Value of Human Milk:
Reducing Morbidities and Necrotizing Enterocolitis
The Value of Human Milk in the NICU: Reducing Morbidities and Necrotizing Enterocolitis

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Introduction

Breast milk is very important if your baby is born early or is sick. Breast milk can help your baby get better faster and develop properly. The nurses or lactation consultant can help you learn how to pump your milk if your baby cannot breastfeed.

Recently, the Joint Commission joined professional organizations, the CDC and the US Surgeon General, in publically promoting the benefits of human milk for all infants, including preterm infants. The evidence supporting the use of human milk in the NICU is both extensive and compelling: laboratory and clinical research demonstrate the value of human milk in reducing multiple disease states of the preterm infant including necrotizing enterocolitis, chronic lung disease, retinopathy of prematurity, and infections. Against this backdrop of information and data, it is easy to lose sight of the most critical and consistent element in all of these diseases: the interface of human milk with the infant gastrointestinal system.

Human milk has evolved to meet the unique needs of human infants. Providing species-specific macro- and micronutrients, human milk is the most appropriate source of infant nutrition. Beyond nutrition, human milk has a significant immunoprotective role, particularly important in preterm infants. By stimulating gut mucosal growth, ensuring intact mucosa barriers and delivering multiple immune factors to the intestine, human milk protects infants from environmental pathogens.

In addition, human milk contains immunomodulatory and anti-inflammatory factors that extend protection beyond the gastrointestinal system to distant organ systems. By downgrading inflammation at the level of the gut, human milk can prevent systemic inflammatory responses associated with necrotizing enterocolitis (NEC) as well as distal organ injury of the brain, lungs, and eyes. Immunomodulatory aspects of human milk impact the immediate health of infants by programming immune responses, but at the same time, establish foundations for healthy immune responses in later life.

In this essay we will explore 3 research studies related to the use of human milk in the neonatal intensive care of preterm infants. The first article for review is a study of the effects of human milk on immature gut permeability. Commentary following a summary of the article focuses on factors in human milk that facilitate maturation of the gastrointestinal system. The second article investigates the role of human milk in reducing of the risk of NEC and death. In the subsequent commentary, we will examine the anti-inflammatory and immunomodulatory aspects of human milk in relation not just to NEC but also to other diseases common in preterm infants. The third article compares the incidence of NEC in preterm infants receiving a human milk-based human milk fortifier to those receiving a standard bovine-based human milk fortifier. Commentary relates study outcomes to the physiology of NEC and the effects of a non species-specific diet.
Key Points

Human milk provides multiple layers of protection to the preterm infant, all beginning at the level of the immature gastrointestinal system.

Human milk contains factors which affect:
- Infant growth and development
- Gastrointestinal maturation
- Gastrointestinal mucosal protection
- Gut microbiota development
- Anti-inflammatory responses of the gut and distal organs
- Immunomodulatory responses to program short and long-term immune responses.

Human milk significantly decreases preterm gut permeability during the first month of life. This benefit occurs in a dose-response manner; that is, higher doses of human milk are associated with greater tight junction closure.

Research evidence suggests a relationship between increased gut permeability associated with formula feeds and an increased risk of NEC.

Human milk feedings during the first two weeks of life can reduce the risk of NEC in preterm infants. As the amount of human milk increases during this brief window of time, the risk of NEC decreases.

Bovine-based products - infant formula and fortifiers - contain proteins that may contribute to the development of NEC in preterm infants.

Extremely premature infants fed an exclusively human milk-based diet have significantly lower rates of medical and surgical NEC and death when compared to infants fed a mother’s milk-based diet that also includes bovine milk-based products.

One hundred percent human milk-based diets are now possible with commercially available human milk-based human milk fortifiers. An initial study of this product suggests potential benefits for preterm infants including reduction of the risk of NEC and death.

While additional study of a 100% human milk-based diet for preterm infants is needed, a preliminary cost analysis of the use of human milk-based human milk fortifiers indicates significant clinical benefits could also produce substantial cost benefits.
Background

At the time of birth, the preterm infant’s gastrointestinal system is anatomically and physiologically immature. As the infant develops, tight junctions between the cells of the intestinal mucosa close, reducing the risk of invasion by pathogens in the environment.

