 1 male) completed the survey. Only one participant reported having a child who was diagnosed with NEC (NEC group, n=1; No NEC group, n=14). Demographic characteristics are shown in **Table 1**. The majority of participants were 30-34 years-old (n=10), resided in Ontario (n=7) or Alberta (n=6), were married (n=15), achieved a high level of education (college certificate/diploma, n=5; university or professional degree, n=10), earned over $100,000 per year and did not identify with an ethnic minority (n=15). Participants’ children, who had been admitted to a NICU for at least 6 weeks, were on average 28.4 ± 17.1 months old (chronological age; range: 4 – 65 months) at the time of the survey, born at 27.9 ± 2.5 weeks gestation and stayed in the NICU for 11.9 ± 3.3 weeks.

Eleven of 15 clinical- and nutrition-related outcomes were rated at least 8/10 for importance (**Figure 1**). Only days to full enteral feeds, days to first enteral feeds, days on parenteral nutrition, and health care costs were rated less than 8/10. Infant death was rated 10/10 and ranked as the most important outcome (**Figures 1** and **2**). Adverse events, gut problems (e.g. feeding intolerance, vomiting, bloody stools, constipation), quality of life and days of ventilation were ranked as next most important. The lowest rated outcome was health care costs. Development, severity and type of NEC were all rated 10/10 and ranked second, third and fourth overall by one parent (NEC group).

Individual outcomes were also grouped into 6 categories. **Table 2** outlines the groupings and mean ratings and rankings for each category. NEC-specific outcomes were rated and ranked as highly important by the participant (n=1) in the NEC group. The No NEC participants rated and

ranked medical outcomes (i.e. infant deaths, adverse events, gut problems and days on a breathing tube) and outcomes involving parents (i.e. quality of life, parent-child bonding, daily function) as being the most important type of outcome categories, followed by length of stay and nutrition-related outcomes. The health care system outcomes, including satisfaction with care and health care costs, were rated and ranked as the lowest priority for participants, that is, least important.

Three participants in the No NEC group identified outcomes important to them that were not on the predefined list. There was no overlap between the added outcomes, so the ratings and rankings of added outcomes were based on one participant's response, as follows:

1. Transition to exclusive breastfeeding, rated 9/10, ranked 13th out of 17 outcomes;
2. Nipple confusion, rated 7/10, ranked 15th out of 17 outcomes;
3. Prevention of oral aversions, rated 7/10, ranked 8th out of 17 outcomes;
4. Early diagnosis of severe or persistent reflux, rated 10/10, ranked 4th out of 17 outcomes;
5. Healthy and appropriate weight gain in the NICU and after being discharged home, rated 10/10 and ranked 1st out of 18 outcomes;
6. Developmental progress in relation to weight gain (e.g. insufficient weight gain causing possible developmental delays), rated 10/10 and ranked 7th out of 18 outcomes; and
7. Parental stress regarding pumping and producing milk, rated 10/10, ranked 8th out of 18 outcomes.

The frequency of primary outcomes reported in the literature on NEC and enteral nutrition, as described in a recent scoping review, was compared to participants' ratings and rankings (**Table 3**). Mortality and NEC were indicated as very important outcomes to participants and were relatively common primary outcomes in the existing research (19.7% of 76 studies, each).

Another similarity involved health care cost, which was infrequently used as a primary outcome (2.6% of 76 studies) and ranked the least important outcome by participants. The main discrepancy between participant priorities and the literature involved nutrition-related outcomes (not including growth). This category was the most common type of primary outcome in the literature (25.0% of 76 studies); however, participants ranked days of parenteral nutrition, time to first enteral feeds and time to full enteral feeds as low priorities (#10, #12 and #13 out of 15 outcomes, respectively). Lastly, only 2 of the 76 studies in the scoping review reported on outcomes involving parents like skin-to-skin contact (secondary outcome), which relates to parent-child bonding. In the survey, parents rated bonding as 9.20/10 for importance (ranking #8).

Discussion

Participants rated the majority of outcomes at least 8/10 for importance, indicating that parents viewed most of the listed outcomes as important. Not surprisingly, infant death was ranked as the most important outcome. Participants also considered adverse events, gut problems (e.g. constipation) and quality of life to be priority outcomes. The occurrence, type and severity of NEC were assessed by one participant (whose infant had NEC) and scored as high-priority outcomes, immediately after infant deaths. Feedback from more parents with NEC experience is needed to determine the importance of NEC-related outcomes to parents.

Health care cost was the least important outcome to participants, but this finding may be influenced by the publically-funded health care system in Canada. Parents who are required to pay for health services and products in the NICU may prioritize health care costs differently. Researchers, hospital administrators and other stakeholders tied to health care financing may also consider cost a higher priority, so balancing priorities across all stakeholder groups is important.

