Necrotizing Enterocolitis (NEC) in Preterm Infants Working Group of the National Advisory Council of Child Health and Human Development (NACHHD)

Report to

Secretary, Department of Health and Human Services

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Executive Summary

In August 2024, at the request of the U.S. Secretary of the Department of Health and Human Services, the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) convened a "Necrotizing Enterocolitis (NEC) in Preterm Infants Working Group" of the National Advisory Council of Child Health and Human Development (NACHHD), an existing Federal Advisory Committee. Membership included laboratory scientists, neonatologists, clinical trials specialists, and representatives from national pediatric organizations and advocacy groups (see Appendix A). The charge of the Working Group is detailed in Box 1. In addition to advising the Secretary, the Working Group was charged with preparing and submitting to the Secretary a report detailing its findings on the state of the science on NEC and nutritional support (i.e., enteral feeding practices) for preterm infants.

Box 1: NEC Working Group Charge

- Assess the scientific evidence regarding enteral feeding practices in premature infants and factors that may protect against or increase risk for NEC.
- Identify important research gaps.
- Make recommendations for potential future research directions.

The Working Group developed 17 recommendations (see Box 2) based on information summarized during three meetings in August 2024 (see Appendices B and C for the agendas and meeting summaries). On September 5, 2024, the co-chairs of the group presented these recommendations to the NACHHD Council, which approved this report and gave concept clearance to implement the recommendations. The recommendations were not prioritized, but are grouped here into six themes: overarching, epidemiology of NEC, mechanisms of NEC, factors affecting risk of NEC, nutritional support for premature infants and NEC, and feeding practices and NEC. Sections 1-3 of this report detail the state of the science about NEC; Section 4 focuses on the recommendations.

NEC is a serious gastrointestinal illness in which the tissue lining of the intestines becomes inflamed and dies off. Symptoms include swelling of the abdomen, bloody stool, vomiting bile, and food not moving through the intestines. NEC is one of the leading causes of illness and death in preterm infants, particularly those born with a very low birth weight (i.e., less than 1,500 grams or 3.3 pounds). NEC may also develop in infants born later, particularly those with heart problems. One infant dies almost every day from NEC in the United States. In 2022, 356 infants died of NEC (CDC[1]), though the number of NEC cases and deaths is thought to be underestimated due to a lack

[1] https://wonder.cdc.gov/lbd-current-expanded.html

of a common definition and limitations in tracking and reporting (e.g., ICD-10 codes need to be more specific to NEC and separate out other similar conditions). NEC is devastating to patients and their families, in part because it can appear suddenly and progress quickly in infants who otherwise may seem to be getting healthier. Survivors of the most severe NEC, especially those who undergo surgery, are at significantly higher risk of lifelong neurodevelopmental problems compared to preterm infants who do not develop NEC (Wang, PMID 38684534[2]).

The Recommendations

The Working Group developed their recommendations to improve the scientific evidence base about NEC. This report does not represent or provide clinical guidance. Please refer to medical practice organizations for specific clinical management recommendations.

Two central themes underpin the recommendations. First, all infants, including those in the neonatal intensive care unit (NICU), must be fed as soon as is medically feasible by whatever means are available. Nutrition is vital for brain and organ development, and prolonged fasting increases risk factors for other serious conditions. Feeding intravenously may increase the risk of bloodstream infections. Therefore, this report focuses specifically on enteral feeding practices of premature infants. Second, the American Academy of Pediatrics recommends that very low birth weight infants receive human milk, preferably from their own parent. If the parent's milk is not available, pasteurized donor human milk is recommended (Parker, PMID 34635582[3]). When infants are born preterm, the parent may not be able to produce enough milk to meet the infant's needs. In these cases, providers usually use either donor milk or preterm infant formula to supplement the parent's milk. In addition, human milk, whether from the parent or donated, often requires fortification to meet the nutritional needs of very low birth weight infants, as detailed in Section 3.

Existing evidence about NEC has substantial limitations. First, other intestinal conditions may mimic NEC, making diagnosis and developing a standardized definition more complicated. Second, much of the current evidence shows only associations, not direct or indirect causation linking biological pathways to risk factors for development and/or progression of NEC. Studies showing an association between a risk factor and the development of NEC do not necessarily mean that the factor causes NEC. Third, some risk factors for NEC identified in observational data have not been confirmed in clinical trials to date (e.g., red cell transfusion, empiric antibiotics), although additional studies are ongoing. In addition, because NEC is not a common outcome in clinical trials, existing data rely on studies that are often observational or small clinical trials. This limits the generalizability of the results, and the certainty of evidence may be low.

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[2] https://pubmed.ncbi.nlm.nih.gov/38684534/
[3] https://pubmed.ncbi.nlm.nih.gov/34635582/

Box 2: Working Group Recommendations

Overarching Recommendations

- Expand research into prevention of premature birth, including ways to delay impending births beyond the window of higher NEC risk.
- Develop a standardized definition of NEC and severity of NEC to improve epidemiologic studies and determine eligibility for trials and treatments.
- Ensure that research on NEC incorporates the perspectives of affected families.

Epidemiology of NEC

- Improve tracking and reporting of NEC cases as well as deaths.
- Expand support for neonatal biorepositories to facilitate research to identify diagnostic, prognostic, predictive, susceptibility, and/or surrogate markers of NEC.
- Explore innovative methods for collecting and analyzing data.

Mechanisms of NEC

- Expand research to describe the biologic mechanisms of NEC development.
- Improve animal and laboratory models to better reflect the disease's complexity.

Factors Affecting Risk of NEC

- Explore exposures that may be positively or negatively associated with risk of NEC development, severity, and mortality.
- Expand research on feeding practices that may affect NEC risk.

Nutritional Support for Premature Infants and NEC

- Support research to identify the optimal nutritional needs of premature infants, how these may vary by gestational age at birth, and the specific nutritional needs of NEC survivors.
- Increase research to support parent's milk production and lactation techniques for preterm and term infants.
- Evaluate how the collection, processing, storage, and dispensing of donor and/or parental milk might impact its nutritional content and impact on intestinal health.

Feeding Practices and NEC

- Support large clinical trials of feeding practices and NEC risk, including timing of onset, severity, mortality, and long-term effects on survivors.
- Explore innovative clinical trial designs to test, via applicable IND regulations, the safety and efficacy of promising drugs and biologics to prevent and/or treat NEC.
- Expand implementation science research to reduce disparities related to NEC and in the availability of parent's own and pasteurized donor milk.
- Support research on parent education both before delivery and in the NICU about feeding practices and risk of specific conditions, including NEC.

The Working Group's consensus was that NEC is a multifactorial disease with many associated risk factors. Maternal and antenatal factors that place preterm infants at a higher risk for NEC include preeclampsia and hypertension. Antenatal steroids and/or tocolysis (e.g., medications used to delay delivery) appear to be partially protective against developing NEC. Infant perinatal factors that increase the risk of NEC include preterm birth, very low birth weight (less than 1,500 grams or 3.3 pounds), and being small for gestational age with infants at lower birthweight and gestational age at the higher risk. Infant postnatal factors that may affect the risk of developing NEC include antibiotic exposure (variable risk) and human milk exposure, which lowers an infant's risk of developing NEC. Infants who receive pasteurized donor human milk during hospitalization may need to transition off donor milk to a strategy consistent with their home feeding plan. The optimal timing to transition off donor milk is uncertain.

The relationship between feeding and development of NEC is unclear. Available evidence supports the hypothesis that it is the absence of any human milk in an infant's diet that is associated with a higher risk of NEC. Additional research is needed to link the underlying mechanisms that cause NEC to the components of human milk that may help prevent it. Research is also needed to determine why some human milk-fed infants still develop NEC. Improved understanding of the mechanisms may help to better define and track the disease, find biomarkers to diagnose NEC earlier, and find and test potential therapeutic targets to prevent and treat it. Gaps in knowledge remain regarding optimal nutrition, optimal feeding methods, and diet fortification practices for infants, particularly very low birth weight infants. Given that a parent may not be able to produce enough milk for a preterm infant, research is needed to find ways to enhance milk production, optimize the beneficial components in human milk (whether from the parent or donor milk), and reduce disparities that impede lactation and pumping for parents and access to donor milk.

Finally, the Working Group agreed that the best way to prevent NEC is the prevention of preterm birth. While some term infants develop NEC, these infants tend to have cardiac conditions that may indicate that the disease they develop is different or has a different cause than NEC in preterm infants. Much more research is needed to reduce the number of preterm births overall. When that is not possible, healthcare professionals need ways to prolong pregnancy past the gestational ages that are at higher risk of developing NEC.

NEC is a devastating disease. One baby dies every day in the United States from NEC, and those that survive may undergo traumatic surgeries, be neurodevelopmentally impaired, and have lifelong consequences. Without more research to improve the evidence base, parents and healthcare practitioners do not have adequate information about what and how best to feed premature infants to maintain a healthy growth rate while preventing or minimizing the risk of developing NEC. Parents, their offspring, and families would greatly benefit from having this essential information. The NEC Working Group of Council urges the Secretary to take action on these recommendations.

Introduction

NEC is a common, serious gastrointestinal illness mostly affecting preterm infants in which the tissue lining the intestine becomes inflamed and dies. More than 90 percent of NEC occurs in very low birth weight preterm infants (VLBW) (i.e., less than 1,500 grams or 3.3 pounds) (Kim[4]). It is one of the leading causes of illness and death among preterm infants and 15-40 percent of infants with NEC die from the disease. NEC usually begins within the first two or three weeks after birth, often in preterm infants who otherwise may seem to be getting healthier.

The CDC's National Center for Health Statistics in 2019 listed NEC as one of the 10 leading causes of infant mortality[5]. For 2022, CDC's Wide-ranging ONline Data for Epidemiologic Research (WONDER)[6] included 356 deaths or 9.7 deaths per 100,000 infants, based on International Classification of Diseases, 10th edition (ICD-10) codes. The overall number of NEC cases per year is not known due to a lack of reliable data and the lack of a standardized definition of the disease. The total cost of cases for NEC per year in the United States is estimated to be $1 billion (Neu, PMID 21247316[7]) for initial hospitalization and care. Mean additional care costs during the age 6-12 months for infants with NEC that did not have surgery were estimated to be $5,112 per infant more than for infants without NEC. For infants with NEC who had surgery, the mean additional incremental healthcare costs were $18,274 (for 6-12 months), $14,047 (for 12-24 months), and $8,501 (for 24-36 months) per infant (Ganapathy, PMID 23962093[8]).

Pathogenesis of NEC

In normal, healthy intestines, the mucosal epithelium, or intestinal lining, has distinct structures called villi that resemble folds. It is made of many different cell types, including goblet and Paneth cells. Goblet cells secrete mucus, which helps to protect the physical barrier of epithelial cells stacked tightly together to protect against bacterial invasion. Goblet cells also actively participate in immunity by sampling antigens passing through the intestine and presenting them to immune cells to decide whether to react. Paneth cells help the intestine maintain homeostasis. They secrete antimicrobial peptides, helping to regulate the intestinal microbiota and defending against intestinal pathogens (Frazer, In Press[9]).

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[4] https://www.uptodate.com/contents/neonatal-necrotizing-enterocolitis-clinical-features-and-diagnosis
[5] https://www.cdc.gov/nchs/data/databriefs/db395-h.pdf
[6] https://wonder.cdc.gov/lbd-current-expanded.html
[7] https://pubmed.ncbi.nlm.nih.gov/21247316/
[8] https://pubmed.ncbi.nlm.nih.gov/23962093/
[9] Frazer, Caplan, Good. Pathophysiology of NEC in Fetal and Neonatal Physiology: Polin and Fox, in press

Microbial dysbiosis is an imbalance in the composition of the microorganisms that live in the intestine. This occurs as one of the necessary and early steps in NEC pathogenesis and is detectable 48-72 hours prior to diagnosis (Bautista, PMID 37082706[10]). The immune system responds to the imbalanced microbiota with an exaggerated, pro-inflammatory response. This inflammatory response and other activated signaling pathways can trigger cell death and break down the tight epithelial barrier of the intestine (Frazer, In Press[11]).

Diagnosis of NEC

NEC is diagnosed based on the presence of the most characteristic clinical features, which are swelling of the belly (abdominal distention), bilious vomiting (vomit that is green or bright yellow due to bile in the stomach), and rectal bleeding (either stools that test positive for blood or grossly bloody stools, without the presence of a tear in the anus or anal canal). In addition, abdominal imaging typically shows gas within the walls of the bowel (intramural gas or pneumatosis intestinalis) or the peritoneal cavity (pneumoperitoneum) or the presence of sentinel bowel loops (a dilation of the small intestine near an inflammatory process in the abdomen). A definitive diagnosis of NEC is made during intestinal surgery or autopsy, based on findings demonstrating intestinal inflammation, infarction (obstruction of the blood supply to an organ or region of tissue), and necrosis (dead body tissue) (Kim[12]).

Treatment for NEC

Treatment for NEC depends on the severity of the condition and the infant's age and general health. It may include stopping feeding, inserting a nasogastric tube (tube from the nose to stomach) to keep the infant's stomach empty, providing intravenous (IV) fluids and nutrition, and providing oxygen or using a breathing machine if the abdomen is so swollen that it affects breathing. Infants with severe NEC may require surgery to remove the section of damaged intestine, an ostomy (connecting part of the intestine to an opening in the abdomen), or draining the abdominal cavity.

Population at Risk for NEC

While newborns of any gestational age can develop NEC, the greatest risk appears to be associated with prematurity. The population at risk for NEC is increasing because the number of moderate preterm infants (i.e., born between 32 and 34 weeks of pregnancy) who survive continues to increase as a result of advances in care. However, the proportion of preterm infants who develop NEC remains unacceptably high (Elisbury, PMID 32173122[13]), revealing a need to better

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[10] https://pubmed.ncbi.nlm.nih.gov/37082706/
[11] Frazer, Caplan, Good. Pathophysiology of NEC in Fetal and Neonatal Physiology: Polin and Fox, in press
[12] https://www.uptodate.com/contents/neonatal-necrotizing-enterocolitis-clinical-features-and-diagnosis
[13] https://pubmed.ncbi.nlm.nih.gov/32173122/

understand the causes, modifiable risk factors, prevention, and treatment of NEC in this population.

Risk factors for infants developing NEC include both maternal and infant characteristics (e.g., delivery, postnatal medical treatment, and feeding). Maternal risk factors include preeclampsia (a pregnancy condition characterized by high blood pressure and sometimes fluid retention and protein in the urine), maternal infection in the uterus, and preterm premature rupture of membranes (PPROM), placental abruption (separation of the placenta from the wall of the uterus), intrauterine growth restriction (IUGR), and African American race. Infant risk factors at delivery include early premature birth (before 34 weeks of gestation), small for gestational age, and low oxygen levels at birth. Postnatally, infants most at risk for NEC in the United States include those with lower oxygen saturation targets in the NICU, intestinal infections, sepsis, assisted ventilation, and intestinal bacterial dysbiosis or viral infections, including rotavirus, cytomegalovirus, and norovirus. Among infants that develop NEC, those most at risk of dying include those with:

- Lower gestational age
- Lower birth weight
- Treatment with assisted ventilation on the day of NEC diagnosis
- Treatment with vasopressors (medication to maintain blood pressure) at the time of diagnosis (Clark, PMID 21593813[14])
- Low Apgar scores (notably at one and five minutes after birth)
- Low hemoglobin concentration
- High lactate level at disease onset and during disease
- Patent ductus arteriosus (Kordasz, PMID 34636956[15])
- African American race (risk of death from NEC is higher for Black infants compared to White infants in the United States, RR 3.1, 95% CI 3.0–3.4 (Seeman, PMID 26946352[16])

The racial disparity in NEC morbidity and mortality supports the notion that social determinants of health need further exploration to improve our understanding of the disease.

Prevention of NEC

The causes of NEC are not well understood, but as gestational age increases, the risk of NEC decreases (Bell, PMID 35040888[17]; Ramani, PMID 23415260[18]). Consequently, supporting maternal

[14] https://pubmed.ncbi.nlm.nih.gov/21593813/
[15] https://pubmed.ncbi.nlm.nih.gov/34636956/
[16] https://pubmed.ncbi.nlm.nih.gov/26946352/
[17] https://pubmed.ncbi.nlm.nih.gov/35040888/
[18] https://pubmed.ncbi.nlm.nih.gov/23415260/

health to reduce preterm birth is of utmost importance in reducing NEC and is the primary prevention strategy.

Given feeding is a risk factor for NEC development, research has focused on what is fed and how food is delivered to infants. Findings suggests that human milk, as compared to formula, reduces the incidence of NEC. The American Academy of Pediatrics (AAP) recommends that preterm infants receive human milk, preferably from their own parents[19]. If parent's milk is not available in sufficient quantity, pasteurized donor human milk is recommended as the second most-preferred option, followed by infant formula. Unfortunately, human breastmilk, including donor milk, is not always available. Additional research is needed to explore the components of formula and compare them to those of breastmilk to identify factors that may be positively or negatively associated with the risk of developing NEC.

Premature infants who are not capable of breastfeeding may need to be provided enteral nutrition (i.e., feeding through a feeding tube) (Brune[20]). While there is guidance for how to perform tube feeding, there is no consensus on the best way to give enteral nutrition to premature infants to prevent NEC. What is known is that when feeding preterm infants, starting with small amounts of food (instead of not feeding at all), beginning feeds early (before day 4), and rapidly advancing feeds (30-35 ml/kg/day instead of 15-20 ml/kg/day) do not make NEC more likely. There is no evidence that continuous tube feeding is better than intermittent tube feeding for these infants. If a preterm infant cannot tolerate tube feeds (they vomit), but shows no other signs of NEC, starting with small amounts of food instead of stopping all feeding may be a good option.

Gaps in Knowledge Regarding NEC

Limited epidemiologic data are available on NEC, and some widely used data may not be representative of the population as a whole. This makes it difficult to explore the full range of potential risk factors. Risk factors may vary by gestational age, so intervention and prevention efforts may need to be tailored accordingly. Comorbid conditions may also impact the risk for NEC. Approaches to filling these knowledge gaps include multidisciplinary collaboration, standardizing the clinical definition of NEC to better characterize the scope of the problem via epidemiological research, mechanistic research, identifying biomarkers for diagnosing NEC, and developing and testing new therapies.

[19] https://www.aap.org/en/patient-care/newborn-and-infant-nutrition/nutrition-for-the-preterm-vlbw-infant-after-nicu-discharge/

[20] https://publications.aap.org/neoreviews/article/19/11/e645/91861/Enteral-Feeding-of-the-Preterm-Infant

Definition of NEC

Defining a disease identifies those features that distinguish it from other diseases, sets boundaries on current knowledge about the disease, helps to identify questions about the disease that need to be answered, and allows clinicians and researchers to determine research methods that will be the most likely to provide pertinent information. Multiple definitions of NEC exist; the three most commonly used definitions are Bell staging, modified Bell staging, and the Vermont Oxford Network definition (Patel, PMID 32855506[21]).

First proposed in 1978 by Bell and colleagues, Bell staging remains the most widely used definition of NEC worldwide. Bell staging classifies infants in one of three stages of NEC, where Stage I indicates symptoms, such as apnea, poor feeding, and abnormal radiographs, which indicate that the infant may have NEC, but additional testing should be considered to rule out other disorders. Stage II was classified as definite NEC, and Stage III is advanced NEC (Bell, PMID 413500). Modified Bell staging was proposed in 1986 and increased the number of stages from three to six, allowing doctors to further differentiate the severity of NEC. The Vermont Oxford Network definition is based on diagnosis at surgery, examination after death, or a combination of clinical criteria and X-ray results. The lack of a single, unified definition of NEC and the use of multiple definitions throughout the field is problematic. For example, whether the incidence of NEC is increasing or decreasing depends on the definition used (Horbar, PMID 28068438). A single standardized definition is necessary to enable the use of common data elements and cross-study comparisons and validation. Consensus on the definition is needed to further our understanding of NEC, advance research, and improve outcomes for infants with NEC.

Causation vs. Association

There are substantial gaps in knowledge about nutritional strategies for preterm infants regarding breastmilk (parent vs. donor), breastmilk handling methods (e.g., preparation, freeze-thaw cycles, and pasteurization), components in breastmilk, genetic factors of breastmilk, pooling methods for donor milk, lactational stage of milk (colostrum, transitional milk, and mature milk), formula preparations, and formula components.

In considering risk factors for NEC, it is important to understand the concepts of causation and association. Studies showing an association between a risk factor and the development of NEC do not necessarily mean that the risk factor causes NEC. Association refers to a relationship between two variables and knowing the value of one variable provides information about the value of the other variable. Causation means that there is a cause-effect relationship. Several criteria (Schunemann, PMID 26425136) are used for determining whether an association is causal. For example, temporality must be established, meaning the cause must precede the effect.

[21] https://pubmed.ncbi.nlm.nih.gov/32855506/

Researchers are also more confident in the causality of a stronger association than a weaker association. Consistency across studies is relevant. In other words, different researchers at different places and times observing the same relationship are important for establishing causality. Dose-response relationships are also helpful—showing, for instance, that as the dose (exposure) increases, the response (outcome) also increases.

Working Group Charge

The Necrotizing Enterocolitis in Preterm Infants Working Group (the "Working Group") was charged with assessing the evidence regarding enteral feeding in premature infants and factors that may protect against or increase risk for necrotizing enterocolitis. The Working Group was also charged with identifying important research gaps and making recommendations for potential future research studies.

Three Working Group meetings were held on August 15th, 22nd, and 28th, 2024, to obtain input from patient advocacy, clinician, and research communities (see Appendices B and C). This report contains materials gathered for the Working Group, as well as the results of the Working Group discussions and meetings.

This report fulfills the Working Group's mandate by providing its findings on the state of the science on NEC and nutritional support in preterm infants and the group's recommendations to identify and address gaps in knowledge. The report's appendices provide further information on the Working Group's membership and procedures, relevant research literature, NIH funding for research, and acronyms used in this report.

Section 1. State of the Science for Risk Factors for NEC

To identify and address gaps in knowledge regarding NEC and the optimal feeding options for preterm infants, this Working Group evaluated the current state of the science regarding risk and protective factors for NEC in premature infants. The Working Group identified key research needed to advance science with the largest impact for this vulnerable population. Feeding factors are briefly listed here at a high level; more details are presented in Section 2.

As discussed in the Introduction, there are multiple definitions of NEC that vary in their clinical and imaging diagnostic requirements. Three definitions (Bell staging, modified Bell staging, and the Vermont Oxford Network) are the most commonly used in the United States. To enable the use of common data elements, cross-study comparisons, and validation, a single standardized definition for NEC is needed. Standardization of data collection would include variables capturing maternal, antenatal, postnatal, and biologically relevant information. Along with standardization of the methods for collecting that data, a standard NEC definition would allow investigators to use large datasets to ask new questions and expand the scientific value of these datasets.

Evidence Regarding Risk and Protective Factors for Developing NEC

Box 1.1: Enteral feeding risk factors in NEC and the current state of the science

- Timing of transition from donor milk to formula
- Slow versus faster feeding: no significant effect on NEC with moderate certainty, 2021 Cochrane review
- Delayed feeding versus early progressive feeding: no significant effect, low certainty, 2022 Cochrane review
- Early trophic feeding versus enteral fasting, significant effect, 2013 Cochrane review
- Standardized feeding regimen - 15 observational studies - marked reduction in risk of NEC, 2017 systematic review
- Enteral feeding strategies – slower versus faster, enteral fasting, trophic feeding – none significantly associated with NEC risk in meta-analysis, 2021 Cochrane review
- Use of standardized feeding regimen associated with decreased risk of NEC in observational studies, Jasani, PMID 28358382.

Risk factors for NEC were last comprehensively identified at a population level in 2018. Since then, several enteral feeding risk factors have been more thoroughly evaluated. An update to the relative risk attributed to several common enteral feeding features is summarized in Box 1.1.

Risk factors for NEC include maternal factors, antenatal factors, postnatal factors, and biological factors involved in the pathogenesis of NEC. These risk factors are listed in Boxes 1.2 and 1.3 and further detailed below.

Box 1.2: Biological and Social Factors Associated with NEC Development and Severity

- Enteral feeding (see Box 1)
- Microbiome and virome
- Immune targets and pathways identified in inflammation and development of necrosis
- Human breastmilk consumption
- Genetic and epigenetic aspects
- Vascular component to NEC
- Social determinants of health
- Hypoxic conditions

Additional discussion of enteral feeding is found in Section 2.

The microbiome and virome represent the complex community of bacteria and viruses that coexist with human cells in and on the human body. The human immune system must learn to identify these passengers as "normal" to keep homeostasis. A disturbance in the microbiome and/or virome has been identified as a risk factor for NEC in premature infants. The immune and digestive systems of premature infants are still developing and actively negotiating what is "normal" versus "harmful". This risk factor spans both the antenatal and postnatal periods, meaning that the balance of bacteria or viruses can become disturbed either before or after birth and contribute to a higher risk for NEC. Several studies have looked at intestinal dysbiosis, the disruptive change in bacterial communities in the gut. Compared to infants who did not have NEC, the bacterial communities in infants who did go on to develop NEC had more bacteria from the Proteobacteria phylum and fewer species from the Firmicutes and Bacteriodes phyla. Viral changes have been studied as well, although there is less definitive association of NEC risk to specific types of viral infections.

The immune system is involved in NEC pathogenesis and development. The pathways involved in inflammation and development of necrosis show increased activity in infants that develop NEC.

Breastmilk has been identified as a protective factor for premature infants. Human milk oligosaccharides (HMOs) may offer some protection from NEC, although research is needed to identify additional components that provide protection. Parent's milk is not always available in sufficient quantity, and its protection is not absolute, as premature infants receiving only human milk can also develop NEC.

Genetic and epigenetic factors may contribute to potential risk, and research is ongoing to understand the specific mechanisms and contributions of these findings.

Risk factors related to the vascular system in both prenatal and postnatal periods have been identified. The evidence suggests there is a vascular component to NEC that is mechanistically related to the rapid pace of necrosis onset and spread.

Social determinants of health may be potential risk factors for NEC. Specifically, Black parents are more likely than White parents to have premature infants who develop NEC. This may present an opportunity for outcomes-based, community-level interventions to reduce the rates of NEC occurrence in these communities.

Box 1.3: Maternal and Infant Factors Associated with NEC Development and Severity

Maternal/antenatal risk factors:

- Increased risk of NEC:
  - Preeclampsia
  - Hypertension
  - Black race
- Decreased risk of NEC:
  - Antenatal steroids and/or tocolysis (e.g., medications used to delay delivery)

Infant factors (Increased risk):

- Younger gestational age
- Very low birth weight
- Small for gestational age

Hypoxic (low oxygen) conditions have also been identified as a potential risk factor for NEC. In preclinical studies, repeated hypoxia-induced exposures, in addition to bacterial challenge and enteral feeding, reliably reproduce a model of NEC. A premature infant may have several hypoxic challenges per day due to the immature nature of their nervous system. Therefore, mechanistic research into animal models has direct clinical relevance to human infants with NEC.

In addition to the risk factors described in Box 1.3, clinical risk factors are broken out into three general categories – maternal/antenatal, infant, and postnatal factors.

Several potential risk factors that were initially identified have not held up to rigorous study and population-level analysis. For example, delayed enteral feeding and trophic-level feeding were originally identified as potential risk factors, but the data revealed that these characteristics are not

associated with increased risk for NEC (Rose and Patel, PMID 30115546[22]; Young, PMID 35049036[23]; Morgan, PMID 23543508[24]). Cesarean delivery has not been consistently associated with increased risk of NEC. Delayed cord clamping was originally suggested as a potential protective factor, but recent analysis suggested that this procedure does not decrease the risk of NEC. With larger, population-level studies, infant sex has also been shown not to be associated with NEC risk.

Box 1.4: Protective Factors against NEC

- Breastmilk diet
- Human milk oligosaccharides (HMO)
- Proper EGF signaling pathway
- Antenatal steroids

In addition to research into risk factors, researchers are interested in identifying potential protective or preventative factors. As described previously, the majority of NEC cases develop in premature infants. Supporting maternal health to delay births beyond the window of higher NEC risk (approximately 34 weeks gestation) is the primary prevention strategy. Additional potential protective factors are listed in Box 1.4 and further detailed below.

A diet containing human breastmilk appears to be protective against the development of NEC. In addition, the use of human milk-based fortifier in the infant's diet, rather than bovine-based fortifier, may be useful in reducing the development of NEC.