Intestinal permeability can be accurately measured by the ratio of lactulose to mannitol in infants’ urine. Mannitol is a small molecule that easily passes through the intestinal epithelial cells by intracellular diffusion. A lactulose molecule is too large to diffuse through the epithelial cells. However, lactulose can pass between the intestinal cells if the junctions between the cells are open. Therefore, the lactulose to mannitol ratio (L/M ratio) is a direct measure of intestinal permeability, indicating the degree of closure of the junctions between intestinal epithelial cells.

The purpose of this study was to examine the relationship between feeding type – human milk and infant formula – on intestinal permeability in preterm infants over the first month of life.

Sixty-two preterm infants less than or equal to 32 weeks gestation were included. The median gestational age at birth was 29.2 weeks (range 24-32 weeks).

Infants were evaluated on postnatal days 7, 14, and 30 for degree of intestinal permeability. On each of these days, a lactulose/mannitol solution was administered by nasogastric tube and the infant’s urine was collected and analyzed for lactulose/mannitol ratio.

The authors collected detailed information on infant feeding, particularly the amounts of human milk and/or preterm infant formula the infants received. Human milk was fortified with standard human milk fortifier once feed volumes reached 120-150 mL/kg/day.

Infants were divided into four groups by the amount of human milk they received over the study period:

- Majority of human milk feeds: received >75% of enteral feeding as mother’s milk
- Partial human milk feeds: received 25-75% of enteral feeding as mother’s milk
- Minimal human milk feeds: received <25% of enteral feeding as mother’s milk
- No human milk feeds: received formula only

Results

Composite data show infants who received any mother’s milk demonstrated significantly lower L/M ratios, indicating reduced intestinal permeability and, therefore, better tight junction closure when compared to infants receiving no human milk (p = 0.006).

Infants who received >75% of feeding as mother’s milk demonstrated a 3.8-fold lower composite median L/M ratio (and decreased intestinal permeability) when compared to infants receiving <25% or no mother’s milk.
Conversely, exclusively formula-fed infants demonstrated a 2.8-fold higher composite median lactulose/ mannitol ratio when compared with those who received any mother’s milk, indicating significantly greater intestinal permeability and risk for infection.

Conclusions

The authors conclude: "Preterm infant intestinal permeability was significantly decreased for those receiving human milk versus formula in a dose-related manner in the first postnatal month" (p.11).

The data suggest a possible relationship between increased gut permeability with formula feeding of preterm infants and a potential increased risk of NEC.

Commentary

This research contributes valuable insights into the question of how human milk protects preterm infants from gastrointestinal disturbances and disease: human milk feedings facilitate the maturation of tight junctions in a dose-response manner over the first month of life. This study also demonstrates the detrimental effect of formula feeding on the immature gastrointestinal system: formula feedings are associated with delayed closing of tight junctions, also in a dose response manner.

The gastrointestinal tract has a dual purpose of absorbing nutrients and protecting the organism from invasion of environmental pathogens. This protection begins in the lumen of the GI tract with functional barriers like mucus and commensal (or protective) bacteria and continues into deeper layers of the mucosa with cells specific to immune response and regulation of inflammation.

The human gastrointestinal tract is comprised of several layers of functional substances overlying the intestinal epithelial absorptive cells, commonly referred to as enterocytes. At the apical end of the enterocyte, several layers of coatings protect the epithelial cells from harmful microbes. The glycocalyx is a thick, mucin-rich glycoprotein matrix lining the entire gastrointestinal system. Together with the mucus layer, it forms a sticky gel-like barrier that lubricates and protects the intestine. Embedded within the mucus layer are antimicrobial inhibitors that help regulate gut colonization. Lastly, a biofilm of symbiotic bacteria develops at the interface with the intestinal lumen. All three layers work in concert to protect the infant from pathogenic bacteria.