We have recently completed a scoping review to map the literature regarding different types of enteral diets and NEC. The scoping review included 76 quantitative studies and recorded the frequency of primary outcome categories used by the included studies. Some primary outcomes, like mortality and NEC, were highly-rated and ranked by participants in this survey. However, there were some discrepancies between the primary outcomes in the literature and outcomes prioritized by the survey participants. Outcomes related to the timing, type or duration of specific feeds, such as days of parenteral nutrition, were the most commonly reported primary outcome in the scoping review. The surveyed participants, on the other hand, ranked this type of outcome as low priority.

Another notable finding was that only 2 of the 76 studies (20, 21) in the scoping review reported outcomes involving parents, such as episodes and duration of skin-to-skin contact. Skin-to-skin contact is linked to parent-child bonding, which was a highly-rated outcome in this study. Based on the outcomes that participants added to the predefined list in the survey, practical outcomes involving parents like nipple confusion and transitioning to exclusive breastfeeding are also important to parents. Therefore, outcomes involving parents have been infrequently reported in the quantitative NEC literature, but these may be outcomes valued by families and should be considered when designing future research.

There has been minimal research done with patient/parent engagement in critical care settings, particularly in the NICU. Menzies et al. (22) completed a narrative review on patient and parent involvement in pediatric intensive care research. The authors found 4 studies on this topic. The included studies used interviews and focus groups to measure perspectives on the importance or relevance of a proposed study, understand beliefs and attitudes toward clinical trials, and develop study protocols or materials. Overall, the review noted that patient and public involvement in pediatric intensive care research is beneficial but the impact of this engagement was unknown. In a similar way, our study highlighted the merits of involving parents in NICU

research; namely, recognizing that priorities of researchers and parents do not always align. Parents may contribute valuable insights to NICU research and should have a voice in determining study outcomes. More work on appropriate methods of effective parent participation and the impact of this engagement would help to bridge this gap. Additional work integrating outcomes identified as priorities by multiple stakeholders (e.g., parents, clinicians, administrators, other decision-makers) also needs to be considered. This is particularly important when stakeholders' priorities are divergent, to ensure all perspectives are respected whilst ensuring that clinical research is conducted efficiently to reach its goal of evaluating the efficacy and effectiveness of different clinical interventions.

Limitations

A limitation of this study was the sample size, particularly for the NEC group that only included one participant. We did not achieve our goal of 20 participants, possibly because the target population was highly specific and our recruitment strategy relied on private social media groups. The demographics of this study sample were also skewed toward Caucasian, married females with a high socioeconomic status. This sample is not representative of all families admitted to the NICU for at least 6 weeks. We recognize that patient/parent-oriented research should, wherever possible, include input from a diverse group of individuals; nonetheless, the data provides a useful starting point for future research regarding preterm nutrition and NEC.

The close-ended questions and cross-sectional nature of this survey precluded parents from justifying their decisions or negotiating priorities in consideration of other stakeholders' perspectives. We note that one parent identified "healthy and appropriate weight gain in the NICU and after being discharged home" as a separate outcome (rated 10/10 and ranked 1st out of 18 outcomes). We would consider this outcome to be related to the pre-specified outcome, "growth", which the same participant rated as 10/10 but ranked as 4th out of 18 outcomes. This

minor discrepancy was not considered to have appreciably affected the overall results, but it does suggest that accompanying the survey with stakeholder discussions may be useful. Higher levels of parent engagement in outcome prioritization, such as focus groups and parent advisory committees, would mitigate this concern.

Lastly, we compared parent priorities with primary outcomes from the quantitative literature on enteral nutrition and NEC, but did not delve into secondary outcomes or qualitative data. Some of the outcomes important to parents may have been addressed by the literature but not comprehensively accounted for here.

Conclusions

Outcome prioritization is an important preliminary step in study development, but it is not the only research opportunity for parents to engage in. Engagement can be integrated into all phases of the research, but may be more effective if done early. In order to produce research that is most relevant to NICU families, researchers could collaborate with veteran NICU parents at multiple levels, such as writing funding applications, formulating the research question, selecting an appropriate study design, prioritizing outcomes and methods of measurement, peer-review and knowledge translation. Collaboration may be achieved through decision-making with parent advisory committees or discussions with focus groups.

We acknowledge that there may be challenges associated with this approach. For instance, a considerable amount of time and resources may be involved in establishing advisory committees. Critical care settings may also compound parent engagement challenges due to the rapid turnover of medical knowledge or practices, necessity for highly-controlled clinical trials, and a risk of inducing stress and psychological trauma. Despite these barriers, parent engagement in the development of NICU studies is feasible. Evaluation and impact of engagement strategies are still needed, but the anticipated benefits, including low attrition rates

(9, 10), increased protocol compliance (7), and enhanced relevance and transparency of the findings (14, 22), may lead to more robust evidence.