One of the components of breastmilk, human milk oligosaccharides (HMO), may be protective against NEC. HMOs act as prebiotics, fostering the growth or activity of beneficial microorganisms. Therefore, screening milk and/or milk fortifiers to ensure that they contain HMOs may help to prevent NEC by preventing the development of microbial dysbiosis. HMOs also help to preserve intestinal architecture, support intestinal perfusion, and decrease proinflammatory cytokines. Animal studies have shown that adding the synthetic HMO, 2'-Fucosyllactose, to mouse formula caused the intestine to maintain its overall architecture even when challenged with NEC-associated bacteria. Fewer proinflammatory cytokines were measured in this setting as well.

Other animal models have shown that normal levels of epidermal growth factor (EGF) and normal functioning of the EGF receptor (EGFR) may be protective against NEC. EGF promotes maturation

[22] https://pubmed.ncbi.nlm.nih.gov/30115546/
[23] https://pubmed.ncbi.nlm.nih.gov/35049036/
[24] https://pubmed.ncbi.nlm.nih.gov/23543508/

of the gut epithelium through the EGFR (Duh, PMID 11102549 [25]). In addition, EGFR helps to protect intestinal barrier integrity, facilitating absorption of nutrients. Breastmilk enhances crypt proliferation via EGF/EGFR. Crypts are the base of the intestine where a lot of stem cells exist. In mouse NEC models, these crypts were unhealthy and did not divide to make new cells. In mice without NEC, these cells divide in a controlled way to maintain the structure of the intestine wall. EGF is now being explored for novel therapeutic targets.

Antenatal steroids and/or tocolysis (medications used to delay delivery) appear to be protective against the development of NEC.

Development of NEC Laboratory Models

Research into NEC is advancing at a fast pace. *In vitro* models of NEC are developing rapidly. NEC-in-a-dish models grow intestine samples in culture. Additionally, cultures on microfluidic chips can be used to grow villus-like structures that mimic the intestine to which a bacteria or microbiome from an infant with NEC can be introduced to examine gene expression and pathophysiology. In these NEC-on-a-chip models, loss of tight junctions was visualized.

To understand the contribution of cell types and how different types of cells interact during NEC pathogenesis, researchers are developing novel *in vitro* experimental models that allow analysis at a single-cell level. These clusters of different cell types from the gut are called enteroids (Frazier[26]; Lanik[27]). When the enteroids are exposed directly to bacteria or to bacterial products from an affected infant's intestine, this model recreates key features of NEC. Specifically, gene expression in the enteroid cells is similar to that in the cells of human infants with NEC. Additionally, the tight junctions between the cells disappear, and fluid can more easily "leak" from the enteroid cell wall.

Gaps in Knowledge

As described previously, it is important to have a standard definition of NEC to enable consistent data collection. This would also enable use of common data elements—precisely defined questions and a set of specific allowable responses that can be used to collect, compare, and analyze data between hospitals, observational studies, and randomized clinical trials.

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[25] https://pubmed.ncbi.nlm.nih.gov/11102549/

[26] Frazer, Author Spotlight: Enhancing Understanding and Treatment Strategies with the NEC-on-a-Chip Model (jove.com)

[27] Lanik, JCI Insight - Microfluidic device facilitates in vitro modeling of human neonatal necrotizing enterocolitis–on-a-chip

Pathogenesis Knowledge Gaps

Current insights into NEC pathogenesis have come largely from preclinical animal models, as the newborn infant is extremely vulnerable and generally unavailable for intensive research studies. Identifying associations between risk factors and the development of NEC has helped researchers develop models of the disease in the laboratory. These models may potentially help identify several factors that are protective against NEC, specifically related to the gut barrier. In humans, the longer infants are on antibiotics, the higher risk of dysbiosis and gut barrier breakdown.

NEC is a complex, multifactorial disease involving an exaggerated pro-inflammatory immune response to bacterial dysbiosis. With the cells of premature infants still developing and learning what a "healthy normal" is, the overgrowth of bacteria or viral products in the intestines can upset the balance of that system, causing the immune system to overreact.

The preclinical work in animal models has allowed researchers to untangle some of these factors in a more precisely controlled setting. Mouse models cannot be delivered prematurely, but other nutritional, environmental, bacterial, and viral factors can be changed to induce NEC. Preclinical models can recreate key components thought to be influential in developing NEC (Nolan, PMID 34816129[28]):

- Hypoxia
- Gut dysbiosis
- Loss of tight junctions
- Vascular involvement
- EGF signaling pathway disruption in crypt cells
- Disruption in intestinal perfusion

Several novel potential therapeutic targets identified in preclinical animal models are currently being explored as future treatments to prevent or lessen the severity of the disease. EGF, heparin-bound EGF, prebiotics, probiotics, amniotic fluid, extracellular vesicle delivery of anti-inflammatory cytokines and other drugs are all being explored as potential therapies.

NEC Risk Factor Knowledge Gaps

More research is needed to understand NEC and its related diseases and common risk factors. Some clinical cases that present similarly to NEC may in fact be different diseases with different pathogenesis. For example, thickening of the meconium (meconium inspissation) in very low birth weight infants may be due to the unique gut biology during the same time period in development. Spontaneous intestine perforation (SIP) also can be confused for NEC, and the differences in

[28] https://pubmed.ncbi.nlm.nih.gov/34816129/

disease initiation and progression between NEC and SIP remain unclear. More research is needed to determine the causes and disease initiation processes to determine whether SIP is similar to NEC in mechanism and/or disease progression.

Milk fortifiers are concentrated supplements that are added to milk to provide extra nutrition for infants who need more calories and protein than milk alone can provide. More research is needed to understand whether the use of a human milk-based fortifier rather than a bovine-based (cow's milk) fortifier may help to reduce the risk of NEC.

Currently, epidemiological research on the prevalence of NEC is limited. Additional research, particularly with the aim of standardizing the clinical definition of NEC, will help to better characterize the scope of the problem at a population level. Epidemiological research can help clarify the risk of NEC in moderate preterm infants and to classify the gestational time point at which the overall risk for development of NEC decreases. Additionally, the current evidence for antibiotic use as a risk factor for NEC is unclear, as several small studies have shown conflicting outcomes. More research is needed to understand the relative risks of antibiotic use in the premature infant population. Additional research is also needed to understand the risk factors affecting the subpopulation of infants that also have cardiovascular complications. The Working Group's consensus was that NEC is a multifactorial disease with many associated risk factors. Maternal and antenatal factors that place preterm infants at a higher risk for NEC include preeclampsia and hypertension. Antenatal steroids and/or tocolysis (e.g., medications used to delay delivery) appear to be partially protective against developing NEC. Infant perinatal factors that increase the risk of NEC include preterm birth, very low birth weight (less than 1,500 grams or 3.3 pounds), and being small for gestational age. Infant postnatal factors that may affect the risk of developing NEC include antibiotic exposure (variable risk) and human milk exposure, which lowers an infant's risk of developing NEC.

Section 2. Evidence Base for Current Approaches to Nutritional Support for Preterm Infants

The American Academy of Pediatrics recommends that very low birth weight infants receive human milk, preferably from their own parent. If parent's milk is not available, pasteurized donor human milk is recommended (Parker, PMID 34635582[29]). When infants are born preterm, the parent may not be able to produce enough milk to meet their infant's needs. In these cases, providers usually use either donor milk or preterm infant formula to supplement the parent's milk. In addition, human milk, whether from the parent or donated, often requires fortification to meet the nutritional needs of very low birth weight infants.

Where do NICUs obtain donor milk?

Most donor milk fed to infants in NICUs in the United States originates from the Human Milk Banking Association of North America (HMBANA), a non-profit organization consisting of 32 member banks serving more than 1,500 hospitals. Milk donors are volunteers and are screened like blood donors. In 2023, HMBANA dispensed approximately 10 million ounces of milk.

Specific Methods and Evidence Related to Using Different Nutritional Support Practices

Outcomes studied

Most studies of NEC are focused on infants who were born extremely preterm or are of very low birth weight. The endpoints examined in recent NEC research tend to focus on the risk of developing NEC with various feeding approaches, the time it takes infants to get to full feeds (i.e., receiving all nutrition as milk feeds either from human milk or formula), and intestinal permeability. Refer to Section 3 for a discussion of base diet and milk fortifiers and the impact on risk for NEC in preterm infants.

Parent's milk vs. donor milk

Human milk contains water, nutritional carbohydrates (including lactose), lipids (nutritional fats), proteins, immune cells, HMOs, lactoferrin, cytokines, probiotic bacteria, antibodies, minerals, and hormones. Parent's milk and donor milk may be delivered differently (breast vs. bottle, nasogastric tube) and may have undergone different storage and processing (e.g., fresh, pumped, refrigerated, frozen, or thawed). This affects the composition of the milk.

[29] https://pubmed.ncbi.nlm.nih.gov/34635582/

The composition of milk that a parent of a preterm infant produces may differ from the milk the parent of a term infant. Moreover, the milk a parent produces shortly after birth (colostrum) will differ from "mature" milk produced weeks or months later. Donor milk banks collect all of these milk variations, blend milk from multiple donors together (pooling), and pasteurize it. The final product is, therefore, not the same as milk from the infant's parent.

Effect of pasteurization on milk composition. Donor milk requires pasteurization, which may inactivate some of the beneficial components of non-pasteurized human milk. Pasteurization is designed to destroy potentially harmful microorganisms by either heating milk to below the boiling point or applying elevated pressure. Various pasteurization methods are used in milk banks and may have differing impacts on macronutrients, micronutrients, and bioactives. Many beneficial properties are retained, although heat-labile enzymes, micronutrients, and other properties of the milk may be altered. There is no evidence of toxicity as a result of pasteurization.

Box 2.1: Formula Use and NEC Risk

Available evidence supports the hypothesis that it is the absence of human milk – rather than the exposure to formula – that is associated with an increase in the risk of NEC.

Formula fed vs parent milk feeding. Current evidence suggests that infants who were fed formula had higher gut permeability compared to those fed parent's milk.

Formula vs (any) human milk. Current evidence suggests that among premature infants, the use of pasteurized donor milk as a supplement from start of feeding is associated with approximately a 50% reduction in NEC risk compared to preterm formula.

It is important to note that even infants that only receive human milk, or even parent's milk, may still develop NEC.

Effect of freezing on milk composition. Human milk—both from the parent and from donors—may be frozen and thawed, in some cases multiple times before use. Studies show that freezing human milk increases unsaturated fatty acids and decreases antioxidant activity, bactericidal activity, cell viability, and milk acidity.

Therapeutic Options for Prevention and/or Treatment of NEC in Premature Infants

Clinical risk factors for NEC have led to several key biological insights into disease pathogenesis. The human intestine includes immune cell receptors and inflammatory detection receptors (such as Toll-like receptor 4 [TLR4] and T helper 17 cells [Th17]) that detect problems in the gut. In the intestine of a premature infant, the signals from these receptors can be excessive (such as increased TLR4 ligands and gut bacterial dysbiosis) and may impact an infant's susceptibility to NEC. Reactive oxygen species and other alterations lead to loosening of the tight junctions between cells lining the intestines, contributing further to inflammation. One cause of dysregulated signaling in the gut is the presence of bacterial dysbiosis or imbalance in the bacterial communities that make up the gut microbiome. Dysbiosis can include too much or too little of certain gut microbes and/or the wrong types of bacteria or viruses. Bacterial signaling has been shown to be a

necessary component of NEC pathogenesis. Mice without exposure to bacteria never develop NEC. In human infants' intestines eventually become colonized with the bacteria necessary to digest food and absorb nutrients. Understanding how and why these bacterial colonies become unbalanced may provide key insights into how NEC develops.

Box 2.2: Highlighted Therapeutic Targets for the Prevention/Treatment of NEC

- Bacterial signaling in the premature gut leads to NEC, likely via the TLR4 pathway.
- Inhibiting TLR4 signaling reduces NEC in animal models and in *ex vivo* tissue from infants with NEC.
- Administration of amniotic fluid in piglets treats NEC.
- PUMA, Notch, and nitric oxide may be therapeutic targets to reduce the severity of NEC.
- Proinflammatory lymphocytes accumulate in NEC, likely via IL-17-mediated pathway.
- HMOs bind to TLR4 and LPS *in silico* and may block TLR4 signaling *in vivo*.
- Enhancing gut motility (potentially with the J11 drug treatment) prevented NEC in mice.

Increased inflammatory signaling in the intestine has been shown to increase the risk of NEC. TLR4 signaling regulates the balance between injury and repair in the intestinal environment. Bacterial dysbiosis in the gut produces a lot of TLR4 ligands, which bind to TLR4 receptors. TLR4 receptors are expressed on several cell types within the intestine. Increased TLR4 signaling in the presence of bacteria can lead to cell death and the inability of stem cells to repair damaged intestinal tissue. Breastmilk does contain TLR4 inhibitors, which dampen the signal from the bacterial ligands.

Removing TLR4 completely from the endothelium cells in the gut protects against NEC. In targeted experiments to understand the mechanism behind this, researchers determined that increased endothelial nitric oxide synthase (eNOS) levels in the blood vessels around the gut protect against NEC. Nitric oxide in blood vessels leads to constriction and brings the gut containing dysbiotic bacteria closer to the blood vessels. Therefore, when eNOS levels are decreased in the blood vessels, NEC is able to spread further and faster through the gut. The bacteria spread up the walls of the intestine, and the vasoconstriction changes the oxygen contents within small areas of the intestine, creating an environment where anaerobic bacteria overgrow compared to healthy levels. Breastmilk can counteract the vasoconstrictive effect of TLR4 signaling through one of its protective components, sodium nitrate. The addition of sodium nitrate to formula also achieves reduced NEC severity and is being explored as a possible therapeutic option. Additional components in breastmilk also act as TLR4 antagonists in intestinal cells, and this is an active area of research for potential therapeutic options.

TLR4 signaling is also important in the stem cells of the gut and in the regulation of the number of goblet cells in the small intestine. When TLR4 signaling is absent, increased goblet cells are found in the small intestine. A lack of goblet cells is also seen in NEC. In researching the mechanism behind this association, researchers found that the TLR4 signaling turns on signaling in the Notch

pathway, which directly regulates goblet cell proliferation. Therapies targeting the Notch pathway have successfully decreased the severity of NEC in animal models.

Breastmilk also inhibits TLR4 signaling directly to prevent NEC by turning on the signaling pathway for EGF, a protein that stimulates cell growth and differentiation. Oligosaccharides—unique combinations of glucose, galactose, N-acetyl-glucosamine, fucose, and sialic acid—in human milk have also been found to reduce NEC risk. The 2'FL oligosaccharide has been clearly demonstrated to protect against NEC, and other oligosaccharides are being investigated as potential therapeutics. Both the 2'-FL and 6'SL oligosaccharides block the lipopolysaccharide, bacterial motif, and TLR4 ligand binding site on the TLR4 molecule. These components in breastmilk can be utilized as a therapeutic option alone or together with other therapies to decrease TLR4 signaling in early premature infants.

A molecule called p53 Upregulated Modulator of Apoptosis (PUMA) is turned on in intestinal stem cells during the process of self-destruction (called apoptosis). Increased PUMA signaling in intestinal stem cells is associated with NEC. Blocking PUMA signaling with a drug prevents the intestinal stem cells from dying and reduces the severity of NEC. Targeting PUMA and other receptors of the Notch signaling pathway stabilizes the dysregulation of the intestinal cell sub-populations and reduces the severity of NEC in animal models.

In addition to bacterial dysbiosis, lymphocyte influx is also required for NEC development. In immunocompromised mice treated to induce NEC, NEC did not develop. When researching the mechanism for this, researchers discovered that interleukin-17 receptor (IL17-R) signaling is responsible for recruiting lymphocytes to the site of inflammation and has a protective effect. Inhibiting IL17-R signaling reverses mucosal damage caused by NEC in animal models. Similarly, recruiting additional adaptive immune cells, called regulatory T (Treg) cells, using retinoic acid treatment prevented NEC in the intestines of animal models. This finding is being explored as a potential therapeutic option for NEC.

Proper motility of the gut allows food movement and nutrient absorption. In premature infants with NEC, gut motility is impaired with little or no smooth muscle contraction to move food and bacteria along the length of the gastrointestinal tract. Motility-increasing therapeutic options have demonstrated a reduction in NEC severity and risk in animal models. Specifically, enteric glial cells help with gut motility and are damaged during the pathogenic processes of NEC. A glia-enhancing drug restored motility and reduced NEC in an animal model.

Research into the pathogenesis and biological mechanisms of NEC has yielded several avenues for potential therapeutic targets to prevent or treat NEC in premature infants. Bacterial signaling through the TLR4 pathway leads to NEC, and therapeutic options that decrease TLR4 signaling confer a protective effect in animal models. One potential drug, Compound 34 (C34), blocks TLR4 signaling upstream, and clinical trials for this potential therapy are currently being planned. One experimental observation that merits follow-up studies is that breastmilk contains TLR4 inhibitors and can block TLR4 signaling via EGF pathway signaling in the mouse model. Interestingly, the administration of amniotic fluid in piglets also treats NEC and merits follow-up to identify potential therapeutic targets. Additional therapeutic targets for NEC include targets within the stem cells

(PUMA) and goblet cells (Notch) of the gut, which decrease in NEC. Counteracting the vasoconstriction seen in NEC via nitric oxide or retinoic acid is another promising avenue of research. Overall, several concurrent avenues for therapeutic options are being explored to provide treatment options for the different clinical presentations of NEC.

Gaps In Research for Nutritional Support in Extremely Premature Infants

The infant gut has evolved to be exposed to amniotic fluid until full-term birth at 40 weeks gestational age and then to human milk. Therefore, the premature infant's gut has less exposure to amniotic fluid than a full-term infant's gut. The consequences of this are not well understood. What we do know is amniotic fluid and human milk share many factors that facilitate normal gut development. As the gut matures, it becomes less permeable and this decrease in permeability is a measure of maturation.

From the Working Group's review, a better understanding of the optimal nutritional needs of preterm infants is needed. Basic science research examining the nutrition provided by the placenta, amniotic fluid, and other sources *in utero* would advance our understanding of the nutritional requirements and supplements needed by premature infants.

Being able to increase human milk available for infants in the NICU may help reduce the risk of NEC. Strategies are needed that can enhance the ability for a parent to produce enough milk for their baby and increase parents' knowledge and support for lactation practices. Relevant social determinants of health, particularly the role of access to parental leave, parent ability and knowledge around pumping practices, and economic factors related to donor milk and fortifiers, require additional research from a community health perspective.

Clinical trials of multi-nutrient fortification versus unfortified human milk have not shown a significant effect on NEC, although the evidence is of low certainty (Cochrane, PMID 35658821[30]). More information is needed to make a definitive conclusion on the current evidence available on fortifiers. Only several hundred infants have been included in studies comparing human milk-derived fortifiers (HMF) to bovine milk-derived fortifiers (BMF), with only a few of these participants developing NEC (Sullivan, PMID 20036378[31]; O'Connor, PMID 29878061[32]; Jensen, PMID 38545091[33]; Christofalo, PMID 23968744[34]; Kumbhare, PMID 36029771[35]). Due to small sample sizes, it is not possible to reach a definitive conclusion from the clinical trials to date. Larger trials

[30] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7268980/
[31] https://pubmed.ncbi.nlm.nih.gov/20036378/
[32] https://pubmed.ncbi.nlm.nih.gov/29878061/
[33] https://pubmed.ncbi.nlm.nih.gov/38545091/
[34] https://pubmed.ncbi.nlm.nih.gov/23968744/
[35] https://pubmed.ncbi.nlm.nih.gov/36029771/

are needed to increase statistical power to answer this question. Trials to develop evidence in this area will be costly.

Though there seems to be sufficient supply of donor human milk to feed the premature infants at highest risk for NEC (e.g., those born very low birth weight or less than 1,500 grams) (Parker, PMID 32103161[36]), we need a better understanding of why some hospitals are not participating in donor milk programs. Expense may be one potential barrier to providing donor milk, as it is not reimbursable in all state Medicaid and private insurance plans, creating financial strains for hospitals and access issues for patients. Hospitals with greater than 75% Medicaid recipients and hospitals with a high proportion of African American or Hispanic patients are less likely to use donor milk than their counterparts (Rose, PMID 35379899[37]). As such, access to donor milk is a healthcare disparity for preterm infants.

Mechanisms to support drug or product development may be another approach to address gaps, especially given that NEC is a rare disease. Public- and private-sector small business and technology transfer research programs can support preliminary work for new product development in this area. In addition, programs focused on orphan therapies and rare diseases could help address some of the need for NEC research.

[36] https://pubmed.ncbi.nlm.nih.gov/32103161/
[37] https://pubmed.ncbi.nlm.nih.gov/35379899/

Section 3. Associations between Feeding Practices and Risk or Severity of NEC

Data Associating Feeding Practice with NEC

As stated previously, current clinical guidance from the American Academy of Pediatrics recommends that human milk, preferably parent's milk, be the first choice for feeding very low birth weight infants. In some cases, parents of preterm infants do not produce milk, or do not produce enough milk for their infants' needs. If parent's milk is not available in sufficient quantity, pasteurized donor human milk is recommended as the second-most preferred option, followed by preterm formula (Parker, PMID 34635582[38]). Parent's milk will likely be provided for a longer period of time than donor milk for a high-risk infant, which likely confers additional benefits. Human milk often requires fortification to meet the nutritional needs of very low birth weight infants.

Base Diet, Milk Fortifiers, and Risk for NEC in Preterm Infants

The Neurodevelopmental Effects of Donor Human Milk vs. Preterm Formula in ELBW infants (MILK) trial, conducted by the NICHD Neonatal Research Network (NRN), found that extremely preterm infants fed donor human milk had half the rate of NEC compared to their formula-fed counterparts (Colaizy, PMID 38497706[39]). Researchers randomly assigned 483 infants born before 29 weeks gestation or who weighed less than 1,000 grams (about 2.2 pounds) at birth to receive formula or donated human milk. Some infants in each group received their own parent's milk; this was in small amounts, as their parents either stopped producing milk before 21 days or could only produce a small supply. NEC developed in 10 infants (4.2%) in the donor milk group, compared to 22 infants (9%) in the formula group. Thus, human milk significantly reduced, but did not eliminate, the risk of NEC compared to formula feeding.

Comparison of composition of human milk (parent/donor) and formula

Human breastmilk contains water, nutritional carbohydrates (including lactose), lipids (nutritional fats), proteins, immune cells, HMOs, lactoferrin, cytokines, probiotic bacteria, antibodies, minerals, and hormones. Infant formula made from cow's milk protein and other ingredients is modified to be similar to human milk. Human milk differs from cow's milk in the levels and/or types of macronutrients (protein, fat, and carbohydrates) and minerals it contains. Human milk also

[38] https://pubmed.ncbi.nlm.nih.gov/34635582/
[39] https://pubmed.ncbi.nlm.nih.gov/38497706/

contains a significantly higher amount of lactose and unique HMOs. HMOs are complex sugars that play a role in infant gut health. They are almost absent in cow's milk.

When considering parent's milk and donor milk in the NICU, it is important to note that colostrum and mature milk are different. Colostrum is the nutrient-rich and antibody-dense milk a parent produces for the first few days after giving birth. Colostrum is concentrated with nutrients and antibodies that strengthens the infant's immune system and is helpful for fighting infection. Parents produce mature milk about two weeks after giving birth.

As mentioned in Section 2, donor milk is expressed by a donor volunteer via pumping and subsequently undergoes pasteurization, processing, storage (refrigeration or freezing), and possibly pooling. These processes can affect the composition of the final milk product. Some, but possibly not all, HMOs can withstand donor milk processing procedures.

Fortification approaches

A critical period of brain growth and gastrointestinal development occurs in the third trimester. For infants born premature, nutrition provided in the NICU promotes this growth. Human milk is often fortified with nutrients to meet the nutritional needs of preterm infants. How this fortification of human milk is done varies across NICUs. Infants can be fed parent's (or donor) milk fortified with a human milk-based fortifier (HMF), parent's (or donor) milk fortified with a bovine milk-based fortifier (BMF), or preterm infant formula.

Box 3.1: Milk Fortifiers

Human milk-based fortifier is made from donated, pasteurized human milk whereas the protein from **bovine milk-based fortifier** is made from cow's milk. Both fortifiers include nutrients and minerals to promote growth and development in preterm infants.

Human milk with HMF may reduce the risk of NEC compared to human milk with BMF. However, as described in Section 2, only a limited number of studies have examined this topic, and the available studies had small sample sizes with too few cases of NEC to draw clear conclusions. In the Canadian Donor Milk for Improved Neurodevelopmental Outcomes (DoMINO) trial, 363 very low birth weight infants were randomized to receive either pasteurized donor milk with BMF or preterm formula whenever parent's milk was not available. Though fewer infants in the donor milk group (1.7%) had NEC than in the formula group (6.6%) (O'Connor, PMID 27825008[40]), these results were based on only 15 cases of NEC. The data on the benefits and risks of using preterm infant formula

[40] https://pubmed.ncbi.nlm.nih.gov/27825008/

are inconclusive and the differential risk needs to be better understood. More studies with larger sample sizes are needed, but a persistent barrier is the high cost of conducting these studies.

How does bovine protein exposure relate to NEC?

It is unknown whether human milk fortified with an HMF, compared to human milk fortified with a BMF, could decrease the risk of NEC. There are few rigorous studies that have addressed these questions, and the study numbers were too small to make definitive conclusions.

In a study by Jensen and colleagues, after examining the effects of HMF vs. BMF in very low birth weight infants fed exclusively human milk, there was no significant difference in the incidence of NEC (Jensen, PMID 38545091[41]). Approximately 250 infants were measured in this randomized controlled trial. Sullivan and colleagues found that among 207 extremely preterm infants, an exclusively human milk-based diet is associated with lower rates of NEC when compared with a parent's milk-based diet that also included bovine milk-based products (Sullivan, PMID 20036378[42]). Maternal milk intake in both bovine and human milk groups was high, which provides an unblinded assessment of fortifier type.

Summary of findings on impact of base diet for NEC risk

As the proportion of parent's milk feeding goes up, the risk of NEC goes down. A 2019 Cochrane review suggests the relative risk of NEC for formula-fed premature infants is higher than for infants fed with fortified donor milk. In Schanler and colleagues' study of 243 extremely premature infants, those with parent's milk had fewer episodes of NEC compared to infants fed with donor milk or preterm formula (Schanler, PMID 16061595[43]). However, 21% of infants receiving donor milk had to be switched to preterm formula due to poor weight gain. Overall, the available research suggests that among infants between 23-28 weeks gestational age, the use of donor milk is associated with an approximate 50% reduction in NEC risk compared to a preterm formula-based diet. The effect size of the reduction in NEC risk is similar whether or not the infants also received parent's milk as part of the base diet.

Gaps in Research Regarding the Relationship between Feeding Practices and NEC

We do not have a full understanding of the optimal nutritional needs of premature infants and how those needs may vary by gestational age at birth. We also need better ways to encourage breastfeeding and therapies to optimize parent's lactation for preterm (and term) infants.

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[41] https://pubmed.ncbi.nlm.nih.gov/38545091/
[42] https://pubmed.ncbi.nlm.nih.gov/20036378/
[43] https://pubmed.ncbi.nlm.nih.gov/16061595/

Further study is needed to optimize how donor and/or parent's milk is processed for safety and for preserving nutritional content (fresh vs. frozen, pasteurized, etc.). We also need to identify barriers to accessing donor milk—why do some hospitals not participate in donor milk programs, what disparities exist in access to donor milk (especially for rural communities, safety net hospitals, and under-represented populations), and how can we overcome these barriers.

A better understanding also is needed of how feeding practices influence NEC risk, timing of onset of NEC, severity, mortality, and long-term effects on survivors. We do not know why some premature infants who receive human milk still get NEC. Specific feeding practices for fortification methods (HMF vs. BMF), as well as the timing and method of transitioning from donor milk to formula need further research.

Finally, nutritional additives to prevent and/or treat NEC need to be developed, and the safety and efficacy of these additives (such as probiotics) need to be tested. Whether such additives work individually, or in combination with others, should be considered.

Section 4. Recommendations to Improve the Evidence Base for NEC Risk Factors and Nutritional Support for Preterm Infants

The NEC Working Group submits the following recommendations to the Secretary of HHS regarding research on NEC Risk Factors and Nutritional Support for Preterm Infants based on information assembled during three meetings. The Working Group developed these recommendations and presented them to the National Advisory Child Health and Human Development Council on September 5, 2024. NACHHD approved these proposed recommendations and provided concept clearance to implement them.