Gut permeability is one of multiple developmental limitations of the preterm infant’s immature gastrointestinal system, all of which can contribute to an increased risk of feeding intolerance as well as short and long-term morbidities. Other aspects of the preterm gastrointestinal system related to immaturity include a need for rapid cellular growth and turnover, decreased peristalsis, decreased gastric acid, decreased proteolytic enzymatic activity, altered intestinal mucus, and an immature inflammatory response.
According to Wagner et al., amniotic fluid and human milk are sources of multiple growth factors important to the continuum of fetal-infant gut development and maturation. Like amniotic fluid, human milk promotes gut maturation by supplying epidermal growth factor as well as other trophic factors. After birth, human milk assumes the role of exogenous source of bioactive substances stimulating cell growth and repair through the synergistic actions of cytokines, insulin-like growth factors, transforming growth factors \( \alpha \) and \( \beta \), insulin, erythropoietin and vasoactive endothelial growth factor. Wagner et al. hypothesize trophic factors in human milk also enhance the development and function of the intestinal mucus barrier. By promoting growth of enterocytes, tight junctions and the mucus barrier, human milk contributes to the overall functioning and integrity of the infant gastrointestinal system.

Human milk provides other benefits related to immaturity of the neonatal gastrointestinal tract. Human milk increases peristalsis, thereby decreasing the build up of toxins and pathogens in the intestinal lumen. Additionally, milk lipases breakdown triglycerides by into anti-microbial free fatty acids promoting an acidic gastric environment essential for nutrient degradation. These are just a few of the numerous protective functions of human milk in the preterm gastrointestinal tract.

As this study by Taylor et al demonstrates, the absence of human milk and the presence of preterm infant formula can impair the system’s ability to mature. The next article and commentary delve deeper into the relationship between infant feedings and clinical outcomes.

**Background**

This study is a secondary data analysis of preterm infants who participated in the National Institute of Child Health and Human Development Neonatal Research Network Glutamine Trial involving 15 Network Centers.

The purpose of the study was to determine the relationship between the intake of human milk during the first 14 days of life and the risk of NEC or death during the study period of birth to discharge or 120 days, whichever came first. The authors evaluated three different measures of human milk intake: 1) the proportion of total intake (enteral and parental); 2) the proportion of enteral intake; and, 3) the cumulative volume of human milk.

NEC and death in preterm infants occur in higher frequency in the first months of life. For a beneficial effect of human milk to be detected, its administration must precede the onset of infant illness. Also, cases of spontaneous intestinal perforation occurring during the first weeks of life can be misclassified as NEC. Therefore, only infant nutrition during the first 14 days was evaluated and infants who died of NEC within the first 14 days were excluded from the study.

The authors analyzed demographic, intrapartum, medical and feeding data from 1272 preterm infants greater than 23 weeks with birth weights between 401 and 1000 grams.

Statistical outcomes were adjusted for birth weight, small for gestational age, race, patent ductus arteriosus, antenatal steroids and duration of mechanical ventilation.

Statistical analyses were reported as hazard ratios. Unlike risk ratios, hazard ratios reflect the analysis of time survived to an event. The “event” in this study was the occurrence of NEC or death and the treatment was human milk feedings. In this study, a hazard ratio of 1.0 would indicate human milk feeds had no effect on the rates of NEC or death.

**Results**

Among the 1272 extremely low birth weight (ELBW) infants, 13.6% died or developed NEC after 14 days of life.

The authors found an inverse relationship between human milk feedings during the first 14 days of life and the risk of NEC or death over hospital stay. Increasing cumulative and proportional amounts of human milk in the first 2 weeks was associated with increased survival time in which the infant was free of NEC.

Infants who developed NEC or died after the first 2 weeks were fed less human milk and had a lower mean daily volume of human milk than infants who survived free of NEC.

The hazard ratio for NEC or death after 14 days of life was 0.87 for every 100 mL/kg increase in volume of human milk.
The hazard ratio of NEC or death after 14 days of life was 0.83 for every 10% increase in proportion of human milk to total feeds.