In conclusion, this study is unique in terms of its focus on parent priorities for research outcomes in the NICU. Mortality was an outcome prioritized by parents and was also commonly used as a primary outcome in the quantitative literature on NEC and enteral nutrition. However, participant prioritization of nutrition-related outcomes like duration of parenteral nutrition, and outcomes involving parents like quality of life did not align with the frequency of those outcomes in the literature. One participant whose child had NEC indicated that the development, severity and type of NEC were very important outcomes. More feedback is needed to determine if NEC-specific outcomes are priorities to parents. Overall, collaborating with parents on outcome prioritization or study development is recommended to ensure that family questions and concerns are brought forward in future research.

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Table 1. Demographic characteristics of the 15 participants who completed the survey

Characteristic	**n (%)**
Female	14 (93.3%)
Age category (years-old)	
25-29	3 (20.0%)
30-34	10 (66.7%)
35-39	1 (6.7%)
40-44	1 (6.7%)
Married	15 (100.0%)
Residing province	
Alberta	6 (40.0%)
British Columbia	1 (6.7%)
Manitoba	1 (6.7%)
Ontario	7 (46.7%)
Highest level of education achieved	
College certificate or diploma	5 (33.3%)
University undergraduate or professional degree	10 (66.7%)
Annual household income ($)	
25,000-49,000	2 (13.3%)
50,000-74,000	1 (6.7%)
75,000-99,000	1 (6.7%)
≥100,000	9 (60.0%)
Prefer not to answer	2 (13.3%)
Ethnic minority	
Yes	0 (0.0%)
No	15 (100.0%)

Table 2. Mean ratings and rankings by outcome category, N=15 participants

Outcome category	**Mean rating out of 10**[a]	**Mean Ranking**[b] (category ranking #)[c]
NEC Development of NEC NEC severity NEC type	10 (NEC group only[d])	2.00 (#1 – NEC group only[c])
Medical Infant deaths Gut problems Adverse events Days on a breathing tube	9.43	3.82 (#1)
Outcomes involving parents Daily function Quality of life Parent-child bonding	9.42	6.53 (#2)
Nutrition Growth Days to full enteral feeds Days to first enteral feeds Days on parenteral nutrition	8.47	8.90 (#3)
Length of stay Days in the NICU/PICU Total hospital length of stay	7.72	10.07 (#4)
Health care system Satisfaction with care Health care costs	7.40	10.40 (#5)

[a] Participants rated individual outcomes from 10 (most important) to 1 (least important) and the mean rating of outcomes in each category was presented.
[b] Participants ranked individual outcomes from most important (#1) to least important (#15 – No NEC group; #18 – NEC group) and the mean ranking of outcomes in each category was presented.
[c] Category rankings were from most (#1) to least (#5) important.
[d] NEC group, n=1. Only the NEC group rated and ranked the NEC-specific outcomes: development of NEC, NEC severity and NEC type. Means of other outcome categories were based on responses from all 15 participants.
Abbreviations: PICU=pediatric intensive care unit; NEC=necrotizing enterocolitis; NICU=neonatal intensive care unit.

Table 3. Comparison of primary outcomes reported in the NEC and enteral nutrition literature, and parent priorities from a survey of 15 participants

Primary outcomes identified by scoping review[a]	**Number and proportion of studies in scoping review with each type of primary outcome, n (%)**	**Mean parent rating out of 10**	**Parent ranking (from #1 to #15)**[b]
Nutritional intake or status (e.g. days of parenteral nutrition, time to full enteral feeds, exclusive or partial human milk feeding at discharge)	19 (25.0%)	7.72	Days on PN - #10, Days to first EN feeds - #12, Days to full EN feeds - #13
Mortality, morbidities or complications	15 (19.7%)	Infant deaths - 10	Infant deaths - #1
NEC	15 (19.7%)	10[c]	#1[c]
Risk factors for NEC	15 (19.7%)	Not measured	Not measured
Growth (e.g. weight gain, length, head circumference)	13 (17.1%)	8.40	#6
Length of stay	3 (3.9%)	8.47	#11
Biochemical outcome (serum folate, serum IGF-1)	2 (2.6%)	Not measured	Not measured
Health care costs	2 (2.6%)	6.20	#15
Other Neurodevelopment Metabolic acidosis Fecal and breast milk analysis Abdominal signs before NEC	 1 (1.3%) 1 (1.3%) 1 (1.3%) 1 (1.3%)	Not measured	Not measured

[a] Some studies reported more than one primary outcome.

[b] There were some differences between the outcomes in the scoping review and online parent survey. Pre-defined outcomes in the online survey were determined by a preliminary review of primary outcomes in the included studies of the scoping review; therefore, not all primary outcomes identified by the final analysis of the scoping review were listed in the online survey (e.g. biochemical outcomes). Outcomes like parent-child bonding were also included in the online survey based on feedback during the pilot testing phase.

[c] Only one parent in the NEC group of the survey rated and ranked NEC as an outcome; the other ratings and rankings were based on responses from all 15 participants.

Abbreviations: EN=enteral nutrition; IGF-1=insulin-like growth factor-1; NEC=necrotizing enterocolitis; PN=parenteral nutrition.