The Working Group reviewed the state of the science for risk factors for NEC, nutritional support (i.e., enteral feeding practices) for preterm infants, and associations between feeding practices and risk or severity of NEC. The recommendations were developed to improve the evidence base and do not represent clinical guidance. Please refer to practice organizations for specific clinical management guidance. The recommendations were not prioritized, but are grouped into six themes: overarching, epidemiology of NEC, mechanisms of NEC, factors affecting risk of NEC, nutritional support for premature infants and NEC, and the relationship between feeding practices and NEC.

Recommendations - Overarching

1. **Expand research on the prevention of premature birth, including ways to delay impending births beyond the window of higher NEC risk.**
    - The best method to prevent most NEC cases is to prevent preterm birth.

2. **Develop a standardized definition of NEC and severity of NEC to improve epidemiologic studies and determine eligibility for trials and treatments.**

3. **Ensure that research on NEC incorporates the perspectives of affected families.**

Recommendations – Epidemiology of NEC

4. **Improve tracking and reporting of NEC cases as well as deaths.**
    - Expand epidemiologic data collection, using a common definition, to better quantify the total burden of disease.
    - Implement updated ICD-10 codes that are more specific to NEC and NEC severity. Separate out spontaneous intestinal perforation and other conditions (e.g., meconium inspissation).

- Track NEC cases by gestational age, as there may be differences between NEC that develop in early preterm infants versus late preterm or term infants.
  - NEC in term infants may be associated with cardiac issues.
- Investigate the mechanisms behind the racial and ethnic disparities in NEC risk.

5. **Expand support for neonatal biorepositories to facilitate research to identify diagnostic, prognostic, predictive, susceptibility, and/or surrogate markers of NEC.**
   - Support collection of biospecimens and nutritional data for NEC research and encourage data and specimen sharing.
   - Improve early diagnosis of NEC by identifying biomarkers prior to disease onset.
   - Once biomarkers are identified, use these as additional diagnostic criteria in clinical studies on NEC.

6. **Explore innovative methods for collecting and analyzing data.**
   - Expand data collection to improve estimates for attributable risk for NEC:
     - Disease rate attributable to exposures (e.g., gestational age, antibiotics)
     - Correct diagnosis rate attributable to a positive predictive result (e.g., Bell Stage 3 diagnosis)
     - NEC rate attributable to a treatment (e.g., specific feeding strategy).
   - Consider whether open-ended artificial intelligence or machine-learning analyses of electronic health records of infants with and without NEC could aid in diagnostic criteria or prediction.

Recommendations – Mechanisms of NEC

7. **Expand research to describe the biologic mechanisms of NEC development.**
   - Identify potential prevention and treatment targets (e.g., activated signaling pathways triggering intestinal cell death or gut barrier breakdown) that more clearly link biological pathways to the development and/or progression of NEC.
   - Explore genetic and epigenetic risk factors and mechanisms involved in NEC, including rare monogenic diseases that might predispose infants to NEC.
   - Explore risk factors during pregnancy and around the time of birth.

8. **Improve animal and laboratory models to better reflect the disease's complexity.**
   - Expand NEC-in-a-dish, NEC-on-a-chip, gastrointestinal organoid systems, and animal models (e.g., stroma, vasculature, and immune cell) to match the complexity of NEC mechanisms and risk factors.

Recommendations – Factors Affecting Risk of NEC

9. **Explore exposures that may be positively or negatively associated with risk of NEC development, severity, and mortality.**
    - Maternal/antenatal factors, such as preeclampsia
    - Nutritional components
    - Medications, including antibiotics
    - Bacterial dysbiosis and viruses.

10. **Expand research on feeding practices that may affect NEC risk.**
    - Timing of initiation/progression and development of NEC.
    - Components of parent's milk, donor milk, fortifiers, and/or formula.

Recommendations – Nutritional Support for Premature Infants and NEC

11. **Support research to identify the optimal nutritional needs of preterm infants, how these may vary by gestational age at birth, and the specific nutritional needs of NEC survivors.**

12. **Increase research to support parent's milk production and lactation techniques for preterm and term infants.**
    - This will benefit human milk availability for both preterm and term infants.

13. **Evaluate how the collection, processing, storage, and dispensing of donor and/or parental milk might impact its nutritional content and impact on intestinal health.**
    - Identify bioactive components of human milk that may be protective for NEC and how to preserve these factors while still protecting against infection.
    - Develop rapid, point-of-care diagnostics to measure these beneficial milk components in NICUs and donor banks.
    - Research potential standards for donor milk – postpartum donation timing, milk composition, pooling practices, and optimization for specific populations (e.g., very low birth weight infants).

Recommendations – Feeding Practices and NEC

14. **Support large clinical trials of feeding practices and NEC risk including timing of onset, severity, mortality, and long-term effects on survivors.**
    - Because NEC is a relatively rare outcome, large trials are needed to have enough statistical power to definitively answer relevant research questions.
    - Evaluate different fortification methods.
    - Determine the best timing and method of transitioning off donor milk.

15. **Explore innovative clinical trial designs to test, via applicable Investigational New Drug (IND) regulations, the safety and efficacy of promising drugs and biologics to prevent and/or treat NEC.**
    - Determine ways to identify, manufacture, and safely test live biotherapeutic products in neonates.
    - Establish ways to test multiple components working together, rather than individual components alone.

16. **Expand implementation science research to reduce disparities related to NEC and in the availability of parent's and donor milk.**
    - Address barriers to access pasteurized donor milk for populations in which it is recommended when a parent's milk is unavailable (e.g., very low birth weight infants), especially for rural communities and underrepresented populations.
    - Enhance community, family, and institutional support for breastfeeding and milk donation.
    - Evaluate economic and workplace interventions for lactation support.

17. **Support research on parent education, both before delivery and in the NICU, about feeding practices and risk of specific conditions, including NEC.**

NEC is a devastating disease. One baby dies every day in the United States from NEC, and those that survive may undergo traumatic surgeries, be neurodevelopmentally impaired, and have lifelong consequences. Without more research to improve the evidence base, parents and healthcare practitioners do not have adequate information about what and how best to feed premature infants to maintain a healthy growth rate while preventing or minimizing the risk of developing NEC. Parents, their offspring, and families would greatly benefit from having this essential information. The NEC Working Group of Council urges the Secretary to take action on these recommendations.

Appendix A. Necrotizing Enterocolitis Working Group of Council Members

Chairpersons

Yvonne (Bonnie) Maldonado, M.D.
Council Member, National Advisory Council of Child Health and Human Development
Senior Associate Dean, Pediatric Infectious Disease
Stanford University

Ravi M. Patel, M.D., M.Sc.
Professor of Pediatrics
Division of Neonatology
Emory University School of Medicine and Children's Healthcare of Atlanta

Members

Steve Abrams, M.D.
Director of Dell Pediatric Research Institute
Dell Medical School
University of Texas Austin

Mandy Brown Belfort, M.D., M.P.H.
Associate Chief of Research
Associate Professor of Pediatrics
Harvard Medical School

Jennifer Canvasser, M.S.W.
Founder, Director
Necrotizing Enterocolitis Society

Tarah T. Colaizy, M.D., M.P.H.
Professor of Pediatrics - Neonatology
University of Iowa Health Care
Stead Family Children's Hospital
Director, Mother's Milk Bank of Iowa

Mark R. Corkins, M.D.
Division Chief of Pediatric Gastroenterology
Professor of Pediatrics
University of Tennessee
Health Science Center

C. Michael Cotten, M.D., M.H.S.
Professor of Pediatrics
Chief, Division of Pediatric Neonatology
Duke University Division of Pediatric Neonatology

Eric C. Eichenwald, M.D.
Chief of the Division of Neonatology
Children's Hospital of Philadelphia
American Academy of Pediatrics Committee on Fetus and Newborn

Misty Good, M.D., M.S.
Division Chief, Neonatal-Perinatal Medicine
Associate Professor
University of North Carolina at Chapel Hill School of Medicine

Munish Gupta, M.D., M.M.Sc.
Assistant Professor in Pediatrics
Harvard Medical School
Beth Israel Deaconess Medical Center
Chair, American Academy of Pediatrics Section on Neonatal-Perinatal Medicine

Jack Moye Jr, M.D.
Maternal and Pediatric Infectious Disease Branch
Eunice Kennedy Shriver National Institute of Child Health and Human Development
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Brenda B. Poindexter, M.D., M.S.
Marcus Professor of Pediatrics
Division of Neonatology
Emory University School of Medicine

David Rowitch, M.D., Ph.D.
Head, Department of Pediatrics
University of Cambridge, United Kingdom
Adjunct Professor of Pediatrics
University of California, San Francisco

Emre Seli, M.D.
Professor of Obstetrics
Gynecology & Reproductive Sciences
Yale School of Medicine
Chief Scientific Advisor
March of Dimes

Lisa Stellwagen, M.D., FAAP
Clinical Professor Emerita of Pediatrics
Medical Director, UC Health Milk Bank
University of California, San Diego
President-Elect, Human Milk Banking of North America

Stephanie Wilson Archer, M.A.
Pregnancy and Perinatology Branch
Eunice Kennedy Shriver National Institute of Child Health and Human Development
National Institutes of Health

Joan Younger Meek, M.D., M.S.
Professor Emerita, Clinical Sciences
Florida State University
College of Medicine

Ex Officio

Andrew Bremer, M.D., Ph.D., M.A.S.
Director
Office of Nutrition Research
National Institutes of Health

Janelle Gunn, Dr.P.H., M.P.H., RDN
Associate Director, Policy, Partnerships, and Communications; Division of Nutrition, Physical Activity, and Obesity
Centers for Disease Control and Prevention

Steven James, M.D.
Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health

David C. Kaslow, M.D.
Director
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration

An N. Massaro, M.D.
Supervisory Medical Officer
Office of Pediatric Therapeutics
Office of the Commissioner
U.S. Food and Drug Administration

L. Clifford McDonald, M.D.
Associate Director for Science in the Division of Healthcare Quality Promotion
Centers for Disease Control and Prevention

Ryan Ranallo, Ph.D.
Program Officer
National Institute of Allergy and Infectious Diseases
National Institutes of Health

Elizabeth V. Schulz, M.D., M.H.P.E., FAAP
Director
Infant Formula and Medical Foods Staff
Office of Nutrition and Food Labeling
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Presenters

Lars Bode, M.S., Ph.D.
Endowed Chair
Collaborative Human Milk Research
Founding Director
University of California, San Diego
Larsson-Rosenquist Foundation Mother-Milk-Infant Center of Research Excellence

David Hackam, M.D., Ph.D.
Surgeon-in-Chief
Co-Director
Johns Hopkins Children's Center

Sarah Taylor, M.D., MSCR
Professor of Pediatrics (Neonatal-Perinatal Medicine)
Chief, Section of Neonatal-Perinatal Medicine, Pediatrics
Yale School of Medicine

Appendix B. Necrotizing Enterocolitis (NEC) Working Group of Council Meeting Agendas

Meeting 1, August 15, 2024

1–1:10 p.m.	Opening Remarks and Charge Dr. Diana Bianchi
1:10–1:15 p.m.	Housekeeping Ms. Laura Berkson
1:15–1:20 p.m.	Introductions and Process Dr. Ravi Patel and Dr. Bonnie Maldonado
1:20–1:40 p.m.	Epidemiology of and Risk Factors for NEC in Premature Infants Dr. Ravi Patel, Emory University Neonatal Research Network
1:40–2 p.m.	Mechanisms Underlying the Development of NEC in Premature Infants Dr. Misty Good, University of North Carolina at Chapel Hill
2–2:20 p.m.	Discussion: Consensus of Risk Factors for NEC in Premature Infants Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado
2:20–2:40 p.m.	Break
2:40–3 p.m.	Portfolio Analysis of NEC from NIH Dr. Sarah Glavin, Deputy Director, Science Policy Analysis & Communication
3–3:45 p.m.	Discussion: Recommendations to Address Scientific Gaps Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado
3:45–4 p.m.	Closing Remarks and Agenda for Next Meeting Dr. Ravi Patel and Dr. Bonnie Maldonado

Meeting 2, August 22, 2024

1–1:05 p.m.	Welcome and Reminder of Charge Dr. Ravi Patel and Dr. Bonnie Maldonado
1:05–1:10 p.m.	Housekeeping Ms. Laura Berkson
1:10–1:25 p.m.	Review of Meeting 1 Dr. Ravi Patel and Dr. Bonnie Maldonado
1:25–1:45 p.m.	Comparison of the Composition of Human Milk and Formula on Risk of NEC in Premature Infants Dr. Sarah Taylor, Yale School of Medicine
1:45–2:05 p.m.	Role of Base Diet and Fortification on Risk of NEC in Premature Infants Dr. Tarah Colaizy, University of Iowa Health Care Stead Family Children's Hospital
2:05–2:25 p.m.	Discussion: Consensus on Enteral Feeding Practices and Risk of NEC in Premature Infants Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado
2:25–2:40 p.m.	Break
2:40–3 p.m.	Therapeutic Targets for Prevention and/or Treatment of NEC in Premature Infants Dr. David Hackam, Johns Hopkins Hospital, Bloomberg Children's Center
3–3:20 p.m.	Discussion: Consensus on Therapeutics/Preventives for NEC in Premature Infants Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado
3:20–3:45 p.m.	Recommendations to Address Scientific Gaps Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado
3:45–4 p.m.	Closing Remarks and Agenda for Next Meeting Dr. Ravi Patel and Dr. Bonnie Maldonado

Meeting 3, August 28, 2024

1:00–1:05 p.m.	Welcome Dr. Ravi Patel and Dr. Bonnie Maldonado
1:05–1:25 p.m.	Review of Meeting Dr. Ravi Patel and Dr. Bonnie Maldonado
1:25–1:45 p.m.	Understanding Human Milk Components and Their Potential Relationship to Infant Intestinal Health and Disease Dr. Lars Bode, University of California, San Diego, Larsson-Rosenquist Foundation Mother-Milk-Infant Center of Research Excellence
1:45–2:05 p.m.	Discussion: Consensus on Milk Processing and Storage Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado
2:05–2:35 p.m.	Break
2:35–3:05 p.m.	Review of Report and Recommendations Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado
3:05–3:50 p.m.	Committee Concurrence Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado
3:50–3:55 p.m.	Closing Remarks and Next Steps Dr. Ravi Patel and Dr. Bonnie Maldonado
3:55–4 p.m.	Thank You Dr. Alison Cernich

Appendix C. Necrotizing Enterocolitis (NEC) Working Group of Council Meeting Summaries

Meeting 1: August 15, 2024

Participants

Co-Chairs

Bonnie Maldonado, M.D.	Stanford University School of Medicine
Ravi M. Patel, M.D., M.Sc. (Presenter)	Emory University School of Medicine

Working Group Members Present

Steve Abrams, M.D.	Dell Medical School
Mandy Brown Belfort, M.D., M.P.H.	Harvard Medical School
Jennifer Canvasser, M.S.W.	NEC Society
Tarah T. Colaizy, M.D., M.P.H.	University of Iowa
Mark Corkins, M.D.	University of Tennessee Health Science Center
C. Michael Cotten, M.D., M.H.S.	Duke University Division of Pediatric Neonatology
Misty Good, M.D., M.S. (Presenter)	University of North Carolina
Munish Gupta, M.D., M.M.Sc.	Harvard Medical School, Boston Children's Hospital
Joan Meek, M.D., M.S.	Florida State University College of Medicine
Jack Moye Jr., M.D.	NICHD
Brenda B. Poindexter, M.D., M.S.	Children's Healthcare of Atlanta and Emory University
David Rowitch, M.D., Ph.D.	Department of Pediatrics, University of Cambridge
Emre Seli, M.D.	March of Dimes, Yale School of Medicine
Lisa Stellwagen, M.D., FAAP	Human Milk Banking Association of North America
Stephanie Wilson Archer, M.A.	NICHD

Working Group Members Absent

Eric C. Eichenwald, M.D.	Children's Hospital of Philadelphia

Others Present

Elizabeth Baden	NICHD
Laura Berkson, J.D.	NICHD
Diana W. Bianchi, M.D.	NICHD

Andrew Bremer, M.D., Ph.D., M.A.S.	NIH Office of Nutrition Research
Amy Buckley	Infinity Conference Group
Nahida Chakhtoura, M.D.	NICHD
Alison Cernich, Ph.D.	NICHD
Helen Fields, Ph.D.	Pathways Research Services
Sarah Glavin, Ph.D. (Presenter)	NICHD
Janelle Gunn, Dr.P.H., M.P.H., RDN	Centers for Disease Control and Prevention
Rohan Hazra, M.D.	NICHD
David Kaslow, M.D.	U.S. Food and Drug Administration
An Massaro, M.D.	U.S. Food and Drug Administration
Clifford McDonald, M.D.	Centers for Disease Control and Prevention
Jamie Newman, Ph.D.	RTI International
G. Stephane Philogene, Ph.D.	NIH Office of Behavioral and Social Sciences Research
Traci Rampton, M.P.P.	Deloitte Consulting
Ryan Ranallo, Ph.D.	NICHD
Rebekah Rasooly, Ph.D.	NICHD
Elizabeth Schulz, M.D., M.H.P.E.	U.S. Food and Drug Administration
Caroline Signore, M.D., M.P.H.	NICHD
Shanard Starke	Infinity Conference Group
Debbie Stein	Infinity Conference Group
Julie Tierney, J.D.	U.S. Food and Drug Administration
Elizabeth Walsh	NICHD
Paul Williams	NICHD
Charlene Wong, M.D., M.S.H.P.	Centers for Disease Control and Prevention
Zahra Younoszai, M.P.H.	NICHD
Kristin Zaterka-Baxter	RTI International

Opening Remarks and Charge

Dr. Diana Bianchi welcomed participants to the first meeting of the NEC Working Group of the National Advisory Child Health and Human Development (NACHHD) Council on August 15, 2024, and introduced the working group co-chairs.

Dr. Bianchi noted that NICHD's mission is to lead research and training to understand human development, improve reproductive health, enhance the lives of children and adolescents, and optimize abilities for all. Understanding more about the risk factors for NEC and the optimal feeding options for preterm infants is within the scope of NICHD's mission.

In providing the charge, Dr. Bianchi noted that the Working Group should assess the evidence regarding enteral feeding in premature infants and factors that may protect against or increase risk for NEC. She said the Working Group will also identify important research gaps and make recommendations for potential future research directions.

Dr. Bianchi thanked the attendees for their participation and for dedicating their valuable time to the meetings. She additionally thanked the speakers for the first meeting and the staff of NICHD for assembling the Working Group and organizing the meetings on very short notice.

Housekeeping

Ms. Laura Berkson reviewed the meeting housekeeping items. She noted that the meetings would not be recorded but would be captured in notes by a science writer with non-attributed comments. All comments entered in the Zoom chat would be captured and included in the meeting record.

She reviewed conflict of interest guidelines, noting that some members identified potential conflicts, including NIH grant support to their institutions of employment and service on scientific committees for nonprofit organizations. Given the focus on the state of the science, she noted that NICHD did not expect these conflicts to limit members' ability to serve on the Working Group. Members could not participate in discussions in which they, their immediate family, or any close professional associates had a financial interest. She noted that, if during the discussion, a member noted a potential conflict, they should identify themselves through the "raised hand" function in Zoom, and they would be placed in the waiting room and readmitted at the end of that discussion.

Ms. Berkson reminded the members that all communication from investigators/press/advocacy groups/the public to working group members about these proceedings should be directed to the Working Group co-chairs. All materials furnished and discussions held during the Working Group meetings were considered privileged information. Remote member attendees were asked to shred all confidential printed material at the conclusion of the review. She also indicated the (NIH Box) site where members could find materials for review and the dates requested for review.

Introductions and Process

Drs. Ravi Patel and Bonnie Maldonado echoed the importance of NEC awareness in neonatal intensive care. They provided a forum for all attendees to introduce themselves and provided guidance on the method and manner of discussion (presentations followed by discussions, recommendations, and scientific gaps). They noted that the first draft of the report would be completed by the following week, and they asked members to be responsive to requests to review it. They noted the goal was to send the report to the U.S. Department of Health and Human Services (HHS) in early September.

Epidemiology of and Risk Factors for NEC in Premature Infants

Dr. Ravi M. Patel, Emory University, Neonatal Research Network (NRN)

Dr. Patel provided the introductory presentation regarding the epidemiology of NEC. He covered the definition of NEC, incidence of NEC, factors associated with NEC, and risk factors for NEC in the population of premature infants.

He explained that there were three commonly used definitions of NEC based on diagnostic criteria: Bell staging, modified Bell staging, and Vermont Oxford Network (VON). Bell staging was

introduced in 1978 and was not intended as a case definition but rather to form a set of criteria for staging NEC severity. Bell staging included various signs, symptoms, and radiographic findings. In earlier stages the criteria were not specific to NEC and resembled many other conditions. In 1984, Walsh and Kliegman introduced modified Bell staging, expanding from three to six stages (IA, IB, IIA, IIB, IIIA, and IIIB) with a focus on disease progression. Stage I was generally not considered NEC, and many studies focused on Stage II and higher. Many in the field called for a better definition and diagnostic criteria for NEC.

The United States did not conduct broad surveillance on NEC in premature infants. Most neonatal intensive care units (NICUs) were involved in VON, which used a more specific and narrower definition, concentrating on one clinical sign and one radiographic sign. The Centers for Disease Control and Prevention (CDC) used a definition similar to VON, but most studies continued to use Bell staging (Battersby et al., 2018).

Recent data from the NICHD NRN, covering the period from 2013 to 2018, used modified Bell stage IIA to define NEC and included infants with gestational age from 22 to 28 weeks who survived more than 12 hours (Bell et al., 2022). Within the NRN, investigators found an overall incidence of NEC of 8.9 percent; of those infants who were diagnosed with NEC, a little less than half receive surgery (3.9 percent).

In NRN centers from 2000 to 2011, of the 22,248 infants born at 22 0/7 weeks to 28 6/7 weeks gestational ages and surviving to 12 hours of age, 6,075 infants died from NEC (275 per 1,000 live births; Patel et al. 2015). U.S. national data from CDC using death certificates tied to the ICD-10 code of NEC as cause of death, from 1999 to 2020, documented 8,951 infants died from NEC, or roughly 1 per day. It was noted that, in the mid-2000s, there was an increase in NEC mortality. It was unclear what contributed to elevated death rates, but the rate decreased after the noted peak (Wolf et al., 2023).

As gestational age at birth increased, the risk of NEC decreased (12-15 percent of infants at 22-23 weeks; 5-4 percent of infants at 28 weeks). Similarly, the proportion of infants that required surgery for NEC was higher for those born earlier. Feeding practices also received less attention in surveillance efforts. However, human milk feeding also increased as gestational age at birth increased, from 73% at 22 weeks to 94% at 28 weeks.

Reported risk factors for NEC in prematurity included maternal/antenatal, infant, and postnatal factors; feeding factors; intestinal dysbiosis; and viral infection (Rose and Patel, 2018). Leading maternal/antenatal risk factors for NEC included demographic factors (e.g., Black race), preeclampsia, hypertension, and tocolysis. Use of antenatal steroids was associated with lower risk of NEC. Three infant risk factors were consistently associated with NEC: lower gestational age, low birth weight, and small size for gestational age. While other factors had been investigated (e.g., cesarean delivery, delayed cord clamping, infant sex at birth), no consistent association had been identified and/or recent analyses demonstrated that these factors did not impact risk of NEC. One consistent postnatal risk factor for NEC was lower oxygen targets, which had been validated in multiple randomized controlled trials, including the Surfactant, Positive Pressure, and Oxygenation Randomized Trial (SUPPORT) led by the NRN.

Feeding factors, and their impact on risk of NEC, would be the focus of subsequent Working Group meetings. These included factors such as a base diet of parent's milk, donor milk, fortification of base diet and approaches, and approaches to feeding (delayed feeding, slow versus fast feeding, trophic feeding, and use of specific feeding protocols). Dr. Patel included a brief introduction to recent systematic reviews of enteral feeding approaches and risk of NEC including: slower versus faster feeding, which showed no significant effect on risk; delayed feeding versus early progressive feeding, which showed no significant effect on risk; early trophic feeding versus enteral fasting, which showed no significant effect on risk; and use of standardized feeding regimens in observational studies, which showed marked reduction in risk.

Another major risk factor was the intestinal environment of the infant, spanning the antenatal and postnatal periods. The microbiome, and relative representation of specific factors in the microbiome, seemed to be strongly associated with NEC. In a systematic review (Pammi et al., 2017), infants with NEC had increased relative abundance of proteobacteria, decreased Firmicutes, and decreased Bacteroides when compared with controls. Viral infections also tended to be associated with NEC, though the certainty of this evidence was low (Mani et al., 2023).

Dr. Patel noted that the Working Group's charge and timeframe did not allow for the application of frameworks to determination causation versus association, or to systematically evaluate the certainty of evidence. He noted that use of criteria, such as the Bradford Hill criteria, may be useful to ensure the Working Group considered consistency of evidence across studies, temporality, evidence of biological gradient (greater dose causing higher or lower risk of disease), specificity of disease, biologic plausibility of evidence, coherence (relation to natural history of disease), inclusion of experiment rather than observation studies, and analogies. He also reminded the group of Grades of Recommendation, Assessment, Development and Evaluation (GRADE) criteria based on the quality of the evidence or study and acknowledged that in studies where there was less rigor, the effect was very uncertain.

In conclusion, Dr. Patel noted that NEC was a multifactorial disease with multiple risk factors spanning antenatal, perinatal, and postnatal periods. He remarked that enteral feeding strategies were not significantly associated with NEC risk in meta-analyses, but that use of standardized feeding regimens decreased risk of NEC in observational studies. He also noted that bacterial dysbiosis and viruses were associated with increased risk of NEC, but these data were concentrated in observational studies.

Mechanisms Underlying the Development of NEC in Premature Infants

Dr. Misty Good, University of North Carolina

Dr. Good noted that the objectives of her talk were to provide a mechanistic overview of NEC pathogenesis, review preclinical models of NEC-like intestinal injury, and discuss prevention and treatment strategies for NEC used in pre-clinical models.

In a healthy intestine, goblet cells secreted mucus and Paneth cells secreted antimicrobial peptides, there was bacterial diversity, and epithelial cells were held together with tight junctions.

Resident immune cells were evident, and breastmilk consumed by the infant delivered a host of components that enrich the gut (growth factors, immunoglobulin). In NEC, microbial dysbiosis was prominent, and there was a bloom of proteobacteria 48 to 72 hours prior to the diagnosis of NEC. If gram-negative bacteria were present in the lumen of the intestine, they began to manufacture polysaccharides. In the setting of a premature infant and the stressors that may be present in the NICU, these factors damaged the tight junctions of the epithelial cells, and the gut became "leaky," allowing bacteria to cross the gut barrier and activate a pro-inflammatory state, leading to a cytokine response.

Several factors were protective against NEC, most related to the gut barrier. For example, the longer babies were on antibiotics, the more likely it was for dysbiosis to develop and to trigger the breakdown of the gut barrier. Hypoxia could also play a significant role in the development of NEC. Studies examined the relationship between the enteric nervous system and gut motility (movement of food through the intestines), which also played a role in NEC development. Using preclinical research, Dr. Good provided an overview of the pathogenesis of NEC, which she noted is complex and multifactorial. Essentially, NEC was an exaggerated pro-inflammatory, immune response to bacterial dysbiosis.

The difficulties in this area of research were related both to the lack of knowledge of what constituted a healthy microbiome and the inability to recapitulate all the risk factors for NEC in animal models. This was specifically problematic for animal models, where different animals can or cannot be delivered prematurely. Current animal models of NEC included mice, rats, piglets, and baboons (though baboons are not as frequently used). In these models, there was evidence for activation of multiple inflammatory responses and signaling pathways, including cell death pathways, all of which can break down the gut barrier.

Dr. Good concentrated on an experimental model of NEC in mice, specifically the model used in the speaker's laboratory. The mice were breastfed by the parent (dam-fed) and then taken away and hand-fed a mixture of human infant formula with puppy formula. This combination was then mixed with lipopolysaccharide/human enteric bacteria or a dysbiotic microbiome from a human infant. Other studies used different formulas (with or without lipopolysaccharides), may add different bacteria, or may add hypothermia or other stressors to augment the breakdown in gut bacteria. In Dr. Good's laboratory, they placed the animals in a hypoxia chamber (5% oxygen, 95% nitrogen) twice a day for 10 minutes to stress the gut and replicate the hypoxic episodes that are relatively common in infants in the NICU. This continued for 3 days and then the intestine was examined. Compared to dam-fed mice who served as controls, the hand-fed animals had an NEC-like injury with yellow tissue and necrosis. Other models induced NEC-like intestinal injury using dithizone and Klebsiella bacteria or clamping of the intestine for 40 minutes to produce ischemia and then allowing reperfusion.