Conclusions

These data suggest a dose-dependent beneficial effect of human milk feeding during the first two weeks of life with a reduction in the risk of NEC or death among extremely low birth weight infants.

These data are consistent with other studies indicating increasing protective effects against NEC with larger doses of human milk.

Commentary

Several studies have demonstrated the protective effects of human milk for preterm infants against the risk of sepsis and NEC.4,8,9,17,18 This particular study by Meinzen-Derr and colleagues evaluates the impact of the dose and total percent of human milk over a short period of time in a large population of infants. In a separate prospective cohort clinical study, also of ELBW infants, Sisk et al19 evaluated the impact of low (<50% of total feeds) and high (>50% of total feeds) doses of human milk. Their results indicated a six-fold decrease in the risk of NEC in infants receiving at least 50% human milk feedings in the first 14 days of life. For mothers who did not plan to provide milk for their infants, this information could positively influence their decisions about initiating pumping.

In the previous commentary, we examined mechanisms by which human milk promotes maturation of the preterm gastrointestinal system. We will now shift our focus to a few of the anti-infective, anti-inflammatory and immunomodulatory aspects of human milk that contribute to the reduction of NEC, sepsis and other morbidities.

Human milk is a well-known source of multiple anti-infective agents including secretory IgA, lactoferrin, lysozyme, macrophages and free fatty acids.12,16 These agents work in concert to inactivate, destroy or bind to specific microbes, preventing their attachment to mucosal surfaces.20 At the same time, human milk contains lactic acid bacteria, primarily bifidobacteria (also referred to as lactobacillus bifidus). These protective commensal bacteria become part of the gut microflora and influence inflammatory and immunomodulatory processes.

The significance of a healthy gut microbiota cannot be understated. Bacterial biofilm along with the glycocalyx and mucus layers form a living, bioactive coating for the intestine. Commensal bacteria prevent the overgrowth of pathogenic bacteria, acidify the gut, ferment lactose, breakdown lipids and proteins, and produce vitamins K and biotin.10,14-16 Colonization of the infant’s GI tract begins at the time of birth with exposure to the mother’s vaginal flora or skin flora, depending on mode of delivery.21 Colonization continues with exposure to the environment and is heavily influenced by type of infant feeding.
Recent discoveries have clarified the symbiotic relationship of human milk oligosaccharides (HMOs) and lactic acid bacteria in the infant’s gut.22-26 HMOs are complex carbohydrate molecules abundant in human milk. However, their purpose has been a mystery because human infants cannot digest HMOs. In the last few years, researchers have discovered HMOs in human milk are digestible by specific bifidobacteria in infants’ gastrointestinal tracts. In this capacity, HMOs function as prebiotics, feeding and stimulating the growth of commensal bacteria. They also act as decoys or receptor analogs to inhibit binding of pathogens – including rotaviruses – to intestinal surfaces.24,26-28

Human milk reduces the risks of NEC,2,3,8,9 sepsis,4,5,8,29 and intestinal disturbances in part by promoting healthy gut microbiota and intact mucosa. Anti-infective agents in mothers’ milk (mentioned above) contribute to these layers of protection against infection. However, localized actions do not explain the ability of human milk to reduce the risk of diseases remote from the GI tract, e.g., chronic lung diseases, retinopathy of prematurity and disorders which lead to neurodevelopmental delays. These diseases, like NEC, are characterized by a systemic inflammatory response triggered by overproduction and release of pro-inflammatory cytokines, such as Interleukin-8 (IL-8).10,15,23,30

Normally, inflammation acts as a healthy defense mechanism to rally immune factors, including leukocytes, to the site of infection or tissue injury. However, preterm infants lack the regulatory mechanisms to keep inflammation in check.31 Caicedo et al.10 hypothesize the release of IL-8 and other pro-inflammatory factors in the preterm gut can cause an exaggerated inflammatory response, leading to intestinal injury (NEC) as well as damage to other organs. Several human milk components interrupt or downgrade inflammatory processes in preterm infants, including interleukin-10 (an anti-inflammatory cytokine), lactoferrin, epidermal growth factor, transforming growth factor-β, HMOs, soluble CD-14 and insulin-like growth factor.10,12,31 These factors work synergistically to protect the preterm infant from over-productive inflammatory responses.