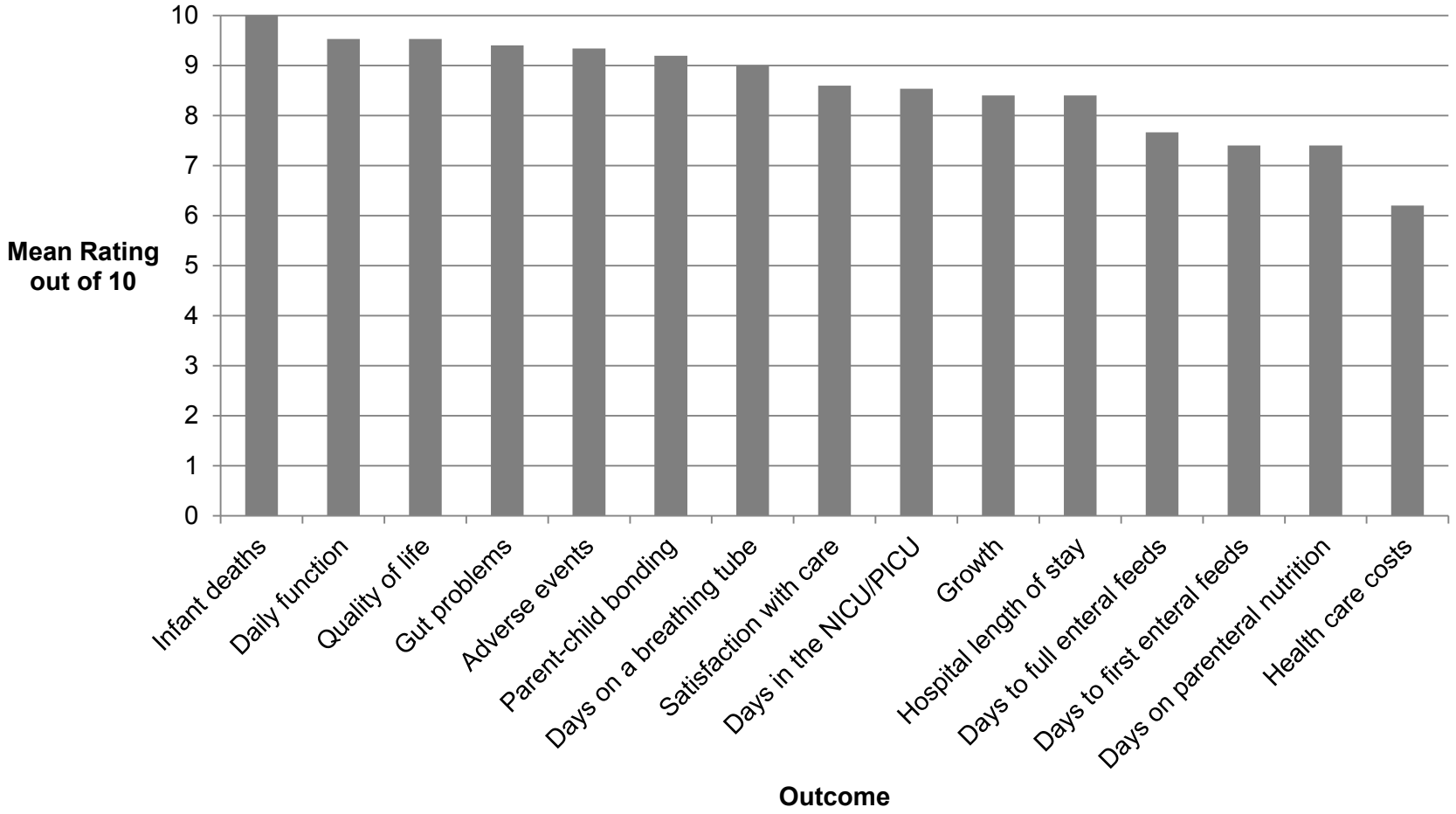

Figure 1. Mean ratings of outcomes on a scale of 1 (not at all important) to 10 (very important) from n=15 parents with previous NICU experience. The 3 NEC outcomes (development, type and severity of NEC) were not included in this figure due to a low response rate (n=1) in the NEC group. Abbreviations: PICU=pediatric intensive care unit; NEC=necrotizing enterocolitis; NICU=neonatal intensive care unit.