Breastmilk was noted to be protective in histology and in knockout models. In animals with NEC versus breastfed controls, the animals demonstrated disrupted microvilli. Using extracted breastmilk in formula-fed mice resulted in protection from a histologic perspective. If the investigators added an epidermal growth factor receptor (EGFR), the animals lost protection from breastmilk. If they only depleted epidermal growth factor (EGF), the animals lost some protection.

In EGFR intestinal-specific knockout mice, they determined that breastmilk provided protection, which suggests that breastmilk may provide protection through EGF signaling. Breastmilk also enhanced the proliferation of crypt at the base of the intestine where stem cells were located likely via EGF/EGFR in mouse models of NEC.

Human milk oligosaccharides (HMO) were prebiotics that fed the microbiome. Adding particular HMOs (2'-Fucosyllactose; 2'-FL) to mouse formula resulted in preservation of intestinal architecture and a decrease in proinflammatory cytokines. Administering HMOs to animals with NEC also improved intestinal perfusion on examination with confocal microscopy. Given the number of pathways involved in NEC, there were multiple potential therapeutic targets and intervention techniques, including: EGF and heparin-bound EGF, vitamins, probiotics, prebiotics, and components found in amniotic fluid (fetuses do not develop NEC *in utero*).

Collaboration drove much of the *in vitro* work. This included the NEC Biorepository, which has samples from 746 infants (controls and infants with NEC). These samples would be used to develop biomarkers and determine if there was a genetic susceptibility to NEC. The Chan-Zuckerberg Initiative provided funding to build a single-cell atlas from patients, utilizing intestine and stool samples, so scientists can understand this disease at the single-cell level. *In vitro* models were developing rapidly. NEC-in-a-dish models used isolated crypts from intestine samples that grow in culture. Additionally, cultures on microfluidic chips could be used to grow villus-like structures that mimic the intestine to which a bacterial slurry or microbiome from an infant with NEC can be introduced to examine gene expression and pathophysiology. In these NEC-on-a-chip models, loss of tight junctions was visualized.

Discussion: Consensus of Risk Factors for NEC in Premature Infants

Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado

The discussion following these two talks was wide ranging. Initially, the discussion focused on feeding practices, particularly late feeding and trophic feeding, and the shift in the evidence in this area. These shifts could result from what was fed in the different time periods. Also, early studies were observational; more recent studies were randomized controlled trials (RCTs), which provided more rigor. It was also possible that delayed feeding exposed babies to more central line time, which can lead to bloodstream infections. Discussion also turned to the challenge of meconium inspissation (a separate disease) in micro-preemies, which is less well measured in studies in the United States. More research is needed to understand bowel pathology in very premature infants.

The group raised questions related to antibiotic use in premature infants and its relationship to NEC. Studies vary in whether prolonged antibiotic exposure increased NEC risk. What was fed was also of concern, with questions about whether preclinical protocols included different products, such as those based on bovine milk. Generally, the models remained the same throughout the years, but some studies looked at specific formula components that could potentially cause injury, especially related to fats included in formula.

On the risk of NEC based on gestational age, there was some consensus that the risk varied based on this variable and that strategies to reduce risk may need to vary as a result. It was noted that most preterm infants born at 28 to 32 weeks were at risk of NEC. Most of the study cohorts limited to smaller size babies, as a previous study from the United Kingdom noted a marked decrease in risk of NEC after 31 weeks. Participants agreed that different subpopulations, such as infants born later in gestation with cardiac disease, also have increased risk but may have different driving factors for that risk. Use of clinical data warehouses and CDC data was discussed as a potential way to examine risk in more mature infants.

The group also discussed feeding methods and NEC, particularly whether enteral feedings were a necessity for developing NEC. The challenge with this topic was that NEC was often confused with intestinal perforation, which can happen without enteral feeding and may be misclassified as NEC. A large percentage of infants had enteral feeding but did not develop NEC. In preclinical models, if animals were not fed, then NEC had to be induced through another mechanism. NEC involved a more pro-inflammatory response, but we did not know if that happens in one or multiple feedings. In animal models, there was an increase of pro-inflammatory cytokines as early as 6 hours after the start of feeding, and it only took a couple of feedings (every 3 hours) to see this response.

The group briefly discussed the need to develop more complex *in vitro* models with representation of stroma, vasculature, innate/adaptive immune cells, and other factors. In discussion, it was noted that in some *in vitro* models this could be done, but it was challenging. It was also noted that there was a team developing a gastrointestinal organoid system.

NIH Portfolio Analysis of NEC Research

Dr. Sarah Glavin, Deputy Director, Science Policy Analysis & Communication, NICHD

Dr. Glavin presented a detailed analysis of NIH funding for and publications resulting from research in preterm infants and NEC. In preparing the analysis, the team relied on terms relevant to the charge of the group. It was noted that these were not official NIH data, as NEC was not reported by NIH's Research, Condition, and Disease Categorization (RCDC) reporting process. However, the natural language processing, thesaurus, and other tools of the RCDC system were used to generate data as accurate and as consistent with NIH reporting as possible.

The analysis examined the following questions:

- What is the level of funding and number of projects that support this area of research across NIH?
- What is the focus of NIH-funded research in NEC and preterm infants?
- What is the landscape of research publications that resulted from NIH funding focused on NEC and preterm infants?

To answer the first question, analysts used the terms and thesaurus from the RCDC system, incorporated relevant items from related categories, then validated the results manually. The team incorporated NICHD NRN studies by including pro-rated funding based on only the NEC-relevant studies, rather than including all NRN funding.

The data encompassed trends from a 6-year period (fiscal years [FY] 2018-2023). The funding for NEC research increased over time, particularly between 2022 and 2023, and early data suggest that this upward trend will probably continue into FY 2024. In FY 2023, the portfolio included about $21.6 million in funding with 62 unique principal investigators (PIs) managing 73 unique projects at 50 unique institutions. The overall trend for the 6-year period represented about $101.3 million in funding, with 100 unique PIs managing 342 unique projects at 61 unique institutions. Of note, this portfolio included both NIH intramural programs as well as extramural funded programs. Institutions conducting NEC research included not only academic medical centers but also children's hospitals. Institutions from states that were historically underrepresented in overall NIH funding were also included in this group.

With respect to the focus of the research funded, the primary NIH institutes providing funding in NEC and preterm infants were (in order of level of funding): NICHD, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of General Medical Sciences (NIGMS), National Institutes of Allergy and Infectious Diseases (NIAID), and National Heart, Lung, and Blood Institute (NHLBI). NICHD had the broadest portfolio including basic to clinical research. NIDDK and NIGMS primarily funded disease mechanism and pathogenesis research. NIAID funding primarily focused on the microbiome, and funding from NHLBI tended to focus on risk of NEC associated with blood transfusion or in infants with cardiovascular anomalies.

The analysis team also looked at how NEC and risk factors associated with NEC were included in research across the portfolio. NEC was the primary or exclusive focus of 40 percent of the research funding, while the remaining 60 percent of the funding supported research studies where NEC was one of multiple outcomes examined. The largest amount of funding focused on disease mechanisms; there was a paucity of epidemiologic research. NIH also funded a significant portfolio of research on infant feeding. About one-half of the identified research focused on feeding with some focusing specifically on feeding only human milk. The microbiome is a major focus of the research in this area, much of which also includes an immune component. Research conducted specifically by those participating in the small business-initiated research programs concentrated on improving the speed, accuracy, and availability of instruments to diagnose NEC at the bedside.

With regard to the landscape of research publications, the analysis team searched PubMed using the same thesaurus terms from the analysis of grants. The initial searches yielded a total of 3,971 publications. Titles and abstracts were reviewed manually to screen out obvious false positives. Reviews, commentaries, and other types of nonoriginal research were removed, leaving 3,262 publications. Of these articles, 867 publications acknowledged external funding, and 541 of those received NIH funding. There has been an increase in publications throughout the last 6 years, and trends suggest that the number will grow in 2024. The locus of research is primarily in the United States; other contributing countries include China, Canada, and the United Kingdom. Of those original research publications, 616 focused on infant feeding. A separate review of clinical trials identified 37 current studies registered in www.clinicaltrials.gov. Few of these trials focused on infant feeding.

In the question-and-answer session following this presentation, a committee member noted it would be helpful to separate parent's milk versus donor milk. Dr. Glavin noted that there were not many studies that focused on donor milk, and very few distinguished between parent's milk and donor milk.

Discussion: Recommendations to Address Scientific Gaps

Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado

As the group moved into recommendations from this meeting, it initially discussed the gestational age range under consideration, potentially inclusive of infants with NEC after 32 weeks. How these factors occur in children born prematurely versus term infants, and how this interacts with risk factors for NEC need to be clarified. Later in the discussion, the group noted that babies born at 26 weeks may not develop NEC until weeks later, and it is often not known why NEC occurs at various time points. Understanding the time course, what is happening that predisposes babies to NEC or puts them at high risk, should be a priority. Current work through the NEC Biorepository, mentioned in a presentation, may be key to this. The wealth of information in the samples obtained (e.g., blood, stool, breastmilk, intestine, saliva) in conjunction with the nutritional data (what is fed and the timing) may move this more quickly. The challenge is that NEC is relatively rare and not all babies in the biorepository have NEC. In addition, maternal samples are not collected, so there may be a gap related to factors arising from the mother.

Risk factors for NEC are a major area in need of investigation. It would be helpful to conduct studies to quantify the risk accounted for by the known risk factors and then drive hypotheses to account for the unknown risk. This led to a discussion related to the potential impact of social determinants of health on risk. For example, disparities in parent's milk provision in the NICU seems significant, including social science to understand methods to encourage and support parents to provide their milk. These supportive programs are a lot of work and developing evidence to support this might be useful. CDC noted they have a survey of maternity hospitals (Maternity Practices in Infant Nutrition and Care (mPINC™) Survey) examining policies that are supportive of breastfeeding, but this is not specifically for preterm infants. While exposure to antibiotics was noted, the group discussed other potentially harmful exposures that premature infants may incur.

There was a concentrated discussion on the need for studies to improve diagnostics. Most studies in this area are relatively small projects that are mostly hypothesis-driven. Open-ended studies, potentially using electronic health records or other data repositories that could provide large-scale data on infants who do not have NEC, might be useful. This could include prenatal data and maternal data to develop predictive models. A key challenge is standardization of data recording or data definitions. There were examples shared of models predicting NEC as early as mid-pregnancy, these need to be tested and validated in larger datasets that include infants without NEC.

Neurocognitive sequelae of NEC are not well understood, especially given the associated risks of neurodevelopmental impairment or cerebral palsy. The NRN has conducted a study, presented this year, that followed infants who experienced NEC for up to 2 years corrected age (2 years after their

original due date). The initial results showed that most survivors of NEC who underwent surgery for the condition have neurodevelopmental impairments and cerebral palsy.

With respect to mechanisms of NEC, the group asked if there was any focus on endothelial development or the microvasculature of the intestine. Some work is ongoing in the endothelial component with identification of a pathway that is modifiable and targetable. The microvasculature and perfusion models are more challenging.

The group then moved to feeding practices, which the co-chairs noted would be a focus for an upcoming meeting. Members had questions related to potential effects of formula components and whether these could increase risk. The general agreement is that the existing guidelines from the American Academy of Pediatrics (AAP) are aligned with the science in this area: parent's milk is the best feeding diet, then donor milk, and then formula. Generally, the consensus was that babies required feeding – nutrition is vital to support neurodevelopmental outcomes in these premature infants. The first 1,000 days of brain development supports scaffolding and if certain steps are missed in brain development at this stage, they cannot be regained. The key is to understand what is protective in breastmilk, what is potentially lacking in formula, and what risks exist if feeding is not provided. Even parenteral nutrition (intravenous feeding) has additional risks and specific conditions that can be equally concerning.

A member asked if feeding reduced or diminished the bacteria bloom or if dysbiosis was reflective of something present in the host. It is unclear whether dysbiosis or NEC occurs first. Infants fed with parent's milk have more diversity in their microbiome, but this does not fully account for other exposures that can modify the microbiome environment (e.g., provider handling, oral medicines). Feeding can impact the microbiome; in mice models, for example, inflammation can be mitigated by adding diverse microbiomes or lactobacillus.

The group noted that even if the infant is solely fed breastmilk, not all breastmilk is the same. Breastmilk varies by parent and contains a genetic component. Even babies who receive only human milk can develop NEC. In addition, parent's milk or donor milk may not meet all nutritional needs, and differences may exist between how parent's milk and donor milk are handled and administered across NICUs and parents. Donor milk is frozen, pasteurized, and processed, which can affect its bioactive factors. The breastmilk may also be subjected to multiple freeze-thaw cycles and preparation, which may have impacts that have not yet been considered. These processes may be more consistent for donor milk. Milk bank processes have changed, and most banks pool milk from multiple donors. Because 23% of women do not have a specific oligosaccharide (disialyllacto-N-tetraose; DSLNT) in their milk, this may need to be considered during pooling of donor milk. In addition, the lactational stage of the milk donation (early versus later after birth) and other aspects of parent-specific biology could be important. Members also raised a knowledge gap related to cases in which the parent's milk has not yet come in and what the timing is to use other methods, including donor milk, to mitigate the risk of NEC. The group requested that NIH include a presentation from suggested experts in this area in the third meeting. NIH representatives requested names from the group for consideration by the co-chairs and agreed to include this presentation in the third meeting.

A member noted that there is a need to consider genomic factors. They cited cases in their clinical practice where infants with fulminant NEC had a genetic risk for inflammation. The group noted that the NRN has done some work using date from its Cytokine study that enrolled infants in 1999 to 2001. This work found one genomic association, but this phenotype has not been validated in an animal or *in vitro* model.

After hearing the discussion, another member noted that linking the epidemiologic and mechanistic work represents a significant gap. They pointed out that it is important to take what is being learned in the animal models and understand, at an epidemiologic level, the contributions of variation in parent's milk or donor milk to the risk of NEC. During discussion, members noted that there are no large studies with breastmilk samples and clinical phenotyping. The group noted that this represents an opportunity to engage in a team science approach with human milk scientists, NEC scientists, and epidemiologists working collaboratively to facilitate this translation effort. Cohort studies conducted with these types of teams were thought to be a vehicle to prioritize what needs to be brought to therapeutic trials.

The co-chairs thanked the group for the discussion, provided a brief summary of the discussion, and identified research gaps. They highlighted that prematurity is one of the most important risk factors for NEC. They then clarified that there is a lack of clear evidence about strategies related to feeding research. The group identified timing of transition from donor milk to formula for those babies not receiving parent's milk as a research priority. There was some consensus that the microbiome is associated with risk, but there is a lack of clarity whether dysbiosis or NEC presents first. There was agreement that there are several immune targets and pathways that might lead to the common endpoint of inflammation. There was also consensus that human milk has protective factors, such as EGF and HMOs, which are important in reducing the risk of NEC. A research gap is whether there are components of formula that influence the risk of inflammation, and it is unclear why some infants who receive human milk develop NEC. There was agreement that additional research that includes genetic and epigenetic methods is important to understanding potential contributors of risk for NEC. The group agreed that team science approaches may be vital to understand the complex risk factors associated with NEC. Finally, the working group noted that social determinants of health are important; there are equity issues that could benefit from outcomes-based interventions. This could include understanding what factors impact the availability and the uptake of milk and could also help to optimize registries and repositories by adding this type of information.

Closing Remarks and Agenda for Next Meeting

The co-chairs again thanked members for the robust discussion and noted the timeline by which members would be asked to review materials from this meeting. The next meeting date was noted, and the agenda was briefly outlined. The co-chairs then moved to close the meeting.

Meeting 2, August 22, 2024

Participants

Co-Chairs

Bonnie Maldonado, M.D. (Presenter)	Stanford University School of Medicine
Ravi M. Patel, M.D., M.Sc. (Presenter)	Emory University School of Medicine

Working Group Members Present

Steve Abrams, M.D.	Dell Medical School
Mandy Brown Belfort, M.D., M.P.H.	Harvard Medical School
Jennifer Canvasser, M.S.W.	Necrotizing Enterocolitis (NEC) Society
Tarah T. Colaizy, M.D., M.P.H.	University of Iowa
Mark Corkins, M.D.	University of Tennessee Health Science Center
C. Michael Cotten, M.D., M.H.S.	Duke University Division of Pediatric Neonatology
Eric C. Eichenwald, M.D.	Children's Hospital of Philadelphia
Misty Good, M.D., M.S.	University of North Carolina
Munish Gupta, M.D., M.M.Sc.	Harvard Medical School, Boston Children's Hospital
Joan Meek, M.D., M.S.	Florida State University College of Medicine
Jack Moye Jr., M.D.	NICHD
Brenda B. Poindexter, M.D., M.S.	Children's Healthcare of Atlanta and Emory University
David Rowitch, M.D., Ph.D.	Department of Pediatrics, University of Cambridge
Emre Seli, M.D.	March of Dimes, Yale School of Medicine
Lisa Stellwagen, M.D., FAAP	Human Milk Banking Association of North America
Stephanie Wilson Archer, M.A.	NICHD

Working Group Members Absent

None

Others Present

Elizabeth Baden	NICHD
Laura Berkson, J.D. (Presenter)	NICHD
Diana W. Bianchi, M.D.	NICHD
Andrew Bremer, M.D., Ph.D., M.A.S.	NIH Office of Nutrition Research
Amy Buckley	Infinity Conference Group
Nahida Chakhtoura, M.D.	NICHD
Alison Cernich, Ph.D.	NICHD
Michelle Engle, NP	Ochsner Medical Center University of Alabama
Helen Fields, Ph.D.	Pathways Research Services
Sarah Glavin, Ph.D.	NICHD
Janelle Gunn, Dr.P.H., M.P.H., RDN	Centers for Disease Control and Prevention

David Hackam, M.D., Ph.D. (Presenter)	Johns Hopkins Hospital-Bloomberg's Children Center
Rohan Hazra, M.D.	NICHD
David Kaslow, M.D.	U.S. Food and Drug Administration
An Massaro, M.D.	U.S. Food and Drug Administration
Clifford McDonald, M.D.	Centers for Disease Control and Prevention
Eunjung Nam	Deloitte Consulting
Jamie Newman, Ph.D.	RTI International
G. Stephane Philogene, Ph.D.	NIH Office of Behavioral and Social Sciences Research
Traci Rampton, M.P.P.	Deloitte Consulting
Ryan Ranallo, Ph.D.	NICHD
Rebekah Rasooly, Ph.D.	NICHD
Elizabeth Schulz, M.D., M.H.P.E.	U.S. Food and Drug Administration
Caroline Signore, M.D., M.P.H.	NICHD
Shanard Starke	Infinity Conference Group
Debbie Stein	Infinity Conference Group
Sarah Taylor, M.D., M.S.C.R. (Presenter)	Yale School of Medicine
Julie Tierney, J.D.	U.S. Food and Drug Administration
Elizabeth Walsh	NICHD
Natasha Williams, Ph.D., J.D., L.L.M., M.P.H.	NICHD
Paul Williams	NICHD
Charlene Wong, M.D., M.S.H.P.	Centers for Disease Control and Prevention
Zahra Younoszai, M.P.H.	NICHD
Kristin Zaterka-Baxter	RTI International

Welcome and Reminder of Charge

Dr. Ravi Patel and Dr. Bonnie Maldonado, the co-chairs of the NEC Working Group of the NACHHD Council, welcomed the Working Group to the second of three meetings on August 22, 2024. They reviewed the charge for the group and agenda and noted that the meeting would primarily focus on specific aspects of human milk and formula, comparison of base diets, fortification, and therapeutic targets for NEC.

Housekeeping

Ms. Laura Berkson reviewed the following meeting housekeeping items for Working Group members who were not in attendance at Working Group Meeting 1:

- Confirmation that the meeting would not be recorded but would have non-attributed notes and a chat record
- Conflicts of interest processes
- Communication protocols for outreach from external parties

- Materials handling and location of materials on NIH Box

Review of Meeting 1

The working group co-chairs reviewed the discussion, outcomes, and recommendations from meeting 1 to set the stage for the current meeting's proceedings (review NEC Working Group of Council Meeting 1 Summary).

Comparison of the Composition of Human Milk and Formula on Risk of NEC in Premature Infants

Dr. Sarah Taylor, Professor of Pediatrics, Yale School of Medicine

Dr. Sarah Taylor provided an overview of various factors in human milk and formula and their relationship to preterm infant gut maturation, inflammation, and development of NEC. She explained that breast or chestfeeding is the standard for healthy, full-term infant nutrition, but that the standard for preterm infant feeding is less clear. Any type of feeding in extremely premature infants was a risk factor for NEC, but fasting was associated with gut atrophy and increased gut permeability. Gut permeability decreased as the gut matured, and both amniotic fluid and nutrition impacted this process, with bioactive milk components playing a role that is not entirely clear in many cases.

Dr. Taylor explained variability in parent's milk versus donor milk versus formula components and handling. Maturity of milk and processing of donor milk or parent's milk via pasteurization or retort processing and variations in usage and storage (e.g., fresh, pumped, refrigerated, frozen) could change milk composition and bioactivity. For example, in pasteurized milk, macronutrient content was sustained, but little was known about the impact on micronutrient content and immunoglobins, water-soluble vitamin activity decreased, and enzymes were completely inactivated. She noted that processing milk could cause harmful reactions that are detrimental to infants, but there are no known toxicity risks. Limited evidence suggested that retort processing can decrease some bioactivity. Freezing milk increased non-esterified fatty acids, decreased antioxidant activity, and reduced milk acidity with minimal impact on amino acid, total protein, IgA, and lactoferrin.

Dr. Taylor next discussed how these bioactive components influence gut maturation and inflammation. She highlighted the natural progression of infant gut exposure to first amniotic fluid and then human milk that facilitates normal gut development. As the gut matured, it became less permeable. Research on different bioactive components in milk examined their influence on gut permeability as a marker of maturation; parent's milk was known to relate favorably to maturation. Additionally, some human milk components modulated toll-like receptors that are connected to gut inflammation. Overall, individual bioactives demonstrated minimal impact on gut maturation, and enteral insulin showed promise as a gut maturation component.

Treatment with human milk components IGF-1 and HMOs did not display significant differences in intestinal permeability overall when examined individually, although IGF-1 demonstrated a significant difference at day 14 that was nullified at later timepoints. HMO function acted partially

via the microbiome, but organoid studies showed HMO impacts independent of the microbiome as well. Enteral glutamine treatment showed intestinal permeability decreased at day 30 but not earlier. Data on HPF was unclear, with one study showing this factor reduces time to full feeds and another suggesting decreased feeding tolerance. Bovine colostrum treatment was associated with decreased gut permeability in one study, but in a randomized controlled trial did not demonstrate improved gut maturation. Lactoferrin also did not seem to decrease NEC risk. A meta-analysis examining enteral insulin showed improved feeding tolerance with fewer days to full feeds, suggesting this component may positively impact gut maturation and feeding tolerance. Supplementation of arginine is related to decreased NEC risk, likely decreasing risk of stage 1 NEC.

Potential harmful exposures to components such as maltodextrin, corn syrup, nutritional fats, and docosahexaenoic acid (DHA) when supplemented alone showed increased risk of NEC in animal models. DHA supplementation with arachidonic acid (ARA) showed no increased risk of NEC. The data on bovine protein exposure and its relation to NEC were unclear. Some literature suggested concern for harm, but a recent clinical observational trial compared hydrolyzed protein preterm infant formula (HPF) to standard protein formula and found lower incidence of NEC in the HPF group. In a pig model, feeding bovine raw milk and colostrum related to less gastrointestinal inflammation. Infants have been receiving more protein over the last two decades without increasing incidence of NEC, so it was unlikely to be a dose-dependent effect of protein content.

Together, these data suggested it may be an absence of human milk rather than exposure to formula that leads to risk of NEC.

Role of Base Diet and Fortification on Risk of NEC in Premature Infants

Dr. Tarah Colaizy

Dr. Colaizy presented an overview of literature and three clinical trials related to the use of donor milk and formula and fortification strategies and how they relate to the risk of NEC. Most of the clinical studies discussed included infants 29 weeks of age or younger who were fed the study diet of interest until 36-40 weeks postmenstrual age (PMA). The effect size demonstrated was similar in all three trials. Trials discussed included the MILK trial, Donor Milk for Improved Neurodevelopmental Outcomes (DoMINO) trial, and Schanler study. Overall, the three trials demonstrated that in the absence of parent's milk relative risk estimates for NEC were between 0.35 and 0.53 in infants exposed to donor milk versus preterm formula. Use of donor milk as a supplement through 36-40 weeks was associated with a 50-percent reduction in NEC relative to feeding preterm formula. The effect size was similar for infants fed substantial amounts of parent's milk and those fed minimal to no parent's milk.

Dr. Colaizy discussed modeling studies that examined various feeding conditions related to the incidence of NEC. In a proportional hazards modeling analysis, she showed that as the proportion of parent's milk feeding went up, the hazard for NEC went down. She noted, however, that infants who received more food in the first 14 days may display differences relative to those who received less food during this time period. In a Monte Carlo simulation examining infants fed more than 98 percent parent's milk, no parent's milk (preterm formula diet), or a mix of both, 848 babies of less

than 1,000 grams survived to 36 weeks PMA. Preterm formula-fed had 11-percent NEC incidence relative to 1.3 percent in the parent's milk group and 8.2 percent in the mixed diet group. There was an adjusted odds ratio of 12 for developing NEC in the exclusively preterm formula-fed infants versus human milk-fed infants but no statistical difference between the exclusively preterm formula-fed and mixed diet infants. Additionally, a Cochrane review suggested that the relative risk of NEC for preterm formula-fed infants is higher than those fed fortified donor milk.

Dr. Colaizy also discussed data from various studies on fortification. Overall, she noted it was difficult to draw conclusions from the studies due to small numbers of infants included (fewer than 400 total in the studies examined) but preliminary data suggest that fortification of base diets with human HMF may decrease risk of NEC compared to bovine HMF even in infants who receive some preterm formula. The studies discussed included various blinding strategies. In an unblinded study comparing human versus bovine HMF, infants were fed either an exclusive human milk diet, including donor milk if needed, with Prolacta HMF fortifier or parent's milk with bovine HMF and preterm formula (if needed). Parent's milk composed 70 percent of the feeds for human HMF group and 82 percent of the bovine HMF group. NEC occurred in 8 percent of the human HMF group (2 percent surgical NEC) and occurred in 11 percent of the bovine HMF infants (8 percent surgical NEC). The unblinded nature of the study may have resulted in an unconscious bias to look for NEC in the bovine group.

In a blinded trial comparing preterm formula versus donor milk, fortified with either human HMF or bovine HMF, NEC occurred in 21 percent of infants whose feeds were fortified with bovine HMF and 3 percent of infants whose feeds were fortified with human HMF, but the number of infants included were too small to draw generalizable conclusions. In another blinded trial, Optimizing Mother's Milk for Preterm Infants (OptiMOM), babies were fed parent's milk and supplemented with donor milk as needed and then randomized to bovine or human HMF fortification. The investigators found no statistically significant outcomes with NEC occurring in 4.7 percent of infants whose feeds were fortified with human HMF relative to 9.8 percent in the bovine HMF group.

Additional studies were needed to draw substantive conclusions, but these studies will likely be very expensive based on the numbers needed.

Discussion: Consensus on Enteral Feeding Practices and Risk of NEC in Premature Infants

Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado

Working Group members discussed understanding of enteral feeding practices and risk of NEC, research gaps, and policy considerations related to feeding practices in hospitals. Members agreed that the protective and other beneficial effects of human milk need to be better understood. Human milk contained positive factors that mechanistically protected against gut injury. There was differential risk for feeding breastmilk versus formula – mechanism for gut injury in feeding formula was not clear. Studies allowed for association rather than causality of NEC in formula-fed infants. Before preterm formula was routinely used, NEC occurred in babies with full-term formula or other diets, suggesting that the absence of human milk rather than amount of protein provided the infant was what matters.