In the context of studies we have already examined – those by Taylor, Meinzen-Derr and Sisk in which the early use of human milk had a significant positive effect on preterm infant outcomes - it should be mentioned that many of these protective milk components are at their highest in colostrum.31 Furthermore, as Meier notes so concisely, it is “during this critical exposure period…that [infant] formula appears to exert an independent, pro-inflammatory effect”31 (p.222). Our last research article moves this discussion into the clinical arena, where the benefits of human milk become complicated by the necessity for human milk fortification.

**Background**

Studies evaluating the efficacy of human milk in reducing the risks of short and long term morbidities are confounded by the need for milk fortification. Once preterm infant feedings progress to volumes greater than 100 mL/kg/day, bovine-based human milk fortifiers are frequently added to human milk – mother’s own or donor milk – to enhance nutrients, including protein, calcium and phosphorous. This practice raises questions about the impact of bovine-based fortifiers on preterm infant health.

Human milk fortifier produced from human milk is now technologically possible. The product used in this study was made by Prolacta Bioscience from pasteurized donor human milk.

The purpose of this study was to compare the health benefits of an exclusively human milk-based diet to a diet of both human milk and bovine-based products in extremely preterm infants.

In this multi-center clinical trial, 207 preterm infants with birth weights between 500-1250 grams were randomized into three study groups. All infants received mothers’ milk.

- Group 1 (HM100) received pasteurized donor human milk-based human milk fortifier when the enteral intake was 100 mL/kg/day. Pasteurized donor human milk was used if no mother’s milk was available.
- Group 2 (HM40) received pasteurized donor human milk-based human milk fortifier when the enteral intake was 40 mL/kg/day (earlier than the usual standard of care). Pasteurized donor human milk was used if no mother’s milk was available.
- Group 3 (BOV) received bovine milk-based human milk fortifier when the enteral intake was 100 mL/kg/day. Preterm formula was used if no mother’s milk was available.

Study protocol included parenteral nutrition within 48 hours of birth with trophic feeds initiated 1-4 days after birth. Feedings were advanced as tolerated per study protocol and study group. Infants remained in the study until they reached one of the following goals: 91 days of age, discharged from the hospital or were taking 50% of feeds (4/day) by mouth.

**Results**

Across all three groups, mother’s own milk comprised more than 70% enteral feeds. Group 3 (BOV) infants received the highest dose of mother’s own milk.

Outcomes of the 3 groups were similar for rates of feeding intolerance, late-onset sepsis, length of hospital stay and weight gain.
Results calculated on group assignment showed NEC rates were significantly lower in the infants who received only a human milk-based diet. After removing infants from Groups 1 and 2 who inadvertently received bovine-based products, further reductions in NEC were realized:

- Infants in Group 1 (HM100) had a 1.7% NEC rate
- Infants in Group 2 (HM40) had a 3.2% NEC rate
- Infants in Group 3 (BOV) had a 15.3% NEC rate

All 8 cases of surgical NEC and the 3 study deaths were in infants who had received bovine-based products, either as part of their group assignment or inadvertently.

**Conclusions**

Extremely premature infants fed an exclusively human milk-based diet have significantly lower rates of NEC and surgical NEC when compared infants fed a mother’s milk-based diet that also includes bovine milk-based products.

The authors estimated that the number of infants needed to treat with an exclusive human milk-based diet to prevent one case of NEC is 10. The number to treat to prevent one case of surgical NEC or death is 8.

The authors conclude the similarity of the 3 groups in terms of other outcomes including other morbidities - length of hospital stay, and weight gain - was probably due to the high doses of human milk across all 3 groups. Ironically, infants in Group 3 received the greatest overall amount of mother’s own milk (mean 5676 mL per study period vs. 4048 mL and 4544 mL in Groups 1 and 2 respectively), yet their incidence of NEC was significantly higher.