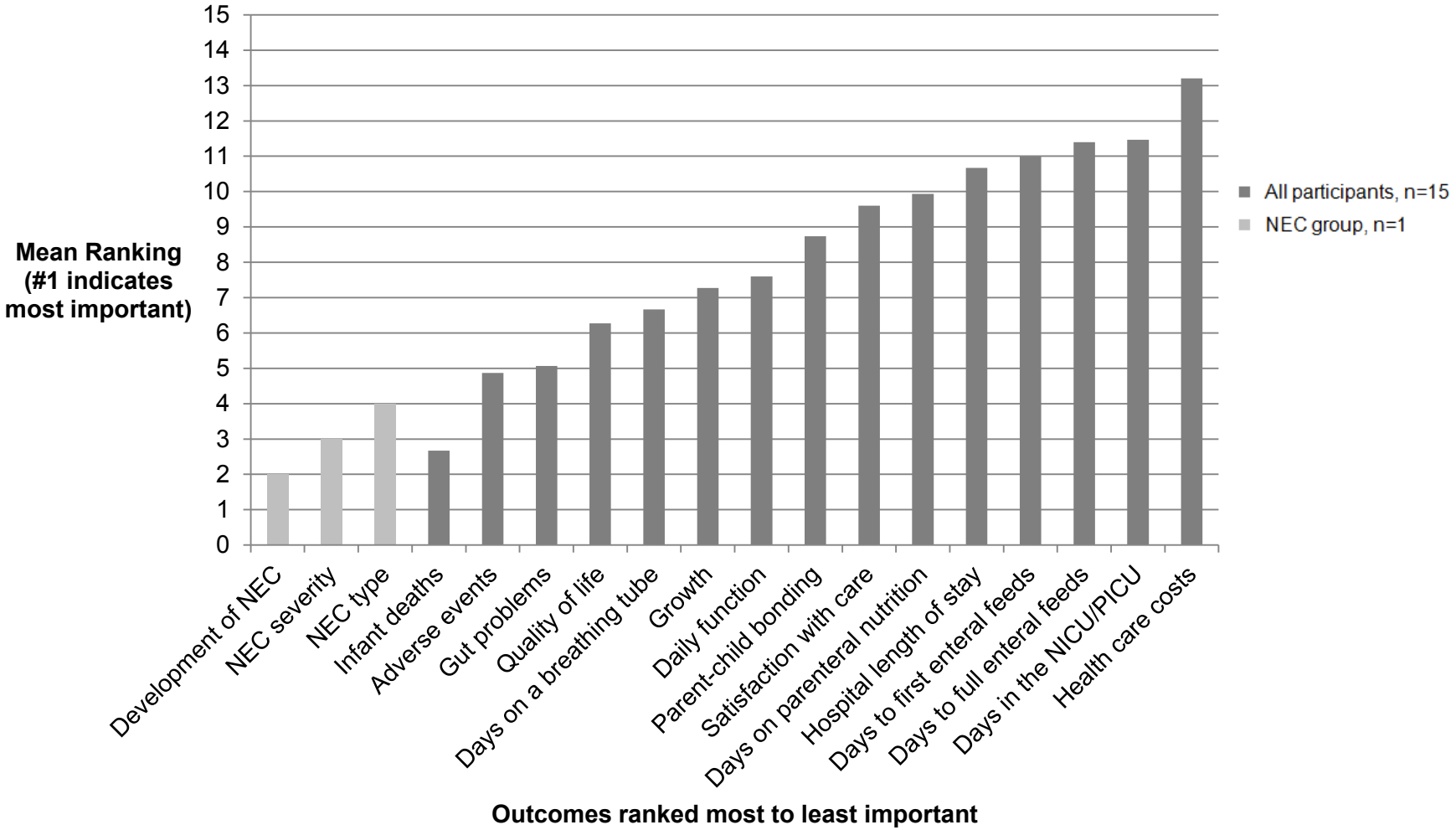

Figure 2. Mean rankings of outcomes from parents with previous NICU experience. The 3 NEC-specific outcomes (light grey) were ranked by one participants (NEC group), and the non-NEC outcomes (dark grey) represent the mean ranking from all 15 participants. The lowest ranking of #1 indicates the most important outcome. Only participants in the NEC group ranked 3 NEC-specific outcomes. Abbreviations: PICU=pediatric intensive care unit; NEC=necrotizing enterocolitis; NICU=neonatal intensive care unit.

Appendix 1. Parent outcome prioritization survey

BACKGROUND

Hi Parents!

Please read the attached information letter before proceeding.

Our topic: We are completing a scoping review exploring different types of nutrition and the effects on a gut disease known as necrotizing enterocolitis (NEC) in infants less than 1-month-old.

What is a scoping review? Our scoping review searches for all the studies looking at different nutrition products for infants (e.g. breast milk, formulas, fortifiers) and the effects on NEC. The scoping review will then summarize and describe what the research has found.

How will this help? The information from our scoping review will help neonatologists, dietitians, nurses, policy makers and families understand which nutrition products have been tested in neonatal intensive care units (NICUs) to help avoid NEC. The review will also look for trends in the findings from these studies, and determine if more research is needed in certain areas.

Why do we need your help? In order to provide the best information to health care professionals and families, we want to include the most important health outcomes in our scoping review. Health outcomes are the results or changes in health status that we would see due to health care activities. For instance, feeding a baby enough calories (health care activity) leads to weight gain (health outcome).

This survey draws on your experience with nutrition support for your newborn in the NICU. We would like to know which outcomes are most important to you when it comes to feeding your newborn through the gut (not intravenous nutrition or TPN).