The group also discussed NEC risk associated with nothing-by-mouth (NPO). There could be a sliding scale of risk based on lack of feeding, milk feeding, exposure to amniotic nutrition, and formula feeding. It was noted that parent's milk and pasteurized donor human milk did not eliminate risk of NEC. Delaying enteral feeding increased risk of NEC based on epidemiological data, but there are little data related to it – most of this data was extrapolated from adults due to ethical considerations. It is not clear whether delayed feeding is better than feeding formula.

Other research gaps identified included understanding the timing of NEC onset based on gestational age and whether transitioning foods at a certain time or feeding a specific diet for a certain duration may reduce NEC risk. Group members suggested existing datasets may be used for meta-analyses to answer these questions. Additionally, the NRN's MILK trial demonstrated risk reduction of NEC when donor milk was fed for a prolonged period of time, but many hospitals only feed donor milk until 32 to 34 weeks post-menstrual age due to cost concerns.

Availability of donor milk and equitable access could also impact NEC risk in infants. Group members suggested that there is an adequate national supply of donor milk available to feed early premature babies at risk for NEC, but usage varied by hospital, likely due to cost concerns and variations in Medicaid and private insurance reimbursement rates. Of course, the preference was feeding parent's milk, but social determinants of health and other factors may prevent a parent from being able to offer it or in enough quantity for the infant's needs. The Donor Milk Act that has been proposed in Congress promoted equitable access to donor milk. In addition, the AAP and advocacy groups offered state toolkits related to Medicaid reimbursement for donor milk. One research gap may be understanding the barriers (e.g., time, awareness, cost) for hospitals to use donor milk. CDC data may indicate which NICUs do not have a donor milk program. The percentage of NICUs that have a program likely varies over time.

The group next considered infant growth related to feeding donor milk versus formula. Infants fed a human milk-based diet in the MILK study grew more slowly versus formula-fed infants. Additional research was needed to understand nutrition in base diets, appropriate volume, protein content, and fortification strategies to optimize infant growth and decrease risk of NEC. The Human Milk Institute had an ongoing effort to review evidence regarding nutrition and donor milk, with consideration of fortification that may be needed. Babies who received donor milk may also require different fortification than those fed parent's milk.

The group also discussed that NEC can occur in full-term infants as well, particularly those with cardiac disease. In these cases, human milk did not seem to provide much protection – suggesting there may be differential physiological pathways for NEC in full-term babies.

Therapeutic Targets for Prevention and/or Treatment of NEC in Premature Infants

Dr. David Hackam, Surgeon in Chief and Co-Director, Johns Hopkins Children's Center

Dr. Hackam provided an overview of potential therapeutic targets to treat NEC in premature infants. He began by outlining some needs to facilitate progress and stratification of patients into clinical trials, including the need to:

- Develop precise definitions of NEC. He observed 5 different presentations of NEC that included pneumatosis that progressed to free air in premature baby, NEC that failed to improve but had no free air, NEC with venous air and necrosis of intestine, staccato NEC where the infant is initially stable but then deteriorated with overwhelming sepsis, and NEC totalis wherein most of small intestine is dead.
- Validate biological links between pathway and development of NEC in more than one model *and* human tissue.
- Consider brain and lung endpoints.

To develop therapies, one needed to understand how the disease presented clinically and commonalities amongst patients. Bacterial signaling was one commonality required for the development of NEC. There was evidence that bacteria accumulate before NEC develops, confirmed via positive blood cultures. Additionally, Toll-like receptor-4 (TLR4) signaling, a cell surface receptor that binds to lipopolysaccharide (LPS) on gram-negative bacteria, was a key receptor by which host intestine can recognize the bacteria seen in NEC. TLR-4 signaling was elevated in intestine from NEC patients. This signaling in the premature gut was thought to lead to NEC. TLR4 signaling elevation in the presence of bacteria led to cell death, the inability to repair tissue, and impaired motility in the gut. Bacteria on the lining of the intestine led to vasoconstriction of the intestine, resulting in NEC.

Dr. Hackam described evidence suggesting that TLR4 signaling (e.g., presence of TLR4 ligands) regulated the balance between injury and repair in the intestine. Under normal developmental circumstances, TLR4 expression rose in the gut earlier in development and then rapidly declined once an infant reached term. TLR4 expression in stem cells of the gut regulated stem cell function. This process is disrupted when an infant is born early. NEC was associated with a loss of intestinal stem cells. Colonizing wild-type infant mice with stool from human NEC patients led to NEC and breakdown of intestinal mucosa. Mice lacking TLR4 expression in the lining of the gut mucosa prevented intestinal structure breakdown and cytokine activation. In humans, TLR4 expression was elevated in patients with NEC and enteric bacteria from NEC patients was rich in TLR4 ligands. Breastmilk contained TLR4 inhibitors and can block TLR4 signaling via the EGF-mediated mechanism as shown in an *in vivo* mouse model. Some probiotics can inhibit TLR4 signaling via TLR9-mediated mechanisms. A prospective, case-control study examined gut bacteria dysbiosis and NEC in very low birthweight infants and determined that a relative abundance of gamma-proteobacteria (e.g., gram-negative facultative bacilli) and lack of strict anaerobic bacteria in the gut preceded NEC in these infants.

Therapeutic targets that block TLR4 signaling may be effective in treating or preventing NEC. Plans for clinical trials were underway for the TLR4 inhibitor Compound 34 (C34), a promising therapeutic candidate with an excellent safety profile. C34 is a master regulator of pathways downstream from TLR4 that may contribute to the development of NEC, including the cell death regulator PUMA, vasoconstriction regulator nitric oxide, and goblet cell formation regulator Notch. Treatment of mice or piglets in NEC models with C34 reduced NEC. In human intestinal tissue, C34 silenced proinflammatory signaling.

Dr. Hackam discussed additional therapeutic targets in the downstream TLR4 signaling pathway. NEC is associated with induction of PUMA, the cell death regulator, in intestinal stem cells in both animal models and human intestinal tissue from NEC patients. Blocking PUMA using short hairpin RNA (shRNA) in a NEC mouse model preserved intestinal stem cells, reduced apoptosis, and attenuated NEC. Adding nitrate to formula to enhance nitric oxide, which breastmilk normally stimulates via sodium nitrate, improved blood flow and reduced vasoconstriction in a NEC mouse model. Inhibiting Notch, another therapeutic target that regulates goblet cell formation, restored goblet cell formation and reduces NEC. NEC is also associated with a lack of gut motility via enteric glia depletion. Brain-derived neurotrophic factor (BDNF) from enteric glia reduced TLR4 signaling in the intestine to prevent NEC. Stimulating production of BDNF via the therapeutic candidate "agent J11" restored motility and prevented NEC in mice. Additional evidence *in silico* suggested HMOs block TLR4 signaling via binding to the TLR4/LPS pocket.

Influx of proinflammatory lymphocytes to the gut is another characteristic of NEC. Antibody blockage of IL-17 receptors prevented accumulation of these deleterious lymphocytes. Feeding retinoic acid to mice inhibited NEC by inducing regulatory T cells in newborn mouse intestine. Additional research is needed to better understand the role immune signaling plays in NEC.

More work still needs to be done to reveal therapeutic pathways for NEC. These include a better understanding of genetic and epigenetic regulators of disease, the impact maternal stressors can have on development of NEC, better methods of diagnosing NEC, and dedicated resources for developing additional NEC drugs.

Discussion: Consensus on Therapeutics/Preventives for NEC in Premature Infants

Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado

The Working Group discussed understanding of the current therapeutics landscape and research needs to advance the pipeline. They agreed that a better understanding of triggers for NEC onset and early disease stages was needed as intervention before NEC progressed to late stages of disease may be critical for successful resolution. This would require development of biomarkers of disease. Working Group members suggested that some of the therapeutic candidates discussed, such as TLR4 signaling elevation or PUMA expression, could be biomarker candidates.

There was also a need to better understand the therapeutic window for intervention and suitability of therapeutic candidates as prophylactic measures. The NRN NEC Surgery Trial (NEST) found that NEC was diagnosed usually between day 11 and 12 of life. Knowing the appropriate therapeutic window and whether a given candidate is more appropriate for one stage of the disease versus another will be critical for successful treatment and clinical trial design for testing therapeutic candidates.

Distinguishing between targets best suited for use as prophylactics versus those to treat ongoing disease is also important. Understanding the factors that infants are exposed to *in utero* during normal development may yield insights into potential prophylactic measures. Mimicking *in utero* exposures after a preterm infant is born could help them be healthy in the extrauterine

environment. Studying components present in amniotic fluid, which a developing infant would normally be swallowing in utero, could help understand regulation of the gut milieu and yield prophylactic targets. For example, EGF is enriched in both amniotic fluid and breastmilk and inhibits TLR4 signaling indirectly. Unintended side effects of using EGF, which is involved in some cancer and inflammatory pathways, and other prophylactic and therapeutic targets would need to be weighed against risk to the infant and their NEC risk or disease stage. If infants were undergoing surgery where mortality can be 30 to 40 percent, this may be used as criteria for entering a study.

The working group discussed if NEC therapeutic targets would need broader applicability to other diseases to get traction and support from companies, but specificity to NEC treatment is the more pressing priority. Additionally, targets need to be easy to manufacture, have low toxicity, and as few synthetic steps as possible to hold appeal to private companies. Finding industry partners with a personal interest may be critical to move targets toward market. It was noted that treatments effective for NEC could potentially also treat NEC-associated brain injury.

The chairs thanked Drs. Taylor and Hackam for their presentations and recused them from the meeting.

Recommendations to Address Scientific Gaps

Moderators: Dr. Ravi Patel and Dr. Bonnie Maldonado

Dr. Patel and Dr. Maldonado led a discussion to highlight scientific gaps related to NEC risk and feeding and nutrition needs of preterm infants. The most effective approach was to prevent premature births from happening; additional research is needed in this area, including the role of diet and stress during pregnancy. Following guidance from professional organizations, such as the AAP, parent's milk was preferred for care of premature infants. Research to improve and enhance the ability of parents to provide human milk for premature infants (e.g., drug development, evidence-based protocols for practices such as pumping frequency and nutritional supplements) and to address social determinants of health that adversely impact parents' ability to provide milk (e.g., parental leave, economic factors related to donor milk and fortifiers) could help reduce NEC risk in these babies. It was also critical to understand the timing and variability in NEC development.

Another key scientific gap was understanding the nutritional needs of premature infants. Additional studies are needed to understand the nutrition provided in utero, the composition of amniotic fluid, and placental physiology and function (e.g., placental immunity) to inform nutritional fortification for premature infants. An evidence base for post-NEC nutrition also needs to be developed. Additionally, understanding the relative efficacy of human milk fortifiers versus bovine milk fortifiers was needed and would require larger studies than those previously conducted. Previous studies included only a handful of babies that developed NEC. The Working Group briefly discussed how these types of products are regulated. Different regulatory practices were in place for nutritional products (regulated as food) versus medical additives (regulated as drugs).

There were also several gaps in knowledge related to human milk handling and storage. It is not known how different milk handling practices (e.g., fresh, frozen, refrigerated) impact the nutritional and bioactive content of milk. For donor milk, practices related to pooling and storage can also impact nutritional content. Duration of donor milk usage and timing and methodology of transition to other diets is another gap that required study.

A better understanding of pathways and mechanisms to support drug and product development would also be beneficial given that NEC is a rare, acquired disease. Programs such as the Small Business Innovation Research program, the Rare Disease Network, Orphan Drug and Rare Pediatric Disease Incentives, and Centers of Excellence in Regulatory Science and Innovation (FDA) may be mechanisms to advance work in drugs and products to address NEC.

The discussion also included other considerations of regulatory and policy considerations, as well as advocacy that may support health of preterm infants (e.g., Medicaid coverage of donor milk). These were considered out of scope of the state-of-the-science report but were noted by federal staff in attendance at the meeting.

Closing Remarks and Agenda for Next Meeting

Dr. Maldonado and Dr. Patel thanked the Working Group members for their discussion and noted the need for interdisciplinary work to address some of the unanswered research questions surrounding NEC. The co-chairs reminded the Working Group that the final working group meeting would occur on August 28, 2024, with a presentation on milk storage and processing as well as discussion to determine the final Working Group recommendations that would be presented at the NACHHD Council meeting on September 5.

Meeting 3, August 28, 2024

Participants

Co-Chairs

Bonnie Maldonado, M.D. (Presenter)	Stanford University School of Medicine
Ravi M. Patel, M.D., M.Sc. (Presenter)	Emory University School of Medicine

Working Group Members Present

Steve Abrams, M.D.	Dell Medical School
Mandy Brown Belfort, M.D., M.P.H.	Harvard Medical School
Jennifer Canvasser, M.S.W.	Necrotizing Enterocolitis (NEC) Society
Tarah T. Colaizy, M.D., M.P.H.	University of Iowa
Mark Corkins, M.D.	University of Tennessee Health Science Center
C. Michael Cotten, M.D., M.H.S.	Duke University Division of Pediatric Neonatology
Misty Good, M.D., M.S.	University of North Carolina
Munish Gupta, M.D., M.M.Sc.	Harvard Medical School, Boston Children's Hospital
Joan Meek, M.D., M.S.	Florida State University College of Medicine
Jack Moye Jr., M.D.	NICHD
David Rowitch, M.D., Ph.D.	Department of Pediatrics, University of Cambridge
Emre Seli, M.D.	March of Dimes, Yale School of Medicine
Lisa Stellwagen, M.D., FAAP	Human Milk Banking Association of North America
Stephanie Wilson Archer, M.A.	NICHD

Working Group Members Absent

Eric C. Eichenwald, M.D.	Children’s Hospital of Philadelphia
Brenda B. Poindexter, M.D., M.S.	Children’s Healthcare of Atlanta and Emory University

Others Present

Elizabeth Baden	NICHD
Laura Berkson, J.D.	NICHD
Diana W. Bianchi, M.D.	NICHD
Lars Bode, Ph.D. (Presenter)	University of California, San Diego, Collaborative Human Milk Research
Andrew Bremer, M.D., Ph.D., M.A.S.	NIH Office of Nutrition Research
Amy Buckley	Infinity Conference Group
Emily Cassio, M.P.H.	NICHD
Patricia Ceger	RTI International
Nahida Chakhtoura, M.D.	NICHD
Alison Cernich, Ph.D.	NICHD
Michelle Engle, NP	Ochsner Medical Center University of Alabama

Helen Fields, Ph.D.	Pathways Research Services
Sarah Glavin, Ph.D.	NICHD
Janelle Gunn, Dr.P.H., M.P.H., RDN	Centers for Disease Control and Prevention
Rohan Hazra, M.D.	NICHD
David Kaslow, M.D.	U.S. Food and Drug Administration
An Massaro, M.D.	U.S. Food and Drug Administration
Clifford McDonald, M.D.	Centers for Disease Control and Prevention
Eunjung Nam	Deloitte Consulting
Jamie Newman, Ph.D.	RTI International
G. Stephane Philogene, Ph.D.	NIH Office of Behavioral and Social Sciences Research
Traci Rampton, M.P.P.	Deloitte Consulting
Ryan Ranallo, Ph.D.	NICHD
Rebekah Rasooly, Ph.D.	NICHD
Elizabeth Schulz, M.D., M.H.P.E.	U.S. Food and Drug Administration
Caroline Signore, M.D., M.P.H.	NICHD
Shanard Starke	Infinity Conference Group
Debbie Stein	Infinity Conference Group
Julie Tierney, J.D.	U.S. Food and Drug Administration
Elizabeth Walsh	NICHD
Natasha Williams, Ph.D., J.D., L.L.M., M.P.H.	NICHD
Paul Williams	NICHD
Charlene Wong, M.D., M.S.H.P.	Centers for Disease Control and Prevention
Zahra Younoszai, M.P.H.	NICHD
Kristin Zaterka-Baxter	RTI International

Welcome and Review of Meeting 2

Drs. Ravi Patel and Bonnie Maldonado reviewed the results of the second meeting.

In the discussion period following the summary, it was agreed that the studies of fortification of human milk, especially those comparing human milk-based fortifiers with bovine milk-based fortifiers, were small and generally inconclusive. Participants stated that as few as one to two cases of NEC could influence the results, and the studies were quite underpowered to study NEC. It was noted that there were two studies conducted by individuals with commercial ties that could present perceived conflicts of interest. Two studies without commercial ties—one done in Europe and one done in Canada—showed no significant differences; the Canadian trial did show a trend towards benefit for the human milk-based fortifier.

It was pointed out that NEC can occur in infants fed exclusively with human milk. In discussing disease mechanism, the TLR4 signaling pathway was viewed as promising, but it was also pointed out that it was probably somewhat downstream, and there were many ways to get to that pathway.

Several individuals stated that there were multiple pathways leading to NEC, and multiple ways to get to each of those pathways. In investigating mechanisms for NEC, caution about bystander effects was urged. It was pointed out that many pathways currently under investigation are non-specific for NEC.

One member stressed the importance of understanding fetal growth and development, especially in the second and third trimesters. This would help develop knowledge around what early preterm babies (and especially those with NEC) need to adapt to life outside the uterine environment.

Understanding Human Milk Components and Their Potential Relationship to Infant Intestinal Health and Disease

Dr. Lars Bode, University of California, San Diego, Collaborative Human Milk Research

Dr. Bode presented an overview of human milk. He explained that human milk has a variety of components, including water, carbohydrates, lipids, proteins, immune cells, bacteria and other microbes, hormones, and other components. Oligosaccharides are a unique and relatively large component of human milk by volume—very few oligosaccharides are found in bovine milk. Oligosaccharides are comprised of 5 building blocks—glucose, galactose, N-acetyl-glucosamine, fucose, and sialic acid. There are approximately 150-200 different oligosaccharides found in human milk, made from different combinations of these building blocks.

Although the relative risk numbers varied across studies, the research literature did indicate that a diet focused on human milk is protective for NEC compared with formula feeding. However, although human milk is protective, infants fed an exclusive human milk diet can still get NEC.

To understand (and perhaps mimic or increase) the protective effect of human milk, scientists studied oligosaccharides in animal models of NEC and in human cohorts. A key question was which specific oligosaccharides contribute to the effectiveness of human milk. In a rat model of NEC, adding oligosaccharides to formula increased the survival of rat pups with NEC. Human milk oligosaccharides were critical – other oligosaccharides were not protective. The results for rats showed that supplementation of formula with human milk oligosaccharides improved outcomes close to those of dam-fed rats.

Although these initial experiments were helpful, additional studies were needed to clarify which specific oligosaccharides might be protective for NEC, and to address the question of why human milk (and oligosaccharides) are only partially protective for NEC. Animal studies, using a multidimensional chromatography approach, suggested that one particular oligosaccharide, disialyllacto-N-tetraose or DSLNT, was uniquely associated with decreased NEC risk. This association was confirmed in 3 independent human studies with separate cohorts in the UK, South Africa, and the United States.

Research on DSLNT suggested why human milk was only partially protective for NEC. Studies of individual variation in human milk composition indicated that there was a very wide range of variation in DSLNT levels from lactating individual to lactating individual. Meta-data suggested that there is a threshold of DSLNT levels needed to confer protection from NEC, and about 23 percent of

lactating individuals did not have enough DSLNT to reach this level. Levels of DSLNT varied by when the milk was expressed—milk expressed later after birth had lower levels of DSLNT, and much donor milk is donated during this period. Older individuals also tended to have lower levels of DSLNT in their milk. Individuals tend to have very similar oligosaccharide profiles when comparing milk from subsequent pregnancies.

Researchers are now looking to develop tools to identify which human milk products (parent's milk or pasteurized donor milk) have sufficient DSLNT concentrations to prevent NEC. A major impediment to this research is the lack of point-of-care technology to quickly measure DSLNT. Currently, it can be done only in a few specialized labs and takes several days to do, which is not useful to prevent a disease like NEC that has a rapid onset and progresses quickly.

Clinical trials of DSLNT are in the planning stages; however, producing DSLNT is currently extremely expensive, making a large-scale trial infeasible. Researchers were at work trying to develop new methods to create or extract DSLNT at a feasible cost.

Discussion: Consensus on Milk Processing and Storage

Moderators: Dr. Patel and Dr. Maldonado

A member inquired – how well might selecting a specific period of time for milk donors work for DLSNT? Dr. Bode stated that early milk from the first month or two after birth has more DLSNT. If a parent is older, DLSNT levels also tended to be lower. So, focusing on early milk might be helpful. He added that when his team used data from a milk biorepository to compare oligosaccharides from parents who have donated from multiple kids, the researchers found that the same parent had the same oligosaccharide levels, even with different kids.

Another member inquired if metabolomics research had been conducted with oligosaccharides. Dr. Bode stated that this has not yet been a major focus. Researchers only knew that DLSNT remained intact in the control group and were "eaten up" in the NEC group. To Dr. Bode, this finding showed that oligosaccharides are not just food for microbes; they acted directly on the host. He was eager to learn which bacteria act on which oligosaccharides in more detail.

Members asked about using probiotics to help protect against NEC. Dr. Bode stated that the choice of probiotic could be driven by which bacteria do not interact with DLSNT. He urged the scientific community to take a close and careful, rigorous look at the relationships between bacteria and oligosaccharides in the presence and absence of NEC, and probably even other conditions. It may be that the possible increased risk of NEC from prolonged antibiotic use is because the antibiotics may act on the bacteria that process DLSNT. More work would be needed to figure that out.

One member suggested that if you found parents who have donated before, and have DLSNT, you could promote them to donate again. Even if you did not know their DLSNT, milk banks could target them to donate during the early postpartum period.

Diagnostics at the point of care to characterize donor milk should be a priority, several participants commented. Members strongly agreed on the need to conduct research that would support developing best practices around donor milk. The milk banking world needed support and funding to improve and standardize practices.

Members discussed in detail issues with analyzing the contents of donor milk. For example, it was pointed out that different measures of lactose can give you very different results, and those results are used to gauge caloric content. That process was simple compared with trying to measure oligosaccharide levels. Milk banks and/or NICUs may not be able to analyze even basic information about components of milk. Some milk banks routinely analyzed all milk, while others did not, and those that did used variable methods. Even when using the same equipment, if it was not calibrated in a standardized way, you get different results.

Pooling methods in donated milk may be important and need to be studied, especially in light of the early postpartum/late postpartum difference in DLSNT levels. The size and volume of the pool and numbers of donors in it were not standardized. Many milk banks did not link volume with donor; in a single pool, they may include 5 oz. from one parent and 45 oz. from another. It may be possible to pool very early milk with late-stage milk and still get a decent level of DLSNT, but some early milk would be needed and a donor pool of at least seven to eight donors may be required.

It was pointed out that there is currently no regulation of nutritional content for donor milk banks. The U.S. Food and Drug Administration (FDA) considered it a food, not a medicine. FDA deals with some additives but does not regulate nutritional content of human milk. Milk Banks received safety inspections, like food. FDA Working Group members commented in the chat: "Since human milk isn't an infant formula, we do not regulate it as such. It is 'regulated' as a food. All the HM facilities get FDA visits from more of a food safety inspection perspective, but not an infant formula type of inspection – and … their nutritional content is not tested at this point in time."

A member reported that milk donors got the same screening as blood donors, both with blood testing (Human immunodeficiency virus, hepatitis B, hepatitis C, Human T-lymphotropic virus type I and II, syphilis) and with an in-depth interview screening for behavioral risk factors for blood-borne diseases. The milk itself was screened post-pasteurization for bacterial contamination, similar to other dairy products. There were concerns to prevent contamination especially with *Bacillus cereus*, which can survive pasteurization.

Experts agreed that there were many other bioactive components in human milk beyond oligosaccharides, and some of those components are destroyed by pasteurization (oligosaccharides are not). So, beyond NEC, donor milk that was pasteurized may be less preferred to fresh parent's milk. It was not known whether components that are destroyed by pasteurization are important or not, and if so at what stage. A more sophisticated method needs to be developed to only remove the harmful components, but leave the rest, rather than apply pasteurization like a blunt instrument. Both animal and human studies are needed to uncover all the important components and protective factors in human milk. Researchers should focus on the function of the components rather than whether a component is intact at any specific point in time.

Donor milk has a very strong safety record. A milk bank is responsible for any "defective" milk, so they need to be able to link donor with the pool from which a baby is fed, and they must keep those records for 21 years. Most banks self-audit, and many also have independent audits. Concern was expressed about a lot of misconceptions about donor milk. For example, some people think babies do not grow well on donor milk, and that is incorrect – growth levels may be lower than with infant formula but are still well above the standard of care.

The chairs thanked Drs. Bode for his presentation and recused him from the meeting.

Review of Report and Recommendations

Moderators: Dr. Patel and Dr. Maldonado

Members discussed potential recommendations in light of the information presented at the three Working Group meetings. Draft slides were presented capturing these discussions and members suggested changes.

Foundational Premises/Starting Points

Members suggested clarifying that the AAP recommendation refers to preterm infant nutrition.

Members stated that an important foundational concept to convey was the lack of information on what the nutritional needs of preterm infants are, and how this varies by gestational age. This was viewed as a key scientific gap that made it difficult to address questions about base diet, feeding methods, and potential preventative interventions related to NEC.

Optimizing Use of Human Milk to Reduce the Risk of NEC

Working Group members agreed that all else being equal, the research supports AAP recommendations that parent's milk is the best base diet for preterm and low birth weight infants at risk for NEC, followed by pasteurized donor human milk, followed by infant formula. They advocated additional research on differences in availability and use of parent's milk, as well as pasteurized donor human milk.

Additional research to support best practices in human milk donation, collection, pasteurization, and processing was viewed as a high priority. Social determinants of health around breastfeeding, lactation, and human milk donation and use should be included. Economic interventions, workplace barriers, community and partner support, and other factors should be studied using rigorous and careful methods. Research on ways to overcome disparities in the availability and uptake of donated pasteurized milk is needed. Working Group members agreed that research was needed to identify and address barriers to wider use of pasteurized donor human milk in hospitals. Reimbursement, staffing, lack of knowledge, and other factors might be possible barriers.

Studies to look at alternatives to pasteurization are also needed. The hope would be to identify NEC-protective components of human milk and optimize the preservation of these components in donor milk, while protecting against contamination and infection

Members discussed the issues involved with fortification of human milk for consumption by preterm infants, and the need for more research in this area. Available literature on this topic was viewed as inadequate, because of small sample sizes, unmasked studies, and the potential for bias and conflicts of interest. The two most robust independent studies—one in Canada and one in Europe—showed no difference in NEC risk based on type of fortification, but these had few NEC cases. The Canadian study had a small trend in favor of human milk-based fortification, but one or two cases of NEC could change the study results. A large-scale pragmatic trial, in real-world NICUs, and independent of commercial interests, was suggested. Ideally, such a trial would be pre-planned to allow its data to pooled with other previous studies. It was noted that the nutrient contents of fortifiers are not regulated under current legislation governing infant formula, and there needs to be better understanding of the content of fortifiers. The FDA is studying this issue. Caution was urged in how we characterize infant formula, especially in making sure that infant formula as such is not confused with fortifiers added to human milk.

Other Recommendations

The Working Group agreed that it was important to address the lived experience of families affected by NEC. They recognized that families were frustrated with not having answers to key questions about what is best for their new family members, although they also acknowledged that it would take a great deal of resources, work, time, and effort to improve the state of the science. Research about how to educate pregnant persons about the risk of NEC and the protective properties of human milk is important, and it is worthwhile to study the best timing and form to convey this information to parents before they are confronted with a case of NEC.

There was support for additional animal model research, although it was recognized that animal models cannot replicate all the exposures an infant will experience in a NICU.

The rapid expansion of whole genome sequencing and the availability of related technologies may create more opportunities to study genetic and epigenetic factors and how they affect the risk and severity of NEC.

It was noted that a workshop related to preterm infant nutrition and NEC is being developed, including collaborations across multiple NIH ICs and the FDA. Efforts to encourage investigator-initiated research applications on NEC were suggested.

Committee Concurrence

Moderators: Dr. Patel and Dr. Maldonado

The committee concurred that the recommendations of the panel had been reported accurately and there was agreement with the recommendations.

Closing Remarks and Next Steps

Drs. Patel and Maldonado summarized the next steps and indicated that committee members would be asked to review the report on a very tight timeframe in order to send the report to the

Secretary of the Department of HHS in the time requested. Drs. Patel and Maldonado thanked the Working Group members for their time, expertise, and effort to carefully address this topic in a challenging time frame.

Thank You

Dr. Alison Cernich expressed the thanks of NICHD, NIH, and HHS for the group's dedication and their excellent, thorough, and detailed work in a challenging timeframe. She also acknowledged the efforts of the federal staff in supporting this important work.