**Commentary**

In a 2009 *Cochrane Database Review*, Kuschel and Harding analyzed the research regarding the use of human milk fortifier in preterm infants. The authors noted an absence of evidence of the long-term benefits of human milk fortification as well as “insufficient evidence to be reassured that there are no deleterious effects…” (p.7). On the other hand, they acknowledged fortification of human milk leads to small but statistically significant increases in infant weight gain, linear growth and head growth. Noting that current practice, research and clinical ethics have moved beyond the discussion of whether or not to fortify human milk, they recommended further research of fortifier components and comparisons of different fortifier preparations.

The study by Sullivan et al suggests a diet that includes non-human proteins can have a significant negative impact on preterm infant morbidity and mortality. The predominant protein in bovine milk - casein - has been identified since the 1970s as a chemoattractant to human leukocytes. Leukocytes have specific receptor sites for binding with casein. In laboratory and animal studies, casein activates movement of leukocytes to the casein molecules. Thus, casein is inherently pro-inflammatory, causing activation of mucosal defenses and the release of inflammatory mediators in the preterm gut, particularly in the presence of pathogenic bacteria.
In 2002 Koivusalo et al. investigated an intraluminal casein model of NEC by injecting casein or normal saline into the intestines of neonatal piglets. NEC was induced only in the casein-injected animals, leading the authors to conclude casein is a key factor in the development of NEC. Along these lines, Clark and Miller developed an intraluminal pathogenesis model of NEC with intestinal bacteria and casein as key triggers for the disease. These authors concluded, “casein may have multiple, potentially deleterious actions, including modification of enteric flora fermentation rates and potent activation of macrophages, neutrophils and leukocytes” (p. S67). These studies demonstrate a significant relationship between NEC and bovine proteins in formula and fortifiers.

Several studies preceded the research by Sullivan et al. on the development and/or use of a human milk-based human milk fortifier. Three studies compared growth rates in preterm infants receiving bovine-based fortifiers to infants receiving human milk-based fortifiers made with ultrafiltrated human milk protein or freeze-dried skimmed human milk. Each of these trials found similar growth rates and feeding tolerance between the groups. However, human milk-based fortifiers outside of the research realm have not been available until recently.

Perhaps in anticipation of concerns about the cost of using human milk-based fortifiers, Ganapathy, Hay and Kim published a revealing cost analysis in 2011. Using Sullivan’s outcomes data, these authors calculated the cost effectiveness of a 100% human milk-based diet comprised of mother’s milk and human milk-based fortifier when compared to a diet of mother’s milk supplemented with formula and standard fortifier. These authors calculated the costs of routine preterm infant care, management of medical and surgical NEC, as well as the costs of donor human milk, infant formula and fortifier products. Their results supported the cost effectiveness of a human milk-based diet; the use of a 100% human milk-based diet could yield a net direct savings of $8,167 per extremely preterm infant.

With the advent of commercially available human milk-based fortifiers, the science of human milk and human milk-based products rises to a new level. In this vein, the Sullivan article enhances our understanding of the potential of human milk-based fortifier not only in promoting growth but also in reducing the risk of disease.

**Concluding Remarks**

We have explored a wide range of topics related to the interaction of human milk, the preterm gastrointestinal system and diseases affecting preterm infants. This review highlighted in a generalized fashion some of the maturational, anti-inflammatory, anti-infective and immunomodulary properties of human milk that work within the neonatal GI tract to facilitate the infant’s transition from intrauterine to extrauterine life.

Of particular importance in this discussion is the potential for human milk and human milk-based fortifiers to decrease the risk of necrotizing enterocolitis in our NICU populations. NEC is an inflammatory bowel disease affecting approximately 10% of preterm infants less than 1500 grams. Mortality rates for NEC are greater than 20%, up to 30-40% if surgical treatment is required. Human milk clearly has an advantage in a dose-response manner of decreasing the risk of NEC. However, until recently, there were few options for promoting adequate preterm infant growth and bone density except to fortify human milk with preterm formula or fortifiers. The studies highlighted in this essay inform our understanding of why human milk is so important and suggest methods to extend its application in the care of preterm infants.
References


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