We would like to get responses from parents of children who were diagnosed with NEC, and from parents of children who did not get NEC. Different views from parents may help us find out what works, which nutrition products families prefer, and which areas in health care and research we need to work on.

This survey should take about 15 minutes to complete.

SECTION 1

During your baby's time in the NICU, was your baby diagnosed with necrotizing enterocolitis (NEC)?

- Yes – *[NEC group. Participants answering yes were provided with 3 additional NEC-specific outcomes (NEC development, type and severity) to assess.]*
- No – *[No NEC group. Participants answering no were not given the 3 NEC-specific outcomes to assess.]*

SECTION 2 – Identifying outcomes

Below is a list of possible outcomes to explore in a scoping review studying the effect of different formulas, fortifiers and human breast milk on necrotizing enterocolitis (NEC) in newborn infants. Are there outcomes not on the list that are important to you? If yes, please specify up to 3 individual outcomes in the spaces provided (one outcome per line). You can choose to not enter additional outcomes by leaving the spaces blank.

NEC outcomes (NEC group only)

- Development of NEC
- Type of NEC (medical versus surgical)
- Severity of NEC (stage I, II or III)

Medical outcomes

- Gut problems, e.g. feeding intolerance, vomiting, bloody stools, constipation
- Infant deaths
- Number of days on a breathing tube
- Adverse events (a side effect or baby's reaction to a medical product), e.g. bloody stools, trouble breathing, blood infection

Hospital stay

- Neonatal or Pediatric Intensive Care Unit admission days
- Total hospital length of stay (in NICU/PICU and other inpatient wards)

Nutrition outcomes

- Number of days food is given into the blood (parenteral nutrition or TPN)
- Number of days until first feeds are given by mouth or feeding tube
- Number of days to reach full feeds by mouth or feeding tube
- Growth, e.g. weight, length, head circumference, rate of weight gain

Health care system

- Healthcare costs, including doctors' fees, inpatient and outpatient costs, formula costs, etc.
- Satisfaction with care

Overall health and well-being

- Quality of life
- Daily function
- Bonding or parent's relationship with infant

Add other outcomes important to you

- Please specify: _____
- Please specify: _____
- Please specify: _____

SECTION 3 – Rating outcomes

Please rate each outcome from 1 (not at all important) to 10 (very important).

NEC outcomes (NEC group only)

- Development of NEC
- Type of NEC (medical versus surgical)
- Severity of NEC (stage I, II or III)

Medical outcomes

- Gut problems, e.g. feeding intolerance, vomiting, bloody stools, constipation
- Infant deaths
- Number of days on a breathing tube
- Adverse events (a side effect or baby's reaction to a medical product), e.g. bloody stools, trouble breathing, blood infection

Hospital stay

- Neonatal or Pediatric Intensive Care Unit admission days
- Total hospital length of stay (in NICU/PICU and other inpatient wards)

Nutrition outcomes

- Number of days food is given into the blood (parenteral nutrition or TPN)
- Number of days until first feeds are given by mouth or feeding tube

- Number of days to reach full feeds by mouth or feeding tube
- Growth, e.g. weight, length, head circumference, rate of weight gain

Health care system

- Healthcare costs, including doctors' fees, inpatient and outpatient costs, formula costs, etc.
- Satisfaction with care

Overall health and well-being

- Quality of life
- Daily function
- Bonding or parent's relationship with infant

Outcomes you added

- _____
- _____
- _____

SECTION 4 – Ranking outcomes

Please rank the outcomes by dragging them to the right-hand column, in order of “Most important” to “Least important”. Outcomes can be re-ordered in the “Most/Least important” column.

- Development of NEC (NEC group only)
- Type of NEC (medical versus surgical) (NEC group only)
- Severity of NEC (stage I, II or III) (NEC group only)
- Gut problems, e.g. feeding intolerance, vomiting, bloody stools, constipation
- Infant deaths
- Number of days on a breathing tube
- Adverse events (a side effect or baby's reaction to a medical product), e.g. bloody stools, trouble breathing, blood infection
- Neonatal or Pediatric Intensive Care Unit admission days
- Total hospital length of stay (in NICU/PICU and other inpatient wards)

- Number of days food is given into the blood (parenteral nutrition or TPN)
- Number of days until first feeds are given by mouth or feeding tube
- Number of days to reach full feeds by mouth or feeding tube
- Growth, e.g. weight, length, head circumference, rate of weight gain
- Healthcare costs, including doctors' fees, inpatient and outpatient costs, formula costs, etc.
- Satisfaction with care
- Quality of life
- Daily function
- Bonding or parent's relationship with infant
- _____
- _____
- _____

SECTION 5 – General information about you

Please indicate your gender
- Female
- Male

What is your age range (years old)?
- <20
- 20-24
- 25-29
- 30-34
- 35-39
- 40-44
- 45-49
- 50-54
- 55-59
- 60-64
- 65-69
- 70+
- I prefer not to answer

Which province or territory do you currently live in?
- Alberta
- British Columbia
- Manitoba
- New Brunswick
- Newfoundland and Labrador
- Northwest Territories
- Nova Scotia
- Nunavut
- Ontario

- Prince Edward Island
- Quebec
- Saskatchewan
- Yukon

What is your marital status?
- Single
- Married
- Common-law
- Separated
- Divorced
- Widowed
- I prefer not to answer

What is your current occupation?