Appendix D. NIH Support for Scientific Research on Necrotizing Enterocolitis (NEC), Fiscal Years (FYs) 2018-2023

Objectives

The objectives of this analysis were to identify the level of NIH support for research focused on NEC, to characterize NIH grants related to NEC, and to describe selected current research studies related to the mission of the Working Group.

Methodology

The NIH uses its Research, Condition, and Disease Categorization (RCDC) system to identify and formally report research projects related to specific research categories. RCDC uses sophisticated text data mining (categorizing and clustering using words and multiword phrases) in conjunction with NIH-wide definitions to match projects to categories. The results of this process are validated by scientific experts at the NIH Institutes and Centers (ICs). The NIH does not expressly budget by category. The annual estimates reflect amounts that may change because of science, actual research projects funded, and the NIH budget. The research categories are not mutually exclusive, and individual research projects can be included in multiple categories.

NEC is not one of the more than 300 categories NIH reports annually, and there is no NIH-wide definition of NEC. However, the tools used by the RCDC system can support internal, unofficial portfolio analyses. NIH grants are indexed to a large series of concepts included in the RCDC's thesaurus used for natural language processing, and NEC and related terms are included. Analysts from NICHD used these tools, in combination with NICHD's internal scientific coding, to generate a project listing of NIH grants related to NEC. This draft project listing was compared with official NIH reports related to the topic (such as the NIH official RCDC report on Preterm Birth, Low Birth Weight, and the Health of the Newborn). The listing was also reviewed and validated by several experts. The NICHD's Neonatal Research Network, which implemented clinical studies on NEC during this time period, was included in the data. However, rather than include all the funding for the entire Neonatal Research Network, internal network data were used so that only the funding attributable to NEC studies was counted.

The resulting final listing used as the basis for this analysis is not an official NIH report and has not been extensively reviewed and vetted by experts from all NIH ICs. It should be viewed as preliminary data and caution should be exercised in drawing firm conclusions.

To complement the analysis of NIH-supported research studies on NEC, a search was conducted on August 9, 2024, for in-progress registered clinical trials related to NEC on www.clinicaltrials.gov. Search results included "necrotizing enterocolitis" in the "condition" category and "currently recruiting" or "not yet recruiting" in the study status category.

Overall NIH Funding for NEC Research

Over FYs 2018 to 2023, NIH invested more than $100 million in NEC research, supporting 342 unique research projects. These projects ranged from large clinical research studies to small, exploratory laboratory experiments, and were designed to last from 1 to 5 years. Research studies were managed by 100 distinct PIs; of those, 18 met NIH's criteria for designation as an "early stage" investigator. A total of 61 unique research institutions—academic institutions, research hospitals, small businesses—received NIH funding for this research, including 7 children's hospitals and 10 institutions located in states that have historically received relatively low NIH funding.

As shown in Figure D.1., NIH research funding for NEC increased over this time period. Funding was relatively steady up until 2023, when a relatively large increase occurred.

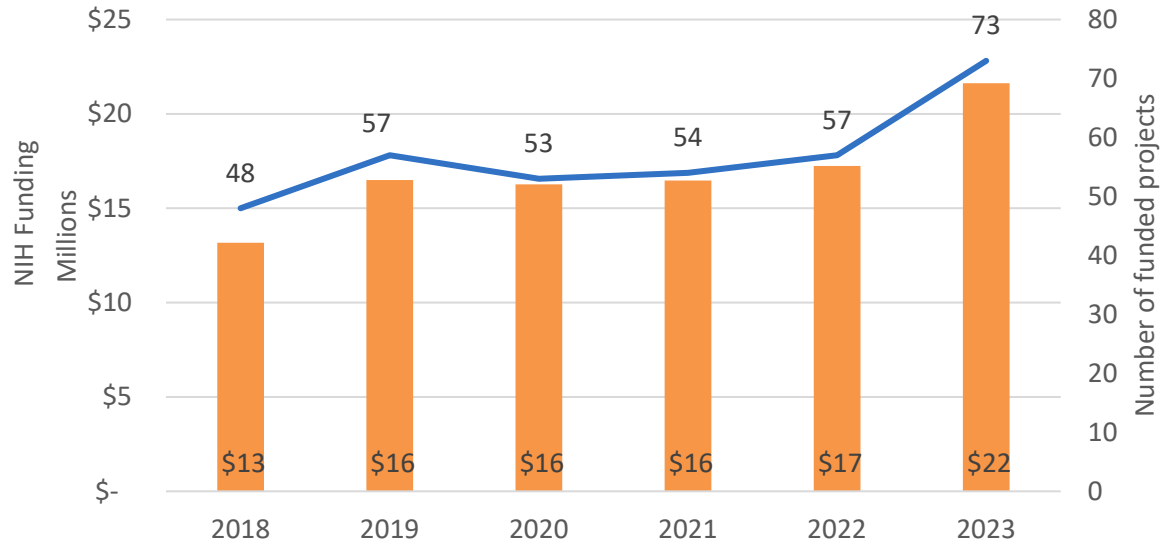
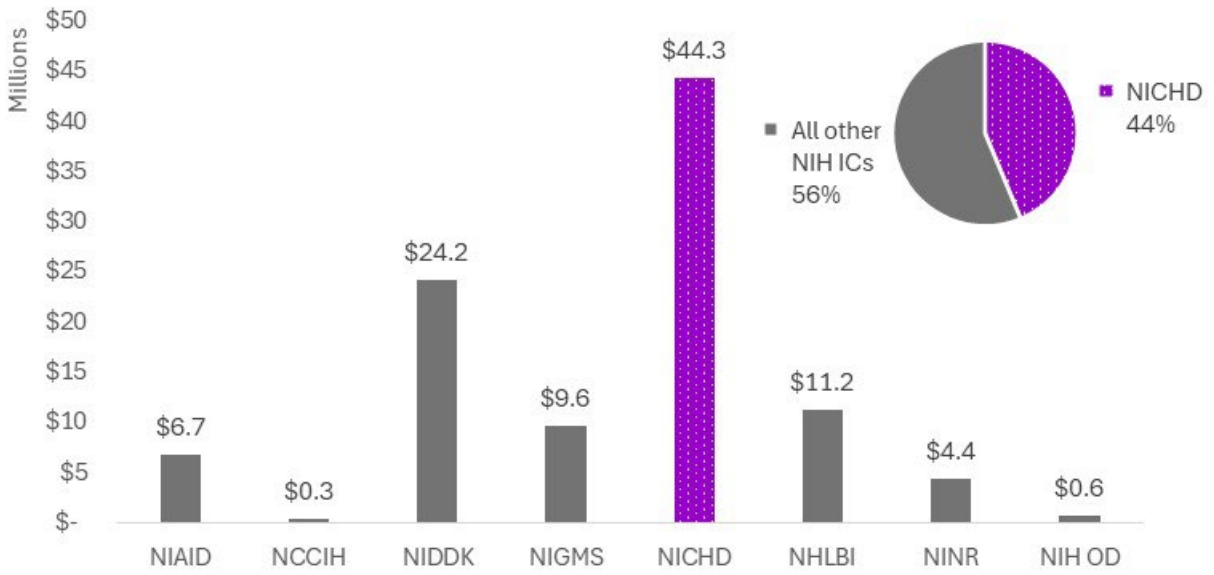


Figure D.1.: NIH Research Portfolio on NEC, by FY: 2018-2023

As shown in Figure D.2., many NIH ICs are involved in NEC research. Each IC funds studies at the intersection of its own mission with NEC research. For example, the National Heart, Lung, and Blood Institute (NHLBI) supports key projects related to NEC risk in preterm infants with congenital heart complications. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute of General Medical Sciences (NIGMS) support a range of research studies on the underlying disease mechanisms and biologic processes related to the risk of NEC. NICHD, which supports the largest share of NEC research at NIH, funds both basic science research on the underlying causes of NEC and clinical studies aimed at improving diagnosis and treatment options.

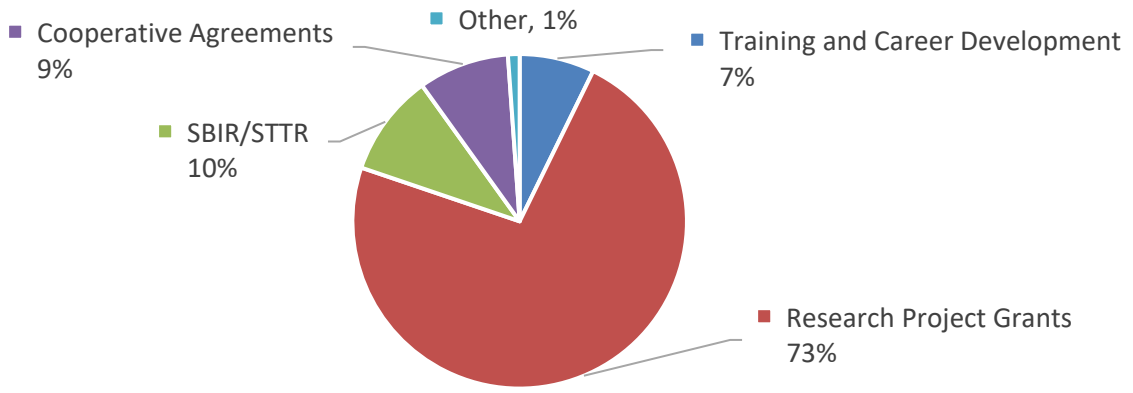
Figure D.2.: NIH Research on NEC, by NIH IC: FYs 2018-2023



The individual projects supported by NIH were reviewed to determine whether NEC was the primary or exclusive focus of the study, or whether NEC was one of several outcomes or focus areas that scientists assessed. In 60 percent of cases, the study was only partially focused on NEC. Many studies centered on outcomes and risk factors for preterm infants generally, and NEC was only one of the outcomes measured. For the remaining 40 percent of the studies, NEC was the primary or sole focus of the research.

As is the case with NIH research as a whole, the majority of projects on NEC were research project grants (see Figure D.3.). Many of these projects are investigator-initiated studies. The NICHD Neonatal Research Network accounted for most but not all of the cooperative agreements related to NEC. Several small business or technology transfer grants focused on developing and testing new methods for early diagnosis of NEC.

Figure D.3.: NIH Research on NEC, by Activity Code Group: FYs 2018-2023



Characteristics of NIH-Supported NEC Research Projects

NIH-supported projects related to NEC were reviewed to identify their primary purpose—that is, whether the project's focus was on identifying and/or assessing risk factors for NEC; uncovering disease mechanism; improving speed and/or accuracy of diagnosis; developing or testing prevention strategies; developing or testing treatment approaches; or developing epidemiological data. (Studies with more than one type of primary aim were included in all categories that were applicable.) Figure D.4. shows the funding level (bars) and percent of funding (circles) by research aims for NIH-supported NEC studies. The largest share of studies and funding focused on disease mechanisms, which accounted for over $61 million in NIH funding. One small study focused on epidemiology. (Some studies included research aims in multiple areas.)

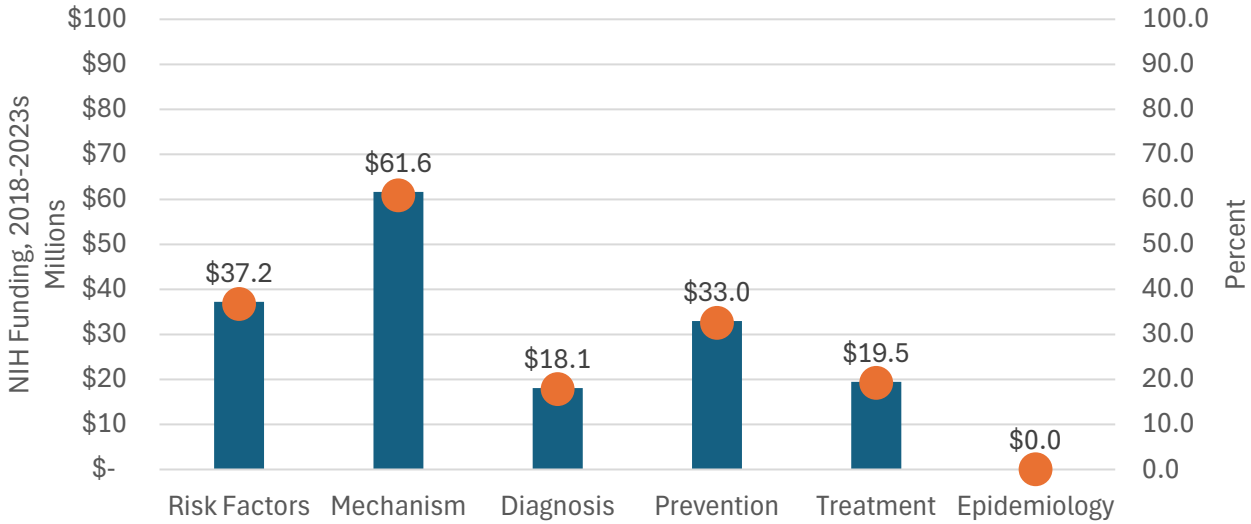


Figure D.4.: NIH Research on NEC, by Research Aims: FYs 2018-2023

Projects were also reviewed in detail to determine whether they were focused on infant feeding or nutrition, and if so, what type of base diet was being studied. Figure D.5. shows that one-half of NIH funding for NEC research went to studies focused on infant feeding or nutrition. This proportion was greater than was observed in NEC publications review (Appendix E). Of the studies that did focus on infant feeding, about one-half included both human milk and formula feeding.

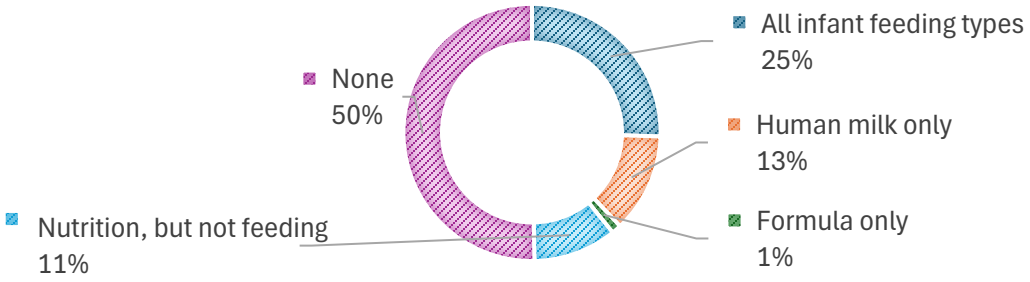


Figure D.5.: NIH Research on NEC, by Focus on Infant Feeding: FYs 2018-2023

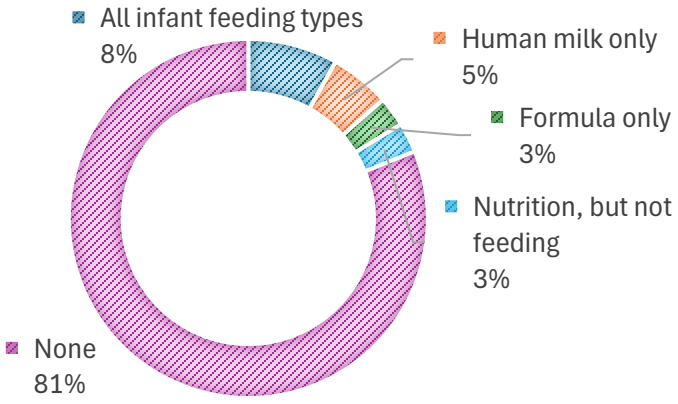
Clinical Trials in Progress Focused on NEC

A search of the clinicaltrials.gov website identified clinical studies related to NEC that were in progress—that is, those actively recruiting or not yet recruiting. The search yielded 37 registered trials. Of these:

- 27 were currently recruiting participants and 10 were not yet recruiting.
- 13 were observational studies, and 14 were intervention trials.
- 9 trials were sponsored by U.S. institutions; 5 by institutions in China; 4 by institutions in France; and 9 studies were supported by institutions in other countries all over the globe.

Figure D.6. shows that few of these clinical studies were focused on infant feeding or nutrition. Of those that did focus on infant feeding, about one-half included both human milk and formula feeding.

Figure D.6.: Studies Registered in ClinicalTrials.gov, as of August 2024, by Infant Feeding or Nutrition Focus



Selected NIH-Supported Grants on NEC, FYs 2018-2024

(grants in alphabetical order by contact PI)

P20GM134973: Oklahoma Center for Microbial Pathogenesis and Immunity. University of Oklahoma, FY2020-2025. Contact PI: Jimmy Ballard (center) and Hala Chaaban (subproject).

> *This research and training center is focused on advancing the careers of junior investigators studying life-threatening infectious microbes and the ways in which the immune system responds to infection. One research project within this center aims to investigate the role of a specific polysaccharide produced naturally in the body in intestinal development, particularly the intestinal microbiome.*

R01AI092531: Population genomic analysis of gut microbial colonization in premature infants. University of California Berkeley, FY2011-2021. Contact PI: Jillian Banfield.

> *Researchers are conducting strain-level analyses of bacterial samples from newborn infants and their parents during the first three years of life, including a subset of preterm infants that developed either NEC or late-onset sepsis. The scientists will use these data to assess whether some early-establishing strains persist beyond the initial colonization period, to examine the strain-level microbial diversity in microbial communities, and to determine whether clinical variables in the newborn period are associated with patterns of strain acquisition in the first three years of life.*

R01HD097327: Targeting human milk fortification to improve preterm infant growth and brain development. Brigham and Women's Hospital, FY2019-2025. Contact PI: Mandy Brown Belfort.

> *Scientists are investigating an individualized approach to human milk fortification, leveraging technology from the dairy industry to analyze human milk composition at the point of care and target fortification to ensure that protein and energy intakes consistently meet recommendations. The research team will conduct a randomized, controlled trial of individually targeted vs. standard fortification among preterm infants receiving human milk (maternal milk plus donor milk if needed, no formula).*

K23HD102580: Cellular components of human milk: An examination of their role in infant health and development and the functional impact of hospital storage practices. University of Massachusetts-Amherst, FY2021-2026. Contact PI: Carrie-Ellen Briere.

> *In this career development study, researchers will: (a) examine how hospital storage practices (refrigeration and freezing) impact the protective mechanisms of human milk cells; and (b) use an animal model to assess if milk cells are integrated as functioning cells specific to vital organs impacted by preterm birth (brain, heart, lungs, and intestines).*

R44HD095779: Noninvasive biomarkers for gastrointestinal disease in preterm infants. Chosen Diagnostics Inc., FY2019-2025. Contact PI: Rebecca Buckley.

Scientists will further develop a biomarker and testing application that could provide direct readout of an individual human's ability to modulate gut homeostasis. Project aims encompass kit prototype development, sample collection, assay validation, and sample analysis for regulatory evaluation.

K08GM127308: The role of extracellular histones and neutrophil extracellular traps in necrotizing enterocolitis. University of Oklahoma, FY2017-2021. Contact PI: Hala Chaaban.

This career development award will support an investigation into the role of specific proteins and processes of cell death in the risk for and severity of NEC, using an animal model.

K08DK125735: Gene-environment interactions in necrotizing enterocolitis: impact of SIGIRR mutation and gut microbiota on intestinal TLR hyperactivity. Children's Mercy Hospital of Kansas City, FY2020-2025. Contact PI: Alain Cuna.

This career development award is designed to lead to a better understanding of how host genetics and intestinal bacterial communities interact with each other to modulate inflammation in the developing neonatal gut.

R01HD097367: Effects of human milk handling practices on peptide release and bioactivity in the preterm infant intestine. Oregon State University, FY2022-2027. Contact PI: David Charles Dallas.

Scientists will use this research grant funding to help determine how handling and pooling practices for human milk fed to preterm infants alter the digestive release of bioactive peptides with gut-health promoting functions. Understanding which milk handling practices will yield the optimal release of bioactive peptides will help guide milk handling practices to best promote healthy development of preterm infants.

R01DK116568: Role of the intestinal microvasculature in necrotizing enterocolitis. Lurie Children's Hospital of Chicago, FY2019-2024. Contact PI: Isabelle DePlaen.

With support from this research grant, scientists will investigate the internal processes that lead to the development of NEC. Using an animal model, they will focus on the hypothesis that, in infants at high risk for NEC, lack of IGF-1 production affects the normal development of intestinal mucosal capillaries and thus makes the intestine prone to NEC under perinatal stresses.

R01HD105301: Neonatal gut-on-a-chip platform for high content drug testing and precision medicine. University of North Carolina Chapel Hill, FY2022-2025. Contact PI: Misty Good.

Researchers will further develop preclinical models of NEC, using both organoids and a NEC-on-a-chip model system derived from premature infant intestinal biopsies with patient- specific microbiota. This research is designed to advance understanding of the multicellular interactions with the microbiome of the developing premature intestine and provide new model systems and preclinical platforms to support the identification and testing of therapeutics for NEC and other intestinal diseases.

R35GM141956: Molecular and metabolic signaling in necrotizing enterocolitis. Johns Hopkins University, FY2021-2025. Contact PI: David Hackam.

With support from this research grant, scientists will conduct a series of studies using animal models to address the causes of NEC, the role of diet and immunometabolism on inflammation in the gut of preterm infants, cellular and molecular factors that contribute to NEC, and causes underlying long-term complications of NEC in the lung, gut, and brain.

R01DK117652: Bile acids in necrotizing enterocolitis. University of Arizona, FY 2018-2024. Contact PI: Melissa Halpern.

This research grant will support researchers' efforts to assess the role of bile acids in the development of NEC. The scientists will define the characteristics of bile acid levels and composition in premature infants that develop NEC, and in an independent cohort with blinded analyses, test the predictive ability of bile acids.

R01DK120697: Mechanisms of maternal Immunoglobulin A control over the neonatal microbiota and the development of necrotizing enterocolitis. University of Pittsburgh at Pittsburgh, FY2020-2024. Contact PI: Timothy Wesley Hand.

Researchers will use this grant to investigate the role of maternal antibodies in human milk play in preventing bacterial invasion of the intestines and NEC. They propose to describe the mechanism via which maternal milk protects the intestine, including shaping the neonatal bacterial flora and specific binding to individual bacterial strains.

K23HL150300: Enteral iron supplementation and intestinal health in preterm infants. University of South Florida, FY2020-2024. Contact PI: Thao Ho.

This career development award will support research to help assess whether high enteral iron supplementation exacerbates intestinal dysbiosis, mucosal inflammation, and permeability in preterm infants, potentially increasing the risk of NEC.

R01HL138714: Impact of anemia and its treatment on gut injury in preterm infants. Johns Hopkins University, FY2020-2025. Contact PI: Cassandra Josephson.

Researchers will use a combination of clinical and pre-clinical approaches to identify and describe key factors responsible for anemia-induced gut injury that may lead to NEC.

R21HD102565: Peripheral immune development in premature infants with and without NEC. Yale University, FY2020-2023. Contact PI: Liza Konnikova.

This grant will support scientists' efforts to identify dysregulation of immune trajectories and develop a model for the immune cell mechanism underlying susceptibility to NEC.

R21HD104481: The therapeutic potential of pasteurized human donor breast milk exosomes. University of Alabama Birmingham, FY2021-2024. Contact PI: Colin Martin.

Based on preliminary data suggesting that exosomes derived from human breastmilk protect intestinal epithelial cells, researchers will investigate the immunologic and other potential protective components in pasteurized donor human milk.

K23HD102554: Early life protein-enriched human milk diets to increase lean body mass accretion and diversity of the gut microbiome in extremely preterm infants: a randomized trial. University of Alabama Birmingham, FY2020-2025. Contact PI: Ariel Salas.

With support from this career development award, scientists will test whether protein-enriched human milk diets during the first 2 weeks after birth in very premature infants result in more lean body mass accretion and more diversity of the gut microbiome before hospital discharge.

K23HD097254: Integrated physiomarker, biomarker and clinical predictive analytics for early warning of sepsis and necrotizing enterocolitis in very low birth weight infants. University of Virginia, FY2019-2024. Contact PI: Brynne Archer Sullivan.

This career development award supports researchers' efforts to develop predictive analytics using biomarkers, physiomarkers, and clinical markers of sepsis and NEC for earlier diagnosis and treatment.

K01DK125633: Therapeutic mechanisms of placental stem cell-based therapy in necrotizing enterocolitis. Wake Forest University, FY2021-2026. Contact PI: Victoria Weis.

This career development grant is supporting research to assess whether perinatal stem cells isolated from placental tissue can facilitate the process of repairing NEC-damaged intestines through regulation of the inflammatory response and re-establishment of the intestinal stem cell niche and epithelial barrier.

R01HD112363: Improving infant health by studying human milk as a biological system: Composition, dynamics, and delivery (at-the-breast vs expressed milk) (BEGIN). University of Rochester, FY2024-2029. Contact PI: Bridget Victoria Young.

With support from this research grant, researchers will assess: 1) how breastmilk changes over time and over the day; 2) how milk dynamics effect infant sleep patterns; 3) how milk dynamics effect infant microbiome dynamics; and 4) how these relationships differ between infants fed directly at-the-breast vs. pumped milk.

Appendix E. Scientific Research Literature on Necrotizing Enterocolitis (NEC), 2018-2024

Objectives

The objectives of this literature analysis were to:

(1) Characterize the recent scientific research literature on NEC
(2) Identify key publications that were most closely related to the relationship between NEC and infant feeding, including describing the results and methodology of those studies

Methodology

The NIH thesaurus and PubMed Medical Subject Headings (MeSH) thesaurus were used to identify terms related to NEC published between January 1, 2018, and August 8, 2024. The same terms were used to search the literature that had been previously used to identify NIH-supported research relevant to NEC.

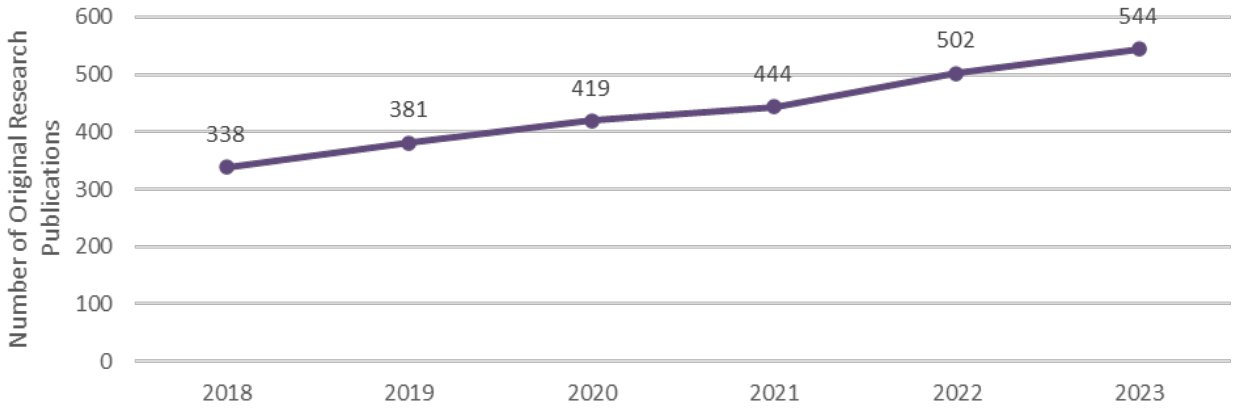
The initial searches yielded a total of 3,971 publications. Titles and abstracts were reviewed manually to screen out obvious false positives; after this review, 3,262 publications remained. Reviews, commentaries, and other types of publications other than original research were identified. Only original research publications (n=3,262) were included in the scan analyzing the characteristics of the research literature. In the summary of key publications on feeding and NEC, 664 publications—including 616 original research publications and 48 reviews—were included.

For the literature scan, publications related to infant feeding were initially identified using a natural language-processing algorithm based on publication titles and abstracts; the classifications produced by this algorithm were then manually validated, resulting in re-classification of 13.4 percent of the publications. Publications focused on infant feeding were manually reviewed. Selected publications with direct relevance to the working group charge were briefly summarized, and the results are provided at the end of this appendix. Information on funding sources came from PubMed's records. The data on funding sources were classified by funding type and country but were not manually validated.

Literature Scan: Publication Characteristics and Funding Support

Figure E.1. shows that, between 2018 and 2023, the number of original research publications related to NEC increased each year.

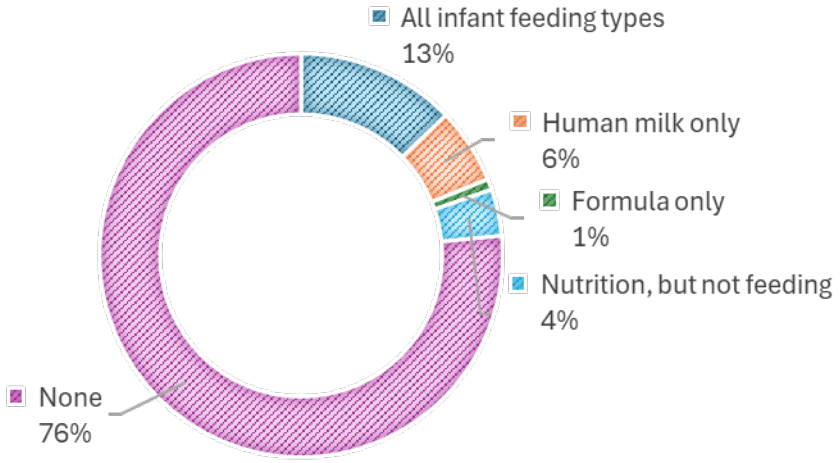
Figure E.1.: Original Research Publications Related to NEC, by year of publication: 2018-2023



The most common journals for publications related to NEC were, unsurprisingly, widely read journals in neonatology and pediatrics. These included: *Pediatric Research*; *Journal of Pediatric Surgery*; *Journal of Maternal, Fetal, and Neonatal Medicine*; *American Journal of Perinatology*; *Journal of Perinatology*; *Journal of Pediatrics*; *BMC Pediatrics*; and *Neonatology*.

As shown in Figure E.2., 24 percent (n=616) of NEC research publications over this timeframe focused on infant feeding. About one-half included all base diet types (human milk and infant formula).

Figure E.2.: NEC Research Publications with a Focus on Infant Feeding: 2018-2024

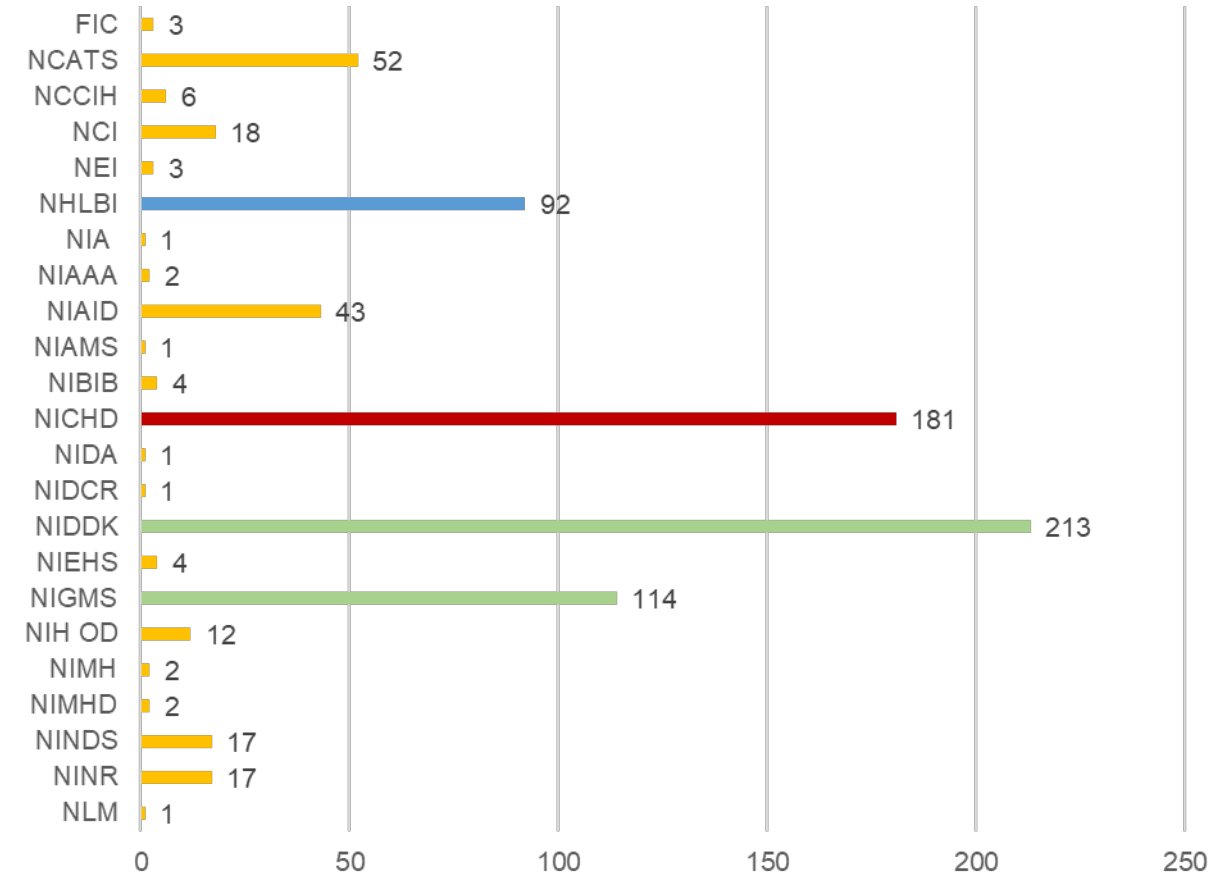


One-third (33 percent) of the original research publications related to NEC acknowledged external funding support in their PubMed records. Of these, a large majority (87 percent) cited only government funding sources. Although funding agencies from 36 countries supported NEC research, the United States supported about two-thirds of publications that acknowledged external funding. Other countries (in order of largest numbers of publications) included China, the United Kingdom, Canada, South Africa, and multiple countries in Europe. NIH accounted for 97

percent of U.S. government supported publications, 63 percent of publications that acknowledged external funding, and 21 percent of original research publications overall.

As shown in Figure E.3., several NIH Institutes and Centers (ICs) involved in NEC research accounted for the majority of the publications acknowledging NIH funding. ICs that supported research focused on disease mechanisms, such as the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute of General Medical Sciences (NIGMS) , had a higher number of publications overall.

Figure E.3.: NEC Publications that Acknowledged NIH Funding, by NIH IC: 2018-2024



Selected Publications on Feeding and NEC, 2018-2024

- With respect to reducing the risk of NEC, there is general agreement within the research literature that parent's milk is preferred for preterm infants, followed by donor milk, followed by formula feeding.
- Studies of fortification yielded mostly negative results concerning the risk of NEC. Systemic reviews point out that the quality of the evidence is low with respect to fortification.

- Enteral feeding methods (early vs. late initiation, volume levels) have generally not been associated with an increased or decreased risk of NEC. Systemic reviews point out that the quality of the evidence is low.

Comparisons of Human Milk, Donor Milk, and Formula Feeding

(articles in alphabetical order by first author)

Berti E, Puglia M, Perugi S, Gagliardi L, Bosi C, Ingargiola A, Magi L, Martelli E, Pratesi S, Sigali E, Tomasini B, Rusconi F. Feeding Practices in Very Preterm and Very Low Birth Weight Infants in an Area Where a Network of Human Milk Banks Is in Place. *Front Pediatr*. 2018 Dec 6;6:387. doi: 10.3389/fped.2018.00387. PMID: 30574473; PMCID: PMC6291747.

> *In an observational study at 25 hospitals of 1,140 early preterm, very low birth weight infants, 74 percent of these infants started enteral feeding during the first 24 hours of life. Overall, 80 percent of newborns were fed exclusive human milk, 67 percent using donor milk and 13 percent starting with exclusive parent's milk. Full enteral feeding was reached earlier in newborns who were fed human milk than in those fed formula, regardless of gestational age. Sixty-four percent of infants were still fed with any human milk at discharge. Starting enteral nutrition with donor milk was associated with early start of enteral feeding and early achievement of full enteral nutrition without affecting parent lactation. This study did not look at NEC as an outcome.*

Boundy EO, Anstey EH, Nelson JM. Donor Human Milk Use in Advanced Neonatal Care Units. United States, 2020. *MMWR Morb Mortal Wkly Rep*. 2022 Aug 19;71(33):1037-1041. doi: 10.15585/mmwr.mm7133a1. PMID: 35980851; PMCID: PMC9400533.

> *CDC's 2020 Maternity Practices in Infant Nutrition and Care (mPINC) survey was used to assess practices for donor milk use in U.S. advanced neonatal care units of hospitals that provide maternity care. Among 616 hospitals with neonatal intensive care units (level III or IV units), 13 percent reported that donor milk was not available for infants with very low birth weight. However, approximately one half of hospitals reported that most very low birth weight infants do receive donor milk. Donor milk availability was more commonly reported among hospitals with a level IV unit, higher annual birth volume, location in the Midwest and Southwest regions, nonprofit and teaching status, and those designated Baby-Friendly.*

Cañizo Vázquez D, Salas García S, Izquierdo Renau M, Iglesias-Platas I. Availability of Donor Milk for Very Preterm Infants Decreased the Risk of Necrotizing Enterocolitis without Adversely Impacting Growth or Rates of Breastfeeding. *Nutrients*. 2019 Aug 14;11(8):1895. doi: 10.3390/nu11081895. PMID: 31416157; PMCID: PMC6722966.

> *In this single-center of 221 early preterm infants, infants fed donor milk had lower rates of NEC compared with formula-fed infants. Rates of breastfeeding and growth were similar across groups.*

Colaizy TT, Poindexter BB, McDonald SA, Bell EF, Carlo WA, Carlson SJ, DeMauro SB, Kennedy KA, Nelin LD, Sánchez PJ, Vohr BR, Johnson KJ, Herron DE, Das A, Crawford MM, Walsh MC, Higgins RD, Stoll BJ; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network; MILK Trial Investigators; Ambalavanan N, Wyckoff MH, D'Angio CT, Bugg GW, Ohls RK, Reynolds AM, Sokol GM, Laptook AR, Olsen SL, White JR, Jadcherla SR, Bajaj M, Parimi PS, Schmidt B, Laughon MM, Barks J, Fisher KA, Hibbs AM, Peralta-Carcelen M, Cook N, Heyne RJ, Cavanaugh B, Adams-Chapman I, Fuller J, Hartley-McAndrew ME, Harmon HM, Duncan AF, Hines AC, Kilbride HW, Richards LA, Maitre NL, Natarajan G, Trembath AN, Carlson MD, Malcolm WF, Wilson-Costello DE; MILK Trial Investigators; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Neurodevelopmental Outcomes of Extremely Preterm Infants Fed Donor Milk or Preterm Infant Formula: A Randomized Clinical Trial. *JAMA*. 2024 Feb 20;331(7):582-591. doi: 10.1001/jama.2023.27693. PMID: 38497706; PMCID: PMC10828950.

Researchers randomly assigned 483 preterm or low birthweight infants whose parents could produce little or no milk to receive formula or donated human milk. The infants fed donor milk had half the rate of NEC as those fed formula. When the infants reached the equivalent of 22 to 26 months of development (corrected for their prematurity) they were tested with the Bayley Scales of Infant Development, a test used to identify children at risk of developmental delays. These scores did not differ significantly across groups.

de Waard M, Li Y, Zhu Y, Ayede AI, Berrington J, Bloomfield FH, Busari OO, Cormack BE, Embleton ND, van Goudoever JB, Greisen G, He Z, Huang Y, Li X, Lin HC, Mei J, Meier PP, Nie C, Patel AL, Ritz C, Sangild PT, Skeath T, Simmer K, Tongo OO, Uhlenfeldt SS, Ye S, Ye X, Zhang C, Zhou P. Time to Full Enteral Feeding for Very Low-Birth-Weight Infants Varies Markedly Among Hospitals Worldwide But May Not Be Associated With Incidence of Necrotizing Enterocolitis: The NEOMUNE-NeoNutriNet Cohort Study. *JPEN J Parenter Enteral Nutr*. 2019 Jul;43(5):658-667. doi: 10.1002/jpen.1466. Epub 2018 Nov 22. PMID: 30465333; PMCID: PMC6531355.

Researchers compared infants from 2 sets of NICUs: (a) 5 NICUs in Guangdong province in China, where providers were mainly using formula feeding and slow feeding advancement, and (b) 8 NICUs from 4 continents, where providers were using mainly human milk with faster advancement rates. Feeding practices varied considerably around the world. Use of formula and long time to full feeding in South China was associated with more use of antibiotics and slower weight gain, but apparently not with more NEC or higher mortality.

Embleton ND, Sproat T, Uthaya S, Young GR, Garg S, Vasu V, Masi AC, Beck L, Modi N, Stewart CJ, Berrington JE. Effect of an Exclusive Human Milk Diet on the Gut Microbiome in Preterm Infants: A Randomized Clinical Trial. *JAMA Netw Open*. 2023 Mar 1;6(3):e231165. doi: 10.1001/jamanetworkopen.2023.1165. PMID: 36857051; PMCID: PMC9978942.

This article reports the results of a randomized clinical trial of gut microbiota in 126 preterm infants hospitalized in 4 centers in the U.K. Researchers compared gut bacteria across 2 groups – infants receiving human milk and infants receiving bovine-based formula. The study

found no effects on overall measures of gut bacterial diversity but there were effects on specific bacterial taxa previously associated with human milk.

Fang L, Zhang M, Wu L, Wang R, Lin B, Yao J, Chen D. Is Preterm Donor Milk Better Than Preterm Formula for Very-Low-Birth-Weight Infants? *Food Nutr Res*. 2021 Sep 24;65. doi: 10.29219/fnr.v65.5346. PMID: 34650391; PMCID: PMC8494261.

This is a single-center, observational cohort study of 304 infants who were identified as having insufficient supply of parent's own milk. Families were given the choice of donor milk supplementation or formula supplementation. Those given donor milk has lower rates of NEC compared with those given formula supplementation.

Goldstein GP, Pai VV, Liu J, Sigurdson K, Vernon LB, Lee HC, Sylvester KG, Shaw GM, Profit J. Racial/Ethnic Disparities and Human Milk Use in Necrotizing Enterocolitis. *Pediatr Res*. 2020 Aug;88(Suppl 1):3-9. doi: 10.1038/s41390-020-1073-5. PMID: 32855505; PMCID: PMC8087165.

Trends in NEC incidence and human milk use at discharge were evaluated by race/ethnicity among 47,112 very low birth weight infants born in California from 2008 to 2017. Annual NEC incidence declined across all racial/ethnic groups from an aggregate average of 4.8% in 2008 to 2.6% in 2017. Human milk use at discharge increased over the time period across all racial groups. Non-Hispanic (NH) black infants received the least human milk each year.

Gupta V, Rebekah G, Sudhakar Y, Santhanam S, Kumar M, Thomas N. A Randomized Controlled Trial Comparing the Effect of Fortification of Human Milk with an Infant Formula Powder Versus Unfortified Human Milk on the Growth of Preterm Very Low Birth Weight Infants. *J Matern Fetal Neonatal Med*. 2020 Aug;33(15):2507-2515. doi: 10.1080/14767058.2018.1554046. Epub 2019 Jan 6. PMID: 30486700.

This was a RCT at a single institution in South India. 148 preterm, low birth weight infants were randomized to receive fortified human milk or human milk only. The fortified milk group had higher weight gain but there was no difference in the rates of NEC.

Harris L, Lewis S, Vardaman S. Exclusive Human Milk Diets and the Reduction of Necrotizing Enterocolitis. *Adv Neonatal Care*. 2024 Jul 10. doi: 10.1097/ANC.0000000000001183. Epub ahead of print. PMID: 38986129.

In this retrospective, single center observational study of 201 preterm, low birth weight infants, researchers found that an exclusive human milk diet reduced the risk of NEC compared with formula feeding.

Hosseini M, Farshbaf-Khalili A, Seyyedzavvar A, Fuladi N, Hosseini N, Talashi S. Short-term Outcomes of Launching Mother's Milk Bank in Neonatal Intensive Care Unit: A Retrospective Study. *Arch Iran Med*. 2021 May 1;24(5):397-404. doi: 10.34172/aim.2021.57. PMID: 34196205.

A single center in Iran that launched a human milk bank observed a subsequent decrease in cases of NEC and sepsis.

Johnson TJ, Berenz A, Wicks J, Esquerra-Zwiers A, Sulo KS, Gross ME, Szotek J, Meier P, Patel AL. The Economic Impact of Donor Milk in the Neonatal Intensive Care Unit. *J Pediatr*. 2020 Sep;224:57-65.e4. doi: 10.1016/j.jpeds.2020.04.044. Epub 2020 Jul 15. PMID: 32682581; PMCID: PMC7484385.

Infants receiving parent's own milk + donor milk had a lower incidence of necrotizing enterocolitis (NEC) than infants receiving parent's own milk + formula. The addition of donor milk was associated with a somewhat lower cost of treatment than the addition of formula. As a result, hospital costs were significantly lower with donor milk, largely due to decreases in NEC.

Li Y, Chi C, Li C, Song J, Song Z, Wang W, Sun J. Efficacy of Donated Milk in Early Nutrition of Preterm Infants: A Meta-Analysis. *Nutrients*. 2022 Apr 21;14(9):1724. doi: 10.3390/nu14091724. PMID: 35565692; PMCID: PMC9105142.

In a meta-analysis of 11 studies, donor milk reduced the risk of NEC compared with formula feeding.

Liu K, Guo J, Yang J, Su Y. The Association of Different Proportions of Human Milk of the Total Enteral Intake on Health Outcomes in Preterm Infants: A Systematic Review. *Breastfeed Med*. 2023 Sep;18(9):666-677. doi: 10.1089/bfm.2023.0128. PMID: 37729036.

In this review of 12 studies, the authors concluded that the proportion of human milk was inversely associated with the risk of NEC.

Miller J, Tonkin E, Damarell RA, McPhee AJ, Suganuma M, Suganuma H, Middleton PF, Makrides M, Collins CT. A Systematic Review and Meta-Analysis of Human Milk Feeding and Morbidity in Very Low Birth Weight Infants. *Nutrients*. 2018 May 31;10(6):707. doi: 10.3390/nu10060707. PMID: 29857555; PMCID: PMC6024377.

In this meta-analysis of 44 studies, human milk was protective against NEC. Compared to exclusive formula feeding, any human milk (donor or parents') was associated with lower risk of NEC.

Ochoa TJ, Mendoza K, Carcamo C, Zegarra J, Bellomo S, Jacobs J, Cossey V. Is Mother's Own Milk Lactoferrin Intake Associated with Reduced Neonatal Sepsis, NEC , and Death? *Neonatology*. 2020;117(2):167-174. doi: 10.1159/000505663. Epub 2020 Feb 13. PMID: 32053823; PMCID: PMC7381382.

In this retrospective review involving 240 infants, researchers found that lactoferrin levels from parent's own milk was associated with lower incidence of a composite outcome of sepsis, NEC, or death.

Parra-Llorca A, Gormaz M, Alcántara C, Cernada M, Nuñez-Ramiro A, Vento M, Collado MC. Preterm Gut Microbiome Depending on Feeding Type: Significance of Donor Human Milk. *Front Microbiol*. 2018 Jun 27;9:1376. doi: 10.3389/fmicb.2018.01376. PMID: 29997594; PMCID: PMC6030370.

Researchers analyzed data from 69 preterm infants in a single NICU to determine the impact of donor human milk upon preterm gut microbiota. No differences in microbial diversity and richness were found, although feeding type significantly influenced the preterm microbiota composition. Donor human milk exhibited an intestinal microbiome more similar to parents' own milk than formula.

Perrin MT, Friend LL, Sisk PM. Fortified Donor Human Milk Frequently Does Not Meet Sodium Recommendations for the Preterm Infant. *J Pediatr*. 2022 May;244:219-223.e1. doi: 10.1016/j.jpeds.2022.01.029. Epub 2022 Jan 31. PMID: 35093320.

As the title states, the authors found that donor milk, with and without fortification, contained less sodium than recommended for preterm infants.

Perrin MT, Belfort MB, Hagadorn JI, McGrath JM, Taylor SN, Tosi LM, Brownell EA. The Nutritional Composition and Energy Content of Donor Human Milk: A Systematic Review. *Adv Nutr*. 2020 Jul 1;11(4):960-970. doi: 10.1093/advances/nmaa014. PMID: 32119744; PMCID: PMC7360450.

In a systematic review, researchers examined the literature regarding research on the fat, protein, carbohydrate, vitamin, and mineral composition of donor human milk obtained through nonprofit milk banks or commercial entities. The 14 studies included had multiple limitations. However, the analysts concluded that existing research suggests the potential for 2-fold and greater differences in the fat, protein, and energy composition of donor human milk, with mean values for energy and fat often below clinical reference values expected for human milk.

Philip RK, Romeih E, Bailie E, Daly M, McGourty KD, Grabrucker AM, Dunne CP, Walker G. Exclusive Human Milk Diet for Extremely Premature Infants: A Novel Fortification Strategy That Enhances the Bioactive Properties of Fresh, Frozen, and Pasteurized Milk Specimens. *Breastfeed Med*. 2023 Apr;18(4):279-290. doi: 10.1089/bfm.2022.0254. PMID: 37071630; PMCID: PMC10124176.

An observational feasibility study analyzed the biochemical and immunochemical characteristics of parent's own milk, both fresh and frozen, and pasteurized banked donor human milk, each supplemented with either human milk-derived fortifier or cow's milk-derived fortifier. Donor human milk, compared with parent's own milk, had reduced bioactive properties, and cow's milk-derived fortifier conferred the least additional bioactive components. Enhancement of bioactivity occurred with human milk derived fortifier.

Pisano C, Galley J, Elbahrawy M, Wang Y, Farrell A, Brigstock D, Besner GE. Human Breast Milk-Derived Extracellular Vesicles in the Protection Against Experimental Necrotizing Enterocolitis. J Pediatr Surg. 2020 Jan;55(1):54-58. doi: 10.1016/j.jpedsurg.2019.09.052. Epub 2019 Oct 25. PMID: 31685268; PMCID: PMC6989376.

In a rat model of NEC, breast milk delivered extracellular vesicles decreased the incidence and severity of experimental NEC.

Rao K, Cuna A, Chavez-Bueno S, Menden H, Yu W, Ahmed I, Srinivasan P, Umar S, Sampath V. Effect of Various Preterm Infant Milk Formulas on NEC-Like Gut Injury in Mice. *Front Pediatr*. 2022 Jul 6;10:902798. doi: 10.3389/fped.2022.902798. PMID: 35874567; PMCID: PMC9299064.

Researchers fed newborn mouse pups with various preterm formulas and found that the different formulas resulted in differing effects on intestinal inflammation, apoptosis, and activation of the pro-inflammatory transcription factor NFκB. ach preterm formula resulted in significant gut microbial alterations that were different from dam-fed controls.

Silano M, Milani GP, Fattore G, Agostoni C. Donor Human Milk and Risk of Surgical NEC: A MetaAanalysis. *Clin Nutr*. 2019 Jun;38(3):1061-1066. doi: 10.1016/j.clnu.2018.03.004. Epub 2018 Mar 10. PMID: 29566974.

In a meta-analysis including 4 studies comparing donor milk to formula, donor milk was not associated with a lower risk of surgical NEC.

Sodhi CP, Fulton WB, Good M, Vurma M, Das T, Lai CS, Jia H, Yamaguchi Y, Lu P, Prindle T, Ozolek JA, Hackam DJ. Fat Composition in Infant Formula Contributes to the Severity of Necrotising Enterocolitis. *Br J Nutr*. 2018 Sep;120(6):665-680. doi: 10.1017/S0007114518001836. PMID: 30176959; PMCID: PMC6126914.

In a mouse model, neonatal mice with NEC fed "pre-digested fat" or "very low fat" diets did not have the accumulation of fat droplets within the intestinal epithelium of the distal ileum that occurred in mice with a "standard fat" diet. This suggested that nutritional formula containing a pre-digested fat system may be helpful in preventing or reducing the severity of NEC.

Strobel NA, Adams C, McAullay DR, Edmond KM. Mother's Own Milk Compared with Formula Milk for Feeding Preterm or Low Birth Weight Infants: Systematic Review and Meta-Analysis. *Pediatrics*. 2022 Aug 1;150(Suppl 1):e2022057092D. doi: 10.1542/peds.2022-057092D. PMID: 35921674.

In this review of 42 studies, parent's own milk was associated with a reduction in NEC compared with formula feeding.

Tongviratskool C, Pongsakul N, Kanaprach P, Supapannachart S, Nuntnarumit P, Chutipongtanate S. How Does Human Milk Protect Against Necrotizing Enterocolitis (NEC)? Targeted Validation and Time-Course Analysis of 35 Gene Responses as NEC-Signature in Fetal Intestinal Epithelial Cells. *OMICS*. 2022 Aug;26(8):440-450. doi: 10.1089/omi.2022.0075. Epub 2022 Jul 27. PMID: 35900271.

In an in vitro study of fetal intestinal epithelial cells, researchers found that human milk may offer protection against NEC through enhancing intestinal EGF production to rescue the inflammatory cell death.

Torres-Muñoz J, Jimenez-Fernandez CA, Murillo-Alvarado J, Torres-Figueroa S, Castro JP. Clinical Results of the Implementation of a Breast Milk Bank in Premature Infants (under 37 Weeks) at the Hospital Universitario del Valle 2018-2020. *Nutrients*. 2021 Jun 25;13(7):2187. doi: 10.3390/nu13072187. PMID: 34202034; PMCID: PMC8308280.

In a single center observational study, compared with formula feeding donor milk was associated with lower risk of NEC and lower risk of sepsis.

Wang C, Zhang M, Guo H, Yan J, Liu F, Chen J, Li Y, Ren F. Human Milk Oligosaccharides Protect against Necrotizing Enterocolitis by Inhibiting Intestinal Damage via Increasing the Proliferation of Crypt Cells. *Mol Nutr Food Res*. 2019 Sep;63(18):e1900262. doi: 10.1002/mnfr.201900262. Epub 2019 Jun 25. PMID: 31207104.

In a mouse model of NEC, administration of human milk oligosaccharides reduces the concentrations of IL-8 in the serum and ileum. This suggests a plausible mechanism for a protective factors of human milk oligosaccharides.

Xiong X, Wang Y, Chen X, Lin B, Zhuang Y, Luo L, Wang H, Yang C. Mixed Feedings and Necrotizing Enterocolitis: The Proportion of Human Milk Matters. *Breastfeed Med*. 2023 Jun;18(6):469-474. doi: 10.1089/bfm.2022.0268. Epub 2023 May 12. PMID: 37184535; PMCID: PMC10282811.

In this retrospective, observational study in a NICU in China, researchers explored the influence of different proportions of human milk on the risk of NEC in very low birthweight babies. All parents were encouraged to provide human milk, and if this was not possible or insufficient, formula supplementation was used (donor milk was not available). The data indicated that the proportion of human milk was inversely associated with the risk of NEC.

Yu F, Cao B, Wen Z, Li M, Chen H, Xie G. Is Donated Breast Milk Better Than Formula for Feeding Very Low Birth Weight Infants? A Systematic Review and Meta-Analysis. *Worldviews Evid Based Nurs*. 2019 Dec;16(6):485-494. doi: 10.1111/wvn.12410. Epub 2019 Nov 19. PMID: 31743577.

Researchers pooled data from 7 clinical trials, including 876 infants, to compare outcomes for infants fed donated breast milk compared with formula. Their pooled analysis indicated that formula showed more advantages in increasing infant weight and length compared with donated breast milk. However, formula was also significantly associated with a higher risk of NEC and a longer length of hospital stay when compared with donated breast milk.

Zhang B, Xiu W, Dai Y, Yang C. Protective Effects of Different Doses of Human Milk on Neonatal Necrotizing Enterocolitis. *Medicine (Baltimore)*. 2020 Sep 11;99(37):e22166. doi: 10.1097/MD.0000000000022166. PMID: 32925782; PMCID: PMC7489715.

In a summary of the literature, researchers stated that exclusive human milk and partial human milk reduced the incidence of NEC in premature infants, especially in the those fed by high proportion of human milk. However, the quality of the existing literature was viewed as low.

Milk Fortification

(articles in alphabetical order by first author)

Agrawal G, Wazir S, Kumar S, Yadav BS, Balde M. Routine versus Selective Fortification of Human Milk with Powdered Human Milk Fortifiers in Very Low Birth Weight (VLBW) Preterm Infants: A

Retrospective Pre-Post Cohort Study. *J Trop Pediatr*. 2019 Oct 1;65(5):439-445. doi: 10.1093/tropej/fmy074. PMID: 30544244.