_____

What is the highest level of education you achieved?
- Elementary school completed, plus some high school credits (no diploma awarded)
- High school diploma or equivalent
- Registered Apprenticeship or other trades certificate or diploma
- College, CEGEP, or other non-university certificate or diploma
- University certificate or diploma
- University undergraduate or professional degree
- University graduate degree (e.g., Master's, PhD)
- Other (please specify) _____
- I prefer not to answer

What is your household income per year?
- under $25,000
- $25,000- $49,000
- $50,000-$74,000
- $75,000 - $99,000
- over $100,000
- I prefer not to answer

Do you identify with an ethnic minority group
- Yes

  If yes, please specify. You may select more than one option.
  - First Nations
  - Chinese
  - South Asian
  - Black
  - Filipino
  - Latin American
  - Southeast Asian
  - Arab
  - West Asian
  - Korean

    - Japanese
    - Caucasian
    - Other (please specify) _____
- No
- I prefer not to answer

What is the current age of your child who was admitted to the NICU for at least 6 weeks? (If you had more than one child in the NICU for at least 6 weeks, please answer for the most recent child.)
Example #1: 0 years, 11 months
Example #2: 2 years, 3 months

_____(years)
_____(months)

What was the gestational age at birth (in weeks) of your child admitted to the NICU for at least 6 weeks? (If you had more than one child in the NICU for at least 6 weeks, please answer for the most recent child.)
_____(weeks gestation)

How many weeks did you spend in a NICU with your child? (If you had more than one child in the NICU for at least 6 weeks, please answer for the most recent child.)
_____(weeks)

Chapter 5: Conclusions

Summary of findings

The first study of this thesis was a narrative review that described NEC and its possible causes, nutrition considerations for preterm and critically ill infants, potential linkages between the type of nutrition fed to infants and the risk of NEC, and a brief appraisal of high-profile studies conducted in this area. The narrative review highlighted that human milk contains many beneficial components, such as immunoglobulins, growth factors, platelet-activating factor acetylhydrolase, lactoferrin, and human milk oligosaccharides, which may provide protection against NEC. Two RCTs and a Cochrane systematic review found that an exclusive human milk diet resulted in fewer cases of NEC than a diet containing bovine milk-based products. However, some questions remain unanswered. The studies did not explain why an exclusive human milk diet did not prevent all cases of NEC (approximately 1-3% of infants fed an exclusive human milk diet developed NEC). In addition, 3 of 4 studies in the meta-analysis of the Cochrane review were published more than 30 years ago. Modern formulas, DHM processing and clinical practice have changed in that time, so the outdated data may no longer be relevant. The narrative review also pointed to the potential benefits of protein-hydrosylated formulas or fortifiers based on cell and animal studies, but there were minimal studies available on human infants. Collectively, research gaps identified by the narrative review led to a comprehensive scoping review on enteral nutrition and NEC, and systematic review of hydrolyzed nutrition products in the second study of the thesis.

The scoping review included 76 studies, mostly case control and cohort studies from the United States. The most common diet comparison was between a predominantly or exclusive human milk diet and partially or exclusively bovine milk-based diet. The majority of these studies reported trends in lower NEC rates for infants fed predominantly or exclusively human milk, with

1/5 RCTs, 5/22 cohort studies and 11/16 case control studies reporting significantly lower NEC rates compared to a diet containing higher amounts of bovine milk-based products. In addition to these studies, other research has used diet as part of multicomponent interventions to reduce NEC rates or improve the quality of care in the NICU. Standardized protocols, education initiatives and programs emphasizing human milk use have been reported as strategies to reduce poor outcomes including NEC. Although the direct effect of diet may not be discerned from the multicomponent interventions, these studies suggest that diet may be used in combination with other interventions or quality improvement initiatives to prevent NEC.

The systematic review of hydrolyzed nutrition products included 8 studies. Two RCTs and 2 cohort studies evaluating hydrolyzed and intact-protein fortifiers were included in a meta-analysis. The confidence intervals of the pooled effect estimates from the RCTs and cohort studies were wide and crossed the line of no effect. Given the small sample size and imprecision of the results, the evidence was graded as low quality. A larger sample size is required to make conclusions about the effect of hydrolyzed fortifiers.