At a single institution in India, researchers compared outcomes for 51 preterm, low birthweight infants under 2 regimes: (1) routine fortification, where a pre-fixed feeding volume and fortification of human milk were used; and (2) selective fortification, where a gradually increasing volume was administered and fortification was used only if weight gain was lower than expected. Infants treated with selective fortification had a comparable growth rate to those treated with routine fortification. There was a nonsignificant trend towards better metabolic parameters and lesser adverse outcomes for selective compared with routine fortification of human milk.

Ahnfeldt AM, Aunsholt L, Hansen BM, Hoest B, Jóhannsdóttir V, Kappel SS, Klamer A, Möller S, Moeller BK, Sangild PT, Skovgaard AL, van Hall G, Vibede LD, Zachariassen G. Bovine Colostrum as a Fortifier to Human Milk in Very Preterm Infants - A Randomized Controlled Trial (FortiColos). *Clin Nutr*. 2023 May;42(5):773-783. doi: 10.1016/j.clnu.2023.03.008. Epub 2023 Mar 15. PMID: 37004355.

In an open-label, multicenter, randomized controlled pilot trial (infants 26-31 weeks' gestation), parent's own milk or donor human milk was fortified with powdered bovine colostrum or a conventional fortifier. There were no differences in the risk of NEC.

Al Mandhari H, Suleman SKM, Al Ghammari Z, Al Qutaiti H, Al Hatmi J, Panchatcharam SM, Ganesh A, Al-Adawi S. Comparison between Efficacy of Human Milk Fortification Using Human Milk Fortifier versus Preterm Formula: A Retrospective Single-institutional Experience. *Oman Med J*. 2023 May 31;38(3):e504. doi: 10.5001/omj.2023.74. PMID: 37476476; PMCID: PMC10354693.

In a retrospective observational study in Oman, researchers found that fortification with human milk fortifier resulted in greater weight gain and lower risk of NEC, compared with preterm formula.

Amissah EA, Brown J, Harding JE. Protein Supplementation of Human Milk for Promoting Growth in Preterm Infants. *Cochrane Database Syst Rev*. 2020 Sep 23;9(9):CD000433. doi: 10.1002/14651858.CD000433.pub3. PMID: 32964431; PMCID: PMC8094919.

This is a Cochrane review with 6 studies and 204 preterm infants. Low-quality evidence showed that protein supplementation of human milk, fed to preterm infants, increased short-term growth. No other conclusions were supportable (including any conclusions about NEC risk). Since protein supplementation of human milk is now usually done as a component of multi-nutrient fortifiers, results of the studies reviewed may not apply to current practices.

Ananthan A, Balasubramanian H, Rao S, Patole S. Human Milk-Derived Fortifiers Compared with Bovine Milk-Derived Fortifiers in Preterm Infants: A Systematic Review and Meta-Analysis. *Adv Nutr*. 2020 Sep 1;11(5):1325-1333. doi: 10.1093/advances/nmaa039. PMID: 32277813; PMCID: PMC7490161.

In a meta-analysis including 6 trials involving 1,071 preterm infants, fortification of milk with human milk fortifiers decreased the risk of NEC but was associated with lower weight gain compared with fortification of milk with bovine milk fortifiers. However, the beneficial effects of human milk-based fortifiers for NEC were no longer significant in sensitivity analyses after excluding studies with high risk of bias. The quality of evidence was determined to be very low to low, and caution was advised in interpreting the results.

Basu S, Upadhyay J, Singh P, Kumar M. Early Versus Late Fortification of Breast Milk in Preterm Infants: A Systematic Review and Meta-Analysis. *Eur J Pediatr*. 2020 Jul;179(7):1057-1068. doi: 10.1007/s00431-020-03677-6. Epub 2020 May 27. PMID: 32458060.

In a pooled analysis with data from 3 studies, there was no statistically significant difference in growth or NEC risk between early and late fortification of breast milk in preterm infants.

Bridges KM, Newkirk M, Byham-Gray L, Chung M. Comparative Effectiveness of Liquid Human Milk Fortifiers: A Systematic Review and Meta-Analysis. *Nutr Clin Pract*. 2021 Dec;36(6):1144-1162. doi: 10.1002/ncp.10663. Epub 2021 Jun 8. PMID:34101248.

Based on 5 studies, very low-quality evidence suggests greater linear growth in VLBW infants fed whey hydrolysate liquid human milk-based fortifier, as well as greater weight gain in those fed casein hydrolysate human milk-based fortifiers, compared with the control of powder human milk-based fortifiers.

Brown JV, Lin L, Embleton ND, Harding JE, McGuire W. Multi-Nutrient Fortification of Human Milk for Preterm Infants. *Cochrane Database Syst Rev*. 2020 Jun 3;6(6):CD000343. PMID: 35658821.

This Cochrane review was designed to assess the positive and negative effects of multi-nutrient fortified human milk, compared with unfortified human milk. From 18 studies involving 1,456 infants, the reviewers concluded that feeding preterm infants with multi-nutrient fortified human breast milk, compared with unfortified human breast milk, was associated with modest increases in in-hospital growth rates. There was no significant increase in the risk in NEC based on fortification. However, the studies reviewed were small and considered methodologically weak. The reviewers concluded that evidence was insufficient to show whether multi-nutrient fortification has any effect on long-term growth or neurodevelopment.

Garg BD, Balasubramanian H, Kabra NS, Bansal A. Effect of Oropharyngeal Colostrum Therapy in the Prevention of Necrotising Enterocolitis among Very Low Birthweight Neonates: A Meta-Analysis of Randomised Controlled Trials. *J Hum Nutr Diet*. 2018 Oct;31(5):612-624. doi: 10.1111/jhn.12585. Epub 2018 Aug 2. PMID: 30073712.

In a meta-analysis of 4 studies, researchers found that oropharyngeal colostrum therapy was not associated with a statistically significant reduction in the incidence of NEC stage ≥2, mortality from any cause, or time to reach full feed.

Grace E, Hilditch C, Gomersall J, Collins CT, Rumbold A, Keir AK. Safety and Efficacy of Human Milk-Based Fortifier in Enterally Fed Preterm and/or Low Birthweight Infants: A Systematic Review and Meta-Analysis. *Arch Dis Child Fetal Neonatal Ed*. 2021 Mar;106(2):137-142. doi: 10.1136/archdischild-2020-319406. Epub 2020 Sep 17. PMID: 32943531.

Researchers reviewed 2 clinical trials. In these trials, the use of human milk-based fortifier compared with cow's milk-based fortifier reduced the risk of NEC. Caution was advised because there was no blinding in one study and the overall quality of the evidence was viewed as very low to low.

Hilditch C, Keir A, Collins CT, Middleton P, Gomersall J. Early Versus Delayed Introduction of Human Milk Fortification in Enterally Fed Preterm Infants: A Systematic Review and Meta-Analysis. *J Paediatr Child Health*. 2022 Jan;58(1):30-38. doi: 10.1111/jpc.15810. Epub 2021 Oct 20. PMID: 34669996.

In this review of 3 studies, there was no difference in the risk of NEC when comparing early and late introduction of human milk fortification.

Huston R, Lee M, Rider E, Stawarz M, Hedstrom D, Pence M, Chan V, Chambers J, Rogers S, Sager N, Riemann L, Cohen H. Early Fortification of Enteral Feedings for Infants <1250 Grams Birth Weight Receiving a Human Milk Diet Including Human Milk-Based Fortifier. J Neonatal Perinatal Med. 2020;13(2):215-221. doi: 10.3233/NPM-190300. PMID: 31707377; PMCID: PMC7369034.

In this single center observational study, researchers compared growth and rates of NEC and chronic lung disease among 394 low birth weight infants who had human-milk-based fortification added to their human milk early in their hospital stay (n=292) as compared to later in their hospital stay (n=102). The scientists found that early human milk fortification was associated with increased growth and with lower rates of lung disease. No differences in NEC were observed.

Jensen GB, Domellof M, Ahlsson F, Elfvin A, Naver L, Abrahamsson T. Effect of human milk-based fortification in extremely preterm infants fed exclusively with breast milk: a randomised controlled trial. *EClinicalMedicine*. 2024 Jan 2:68:102375. PMID: 38545091.

In this clinical trial at 24 neonatal units in Sweden, extremely preterm infants fed exclusively human breast milk were randomized to the use of human milk-based fortifiers or bovine milk-based fortifiers. Infants fed the human milk-based fortifiers and bovine milk-based fortifiers had comparable outcomes. Supplementation with human milk-based fortifiers, as compared with bovine milk-based fortifiers, did not reduce the incidence of the composite outcome of NEC, sepsis, or death.

Lin R, Shen W, Wu F, Mao J, Liu L, Chang Y, Zhang R, Ye X, Qiu Y, Ma L, Cheng R, Wu H, Chen D, Zheng Z, Lin X, Tong X; National Multicenter EUGR Collaborative Group. Human Milk Fortification in Very Preterm Infants in China: A Multicenter Survey. *Front Pediatr*. 2022 Feb 23;10:795222. doi: 10.3389/fped.2022.795222. PMID: 35281235; PMCID: PMC8905358.

This is a single center trial in a Chinese NICU involving 985 early preterm, very low birth weight infants. All were on a human milk diet, and they were assigned to different fortification groups – no fortification, early, middle, and late fortification. Rates of NEC at stage II or higher did not differ across groups.

Modi N. The Implications of Routine Milk Fortification for the Short and Long-Term Health of Preterm babies. *Semin Fetal Neonatal Med*. 2021 Jun;26(3):101216. doi: 10.1016/j.siny.2021.101216. Epub 2021 Feb 25. PMID: 33714708.

This review concludes that randomized controlled trials of multi-nutrient fortification to-date are inadequate. No trial has had power to detect important functional effects; the majority are methodologically weak and focus primarily upon short-term growth.

O'Connor DL, A Kiss, C Tomlinson, N Bando, A Bayless, DM Campbell, A Daneman, J Francis, K Kotsopoulos, PS Shah, S Vaz, B Williams, S Unger, the Optimom Feeding Group. Nutrient Enrichment of Human Milk with Human and Bovine Milk-Based Fortifiers for Infants Born Weighing <1250 g: A Randomized Clinical Trial. *Am J Clin Nutr*. 2018 Jul 1;108(1):108-116. PMID 29878061. Also see Errata -- Corrigendum for O'Connor et al. Nutrient Enrichment of Human Milk with Human and Bovine Milk-Based Fortifiers for Infants Born Weighing < 1250 g: A Randomized Clinical Trial. *Am J Clin Nutr*. 2018;108:108-16, PMID 31367755; and Corrigendum for O'Connor et al. Nutrient Enrichment of Human Milk with Human and Bovine Milk-Based Fortifiers for Infants Born Weighing < 1250 g: A Randomized Clinical Trial. *Am J Clin Nutr*. 2018;108:108-16, PMID 32367115.

In this study of infants in Canada born weighing <1250 g and exclusively fed human milk, infants were randomized to the use of human milk-based fortifiers or bovine milk-based fortifiers. Infants fed the human milk-based fortifiers and bovine milk-based fortifiers had comparable outcomes. Human milk-based fortifiers did not improve feeding tolerance or reduce mortality and morbidity compared with bovine milk-based fortifiers.

OuYang X, Yang CY, Xiu WL, Hu YH, Mei SS, Lin Q. Oropharyngeal Administration of Colostrum for Preventing NECand Late-Onset Sepsis in Preterm Infants with Gestational age ≤ 32 Weeks: A Pilot Single-Center Randomized Controlled Trial. *Int Breastfeed J*. 2021 Aug 21;16(1):59. doi: 10.1186/s13006-021-00408-x. PMID: 34419090; PMCID: PMC8379587.

In a pilot, single-center trial of 252 preterm infants in China, colostrum supplementation was associated with lower rates of NEC.

Pammi M, Suresh G. Enteral Lactoferrin Supplementation for Prevention of Sepsis and Necrotizing Enterocolitis in Preterm Infants. *Cochrane Database Syst Rev*. 2020 Mar 31;3(3):CD007137. doi: 10.1002/14651858.CD007137.pub6. PMID: 32232984; PMCID: PMC7106972.

This is a Cochrane review of lactoferrin supplementation with 12 studies included. Lactoferrin supplementation to enteral feeds was not associated with risk of NEC at stage II or higher, although there was some evidence to suggest a reduction in sepsis. Low- to very low-certainty evidence suggested that lactoferrin supplementation of enteral feeds in combination with probiotics may decrease the risk of late-onset sepsis and NEC ≥ stage II in preterm infants

without adverse effects. However, caution was advised because there were few included studies of poor methodological quality.

Premkumar MH, Pammi M, Suresh G. Human Milk-Derived Fortifier Versus Bovine Milk-Derived Fortifier for Prevention of Mortality and Morbidity in Preterm Neonates. *Cochrane Database Syst Rev*. 2019 Nov 7;2019(11):CD013145. doi: 10.1002/14651858.CD013145.pub2. PMID: 31697857; PMCID: PMC6837687.

This is a Cochrane review where only 1 study, with 127 infants, met inclusion criteria. There was insufficient evidence evaluating human milk-derived fortifier with bovine milk-derived fortifier in exclusively breast milk-fed preterm infants. Low-certainty evidence from one study suggested that in exclusively breast milk-fed preterm infants, human milk-derived fortifiers in comparison with bovine milk-derived fortifier may not change the risk of necrotizing enterocolitis, mortality, feeding intolerance, infection, or improve growth.

Sadeghirad B, Morgan RL, Zeraatkar D, Zea AM, Couban R, Johnston BC, Florez ID. Human and Bovine Colostrum for Prevention of Necrotizing Enterocolitis: A Meta-Analysis. *Pediatrics*. 2018 Aug;142(2):e20180767. doi: 10.1542/peds.2018-0767. Epub 2018 Jul 10. PMID: 29991526.

In this meta-analysis involving 8 RCTs and 385 infants, bovine or human colostrum had no effect on severe NEC, mortality, culture-proven sepsis, feed intolerance, or length of stay. The number of cases of NEC was low.

Enteral Feeding Methods

(articles in alphabetical order by first author)

Abiramalatha T, Thomas N, Thanigainathan S. High versus standard volume enteral feeds to promote growth in preterm or low birth weight infants. *Cochrane Database Syst Rev*. 2021 Mar 9;3(3):CD012413. doi: 10.1002/14651858.CD012413.pub3. PMID: 33733486; PMCID: PMC8092452.

This is a Cochrane review where 2 studies, with 283 infants, met inclusion criteria. Although high volume feeding "probably" promoted short term weight gain, the available data were inadequate to draw conclusions on the effect of high-volume feeds on other growth and clinical outcomes. Analysis of NEC showed no effect, but the studies were not powered to draw conclusions about the risk of NEC.

Arbra CA, Oprisan A, Wilson DA, Ryan RM, Lesher AP. Time to Reintroduction of Feeding in Infants with Nonsurgical Necrotizing Enterocolitis. *J Pediatr Surg*. 2018 Jun;53(6):1187-1191. doi: 10.1016/j.jpedsurg.2018.02.082. Epub 2018 Mar 7. PMID: 29622398.

Researchers conducted a chart review of 149 cases of non-surgical NEC. After adjusting for Bell's stage, the odds of NEC recurrence, death, and the composite outcome of recurrence or stricture or death were not significantly different between early and late refeeding groups.

Barr PA, Mally PV, Caprio MC. Standardized Nutrition Protocol for Very Low-Birth-Weight Infants Resulted in Less Use of Parenteral Nutrition and Associated Complications, Better Growth, and Lower Rates of Necrotizing Enterocolitis. *JPEN J Parenter Enteral Nutr*. 2019 May;43(4):540-549. doi: 10.1002/jpen.1453. Epub 2018 Nov 9. PMID: 30414179.

This was a prospective analysis of 136 VLBW infants born at a single institution, before and after the initiation of a nutrition protocol. The protocol included trophic feeds, feeding advancement, fortification guidelines, parameters on the concentration of parenteral nutrition (PN), and the discontinuation of PN and central lines. After initiation of the protocol, the researchers observed significantly improved growth, reduced PN use, and improved patient outcomes including incidence of NEC.

Bozkurt O, Alyamac Dizdar E, Bidev D, Sari FN, Uras N, Oguz SS. Prolonged minimal enteral nutrition versus early feeding advancements in preterm infants with birth weight ≤1250 g: a prospective randomized trial. *J Matern Fetal Neonatal Med*. 2022 Jan;35(2):341-347. doi: 10.1080/14767058.2020.1716723. Epub 2020 Jan 29. PMID: 31994953.

In this single center trial of 200 preterm low birth weight infants, researchers compared growth and outcomes across 2 groups – (a) infants on a "prolonged minimal enteral feeding" regimen, where they get minimal feeding for 5 days before volume is increased, and (b) "advancing feeding" regimen, where volume is increased daily from the start. There were no outcome differences. There was a trend towards fewer cases of NEC in group (a) (the prolonged minimal feeding), but few cases were observed overall.

Chitale R, Ferguson K, Talej M, Yang WC, He S, Edmond KM, Smith ER. Early Enteral Feeding for Preterm or Low Birth Weight Infants: A Systematic Review and Meta-Analysis. *Pediatrics*. 2022 Aug 1;150(Suppl 1):e2022057092E. doi: 10.1542/peds.2022-057092E. PMID: 35921673.

Researchers analyzed pooled data from 14 studies, including 1,505 preterm or low birth weight infants. They concluded that there was little or no association between early or late enteral feeding and the risk of NEC.

Dorling J, Abbott J, Berrington J, Bosiak B, Bowler U, Boyle E, Embleton N, Hewer O, Johnson S, Juszczak E, Leaf A, Linsell L, McCormick K, McGuire W, Omar O, Partlett C, Patel M, Roberts T, Stenson B, Townend J; SIFT Investigators Group. Controlled Trial of Two Incremental Milk-Feeding Rates in Preterm Infants. *N Engl J Med*. 2019 Oct 10;381(15):1434-1443. doi: 10.1056/NEJMoa1816654. PMID: 31597020.

In a randomized clinical trial of 2,804 preterm infants in the UK/Ireland, researchers compared faster and slower feeding regimens. They found no difference in two-year survival without disability, or NEC, according to feeding regimen. The researchers did not standardize what the infants were fed, but only a small number received only formula, with the majority receiving a combination of formula and breast milk. Although the group fed exclusively with formula tended to have more adverse outcomes, the small number of infants in this group did not allow for definitive conclusions comparing formula and breast milk.

Gao L, Shen W, Wu F, Mao J, Liu L, Chang YM, Zhang R, Ye XZ, Qiu YP, Ma L, Cheng R, Wu H, Chen DM, Chen L, Xu P, Mei H, Wang SN, Xu FL, Ju R, Zheng Z, Lin XZ, Tong XM; Chinese Multicenter EUGR Collaborative Group. Effect of Early Initiation of Enteral Nutrition on Short-Term Clinical Outcomes of Very Premature Infants: A National Multicenter Cohort Study in China. *Nutrition*. 2023 Mar;107:111912. doi: 10.1016/j.nut.2022.111912. Epub 2022 Nov 12. PMID: 36577163.

In this multi-center observational study in China, no differences in NEC risk were observed when comparing early and later initiation of enteral nutrition in early preterm infants.

Hock AM, Chen Y, Miyake H, Koike Y, Seo S, Pierro A. Initiation of Enteral Feeding After Necrotizing Enterocolitis. *Eur J Pediatr Surg*. 2018 Feb;28(1):44-50. doi: 10.1055/s-0037-1604436. Epub 2017 Aug 24. PMID: 28837997.

This systematic review included 2 studies with 91 infants. Neither study was a RCT. The recurrence rates of NEC were not statistically different between early (5.4%) and delayed (8.6%) enteral feeding groups when combining data across the two observational studies.

Ibrahim NR, Van Rostenberghe H, Ho JJ, Nasir A. Short versus long feeding interval for bolus feedings in very preterm infants. *Cochrane Database Syst Rev*. 2021 Aug 19;8(8):CD012322. doi: 10.1002/14651858.CD012322.pub2. PMID: 34415568; PMCID: PMC8407504.

This is a Cochrane review including 4 studies and 417 preterm infants (the majority were in one study). There was no difference in outcomes when comparing short and long feeding intervals.

Modi M, Ramji S, Jain A, Kumar P, Gupta N. Early Aggressive Enteral Feeding in Neonates Weighing 750-1250 Grams: A Randomized Controlled Trial. *Indian Pediatr*. 2019 Apr 15;56(4):294-298. PMID: 31064897.

In this single center study of 131 very low birth weight infants, an "aggressive" (higher volume and speed) feeding regimen was compared with a "conservative" feeding regimen. No differences in outcomes, including NEC, were observed.

Oddie SJ, Young L, McGuire W. Slow Advancement of Enteral Feed Volumes to Prevent Necrotising Enterocolitis in Very Low Birth Weight Infants. *Cochrane Database Syst Rev*. 2021 Aug 24;8(8):CD001241. doi: 10.1002/14651858.CD001241.pub8. PMID: 34427330; PMCID: PMC8407506.

This is a Cochrane review with 14 studies and 4,033 preterm infants (about 70 percent of the infants were in one trial). The available trial data indicated that advancing enteral feed volumes slowly (daily increments up to 24 mL/kg) compared with faster rates was not associated with a reduction in the risk of NEC, death, or feed intolerance in very preterm or VLBW infants.

Patel S, Chaudhari M, Kadam S, Rao S, Patole S. Standardized Feeding and Probiotic Supplementation for Reducing Necrotizing Enterocolitis in Preterm Infants in a Resource-Limited Set Up. *Eur J Clin Nutr*. 2018 Feb;72(2):281-287. doi: 10.1038/s41430-017-0040-7. Epub 2017 Dec 19. PMID: 29255185.

In a case series at a single institution, NEC incidence declined after the introduction of a standardized feeding and probiotic supplement protocol.

Ramaswamy VV, Bandyopadhyay T, Ahmed J, Bandiya P, Zivanovic S, Roehr CC. Enteral Feeding Strategies in Preterm Neonates ≤32 weeks Gestational Age: A Systematic Review and Network Meta-Analysis. *Ann Nutr Metab*. 2021;77(4):204-220. doi: 10.1159/000516640. Epub 2021 Jul 9. PMID: 34247152.

In this review of 29 studies, early initiated, moderately early, or late advanced with moderate volume increment feeding regimens may help decrease the risk of NEC stage ≥II. However, the quality of the evidence was viewed as low.

Razak A. Two-Hourly Versus Three-Hourly Feeding in Very Low-Birth-Weight Infants: A Systematic Review and Meta-Analysis. *Am J Perinatol*. 2020 Jul;37(9):898-906. doi: 10.1055/s-0039-1691767. Epub 2019 Jun 4. PMID: 31163479.

To analyze 2-hour versus 3-hour feeding regimens, researchers pooled data on 952 infants from 7 studies – 4 RCTs and 3 observational. The pooled analyses of RCTs showed no significant differences in the outcomes: time to reach full enteral feeding, necrotizing enterocolitis, feed intolerance, and hypoglycemia.

Shah SD, Booth N, Nandula P, Makker K, Cortez J, Sharma R, Smotherman C, Hudak ML. Effects of Standardized Feeding Protocol on Growth Velocity and Necrotizing Enterocolitis in Extremely Low Birth Weight Infants. *J Perinatol*. 2021 Jan;41(1):134-139. doi: 10.1038/s41372-020-00892-9. Epub 2020 Dec 6. PMID: 33281186.

In a single center observational study, initiation of a standardized feeding protocol was associated with improved growth velocity and lower adjusted rate of NEC at 36 weeks' post-menstrual age.

Walsh V, Brown JVE, Copperthwaite BR, Oddie SJ, McGuire W. Early Full Enteral Feeding for Preterm or Low Birth Weight Infants. *Cochrane Database Syst Rev*. 2020 Dec 27;12(12):CD013542. doi: 10.1002/14651858.CD013542.pub2. PMID: 33368149; PMCID: PMC8094920.

This is a Cochrane review where 6 studies, with 526 infants, met inclusion criteria. The studies provided insufficient data to determine with any certainty how early full enteral feeding, compared with delayed or progressive introduction of enteral feeds, affects growth in preterm or low birth weight infants. Analysis of whether early full enteral feeding affects the risk of NEC showed no effect, and no conclusions were possible because of the risk of bias in the trials (due to lack of masking), inconsistency, and imprecision.

Yeo KT, Kong JY, Sasi A, Tan K, Lai NM, Schindler T. Stopping Enteral Feeds for Prevention of Transfusion-Associated Necrotising Enterocolitis in Preterm Infants. *Cochrane Database Syst Rev*. 2019 Oct 28;2019(10):CD012888. doi: 10.1002/14651858.CD012888.pub2. PMID: 31684689; PMCID: PMC6815687.

This is a Cochrane review where only 1 study, with 22 infants, met inclusion criteria, although there were 7 studies related to the topic. Care personnel were not blinded in the one study and no cases of NEC were reported. No conclusions could be drawn, and the overall GRADE rating was very low.

Young L, Oddie SJ, McGuire W. Delayed Introduction of Progressive Enteral Feeds to Prevent Necrotising Enterocolitis in Very Low Birth Weight Infants. *Cochrane Database Syst Rev*. 2022 Jan 20;1(1):CD001970. doi: 10.1002/14651858.CD001970.pub6. PMID: 35049036; PMCID: PMC8771918.

This is a Cochrane review including 14 studies and 1,551 infants. A meta-analysis indicated that delaying the introduction of progressive enteral feeds beyond four days after birth (compared with earlier introduction) was not associated with a reduced risk of NEC or death in very preterm or very low birth weight infants.

Appendix F. Acronyms and Abbreviations

AAP	American Academy of Pediatrics
ARA	Arachidonic Acid
BDNF	Brain-derived neurotrophic factor
BMDF	Bovine milk-derived fortifier
BMF	Bovine milk fortifiers
C34	Compound 34
CDC	Centers for Disease Control and Prevention
DHA	Docosahexaenoic acid
DoMINO	Donor Milk for Improved Neurodevelopmental Outcomes trial
DSLNT	Disialyllacto-N-tetraose
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ELBW	Extremely low birth weight
eNOS	Endothelial nitric oxide synthase
FDA	U.S. Food and Drug Administration
GRADE	Grades of Recommendation, Assessment, Development and Evaluation
HMBANA	Human Milk Banking Association of North America
HM	Human milk
HMDF	Human milk-derived fortifier
HMF	Human milk fortifier
HMO	Human milk oligosaccharide
HPF	Hydrolyzed protein preterm infant formula
HHS	U.S. Department of Health and Human Services

ICD-10	International Classification of Diseases, 10th edition
IgA	Immunoglobulin A
IGF-1	Insulin-like Growth Factor 1
IL-17	Interleukin 17
IL-17R	Interleukin 17 receptor
IND	Investigational New Drug
IUGR	Intrauterine growth restriction
IV	Intravenous
LPS	Lipopolysaccharide
MILK Trial	Neurodevelopmental Effects of Donor Human Milk vs. Preterm Formula in ELBW Infants Trial
mPINC™	Maternity Practices in Infant Nutrition and Care survey
NACHHD	National Advisory Child Health and Human Development
NEC	Necrotizing Enterocolitis
NEST	Necrotizing Enterocolitis Surgery Trial
NIAID	National Institute of Allergy and Infectious Diseases
NICHD	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
NICU	Neonatal intensive care unit
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIGMS	National Institute of General Medical Sciences
NIH	National Institutes of Health
NHLBI	National Heart, Lung, and Blood Institute
NPO	*Nil per os:* Latin for nothing-by-mouth
NRN	Neonatal Research Network
OptiMOM	Optimizing Mothers' Milk for Preterm Infants
PI	Principal Investigator

PMA	Postmenstrual age
PPROM	Preterm premature rupture of membranes
PREEMIE Act	Prematurity Research Expansion and Education for Mothers Who Deliver Infants Early Act (Public Law 109-450)
PUMA	p53 Upregulated Modulator of Apoptosis
RCDC	NIH Research, Condition, and Disease Categorization
RCT	Randomized controlled trial
shRNA	Small hairpin ribonucleic acid
SIP	Spontaneous intestine perforation
SUPPORT	Surfactant Positive Airway Pressure and Pulse Oximetry Trial in Extremely Low Birth Weight Infants
Th17	T-helper 17 cell
TLR4	Toll-like receptor 4
Treg	Regulatory T-cell
VLBW	Very low birth weight
VON	Vermont Oxford Network
WG	Working Group
WONDER	Wide-ranging ONline Data for Epidemiologic Research