The scoping and systematic review underscored the need for additional clinical trials on enteral diet, especially hydrolyzed nutrition products, and NEC in order to make recommendations for clinical practice. Collaborating with parents on the development of research protocols is expected to enhance the relevance of research results, and may also lead to efficiencies in the conduct and uptake of research. Therefore, the third study of this thesis was an outcome prioritization survey asking parents with previous NICU experience about research outcomes most important to them. Parent priorities were also compared to commonly reported primary outcomes in the literature. The most important outcome to the 15 parent respondents was infant deaths. Other prioritized outcomes included adverse events, gut problems (e.g. constipation) and quality of life. NEC development, type and severity were assessed by one participant whose child had been diagnosed with this disease, and were identified as important outcomes

to the participant. These preliminary findings suggest that collaboration with parents on outcome prioritization and study planning may ensure that future research addresses priority concerns of families as well as clinicians and researchers.

Implications for clinical practice

In consideration of the evidence on enteral diet and NEC, a predominantly or exclusively human milk diet may help protect against NEC. This work did not systematically review or meta-analyze the data on human milk versus bovine milk-based products, nor did it establish a dose-response relationship for human milk and NEC. However, the majority of studies in the scoping review comparing human milk to bovine milk-based products showed trends toward lower rates of NEC when human milk was more predominant in the diet. Encouraging mothers to breastfeed and express breast milk and, if needed, supplement with DHM for infants at risk of NEC remains the best recommendation for clinical practice.

To meet a preterm infant's high demands for protein, energy and micronutrients, many NICUs add an intact-protein bovine milk-based HMF to MOM and DHM. The addition of this fortifier exposes infants to bovine milk-based products, which may diminish the benefits of an exclusive human milk diet. Transitioning to a DHM-based HMF (Prolact+ $H^2MF^{®}$) for very or extremely low birth weight infants, however, is not currently feasible due to the cost of this product. Further, the evidence on Prolacta comes from two industry-funded trials (1, 2) including infants born 500-1250 g that used a comparator no longer applied in practice (the trials used bovine milk-based preterm formula if MOM was unavailable in the control group, whereas local NICUs currently provide DHM) and the proportion of infants with NEC in the control group were considerably higher (16% and 21%) than the incidence of the disease (5%). These discrepancies question the reliability of the evidence on Prolacta. Hydrolyzed fortifiers may be another option, but more evidence is needed to determine if this type of fortifier is an effective alternative.

Future directions

NEC is a complex disease and its etiology is still unclear. Animal studies may be helpful to better describe the pathogenesis of NEC, and the mechanism by which different types of enteral feeds protect against or instigate NEC. Findings from animal work may offer new evidence to proceed with large RCTs in the clinical setting.

For clinical practice decisions, research on a dose-response relationship for human milk versus bovine milk-based diet would be helpful to understand if there is a threshold amount or proportion of human milk that results in the best protection against NEC. As discussed in the systematic review of hydrolyzed nutrition products, a larger trial on hydrolyzed fortifiers compared to intact-protein bovine milk-based fortifiers is also needed to determine which type of fortifier should be used for infants at risk of NEC. Ideally, an adequately powered 3-armed RCT comparing the effect of a hydrolyzed fortifier, bovine milk-based HMF and Prolact+ $H^2MF^®$ on NEC, accompanied by a cost-effectiveness analysis would help clinicians decide which product is optimal. This type of trial, however, would be expensive and difficult to conduct.

Several challenges of conducting research on enteral nutrition and NEC were identified by the scoping and systematic review, particularly sample size and cost. Because NEC is a relatively rare disease measured as a dichotomous outcome, a clinical trial powered on NEC as the primary outcome would need thousands of participants. For example, assuming a 2-armed superiority trial, 5% NEC control event rate, 35% reduction in NEC as the minimal clinically important difference, 5% type I error and 80% power, a total of 4052 infants would be needed (3). Funding a trial of this magnitude, including the cost of nutrition products and multicenter coordination, would be difficult.

As outlined in the third study of this thesis, collaboration with parents on the development of future studies may help make research more efficient and impactful. Methodologically rigorous

clinical trials rely on high recruitment rates, strict protocol compliance and low attrition. NICU parents are often very knowledgeable about their child's care and can advise on practical barriers (e.g. stress of establishing milk supply, or attitudes toward enrolling in a clinical trial), which may offer strategies to improve recruitment, compliance and follow-up. As such, parent advisory committees or focus groups are recommended to engage parents in the planning and design of future trials. Parent participation in tasks like outcome prioritization may enhance the quality of studies and generate knowledge relevant to families, as well as clinicians and researchers.

Concluding remarks

Continuing to study the type of enteral diet that offers the most protection against NEC and that is practically and economically sustainable is needed. This may be a moving target as clinical practice, nutrition products and technologies advance. Furthermore, given the complexity of NEC, it is possible that diet may be only one piece of an effective NEC prevention strategy. Antibiotic stewardship, probiotics, feeding tube maintenance, and initiation and advancements of enteral feeds are a few of the many practices that may play a part in preventing this disease. Building on the work that has already been done and engaging parents in the development of research that is meaningful to all stakeholders will hopefully resolve the uncertainties of NEC in the near future.

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