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The preterm infant stomach actively degrades milk proteins with increasing breakdown across digestion time

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Abstract

Aim: This study investigated the effect of time post-ingestion on gastric digestion and gastric hormones after feeding preterm infants unfortified and fortified human milk.

Methods: Human milk and infant gastric samples were collected from 14 preterm (23–32 wk birth gestational age) mother-infant pairs within 7–98 days postnatal age. Gastric samples were collected 1, 2 and 3 h after beginning of feeding. Samples were analyzed for pH, proteolysis, general protease activity and the concentrations of pepsin, gastrin and gastrin-releasing peptide. One-way ANOVA with repeated measures followed by Tukey's multiple comparisons test was used.

Results: Gastric pH was significantly decreased after each hour in the preterm infant stomach from 1 to 3 h postprandial. Proteolysis increased significantly from human milk to gastric contents at 1, 2 and 3 h postprandial (by 62, 131% and 181%, $p < 0.05$). General protease activity increased significantly by 58% from human milk to the gastric contents at 2 h postprandial. Gastrin-releasing peptide was present in human milk whereas gastrin was produced in the infant stomach.

Conclusion: Though preterm infants may digest human milk proteins to a lesser extent than term infants, we demonstrated that the preterm infant stomach actively degrades milk proteins with increasing breakdown over digestion time.

Keywords

enzyme; gastric proteolysis; gastrointestinal hormones; pepsin; preterm milk

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CONFLICTS OF INTEREST

The authors have no conflicts to declare.

INTRODUCTION

The preterm infant stomach is thought to be poorly equipped for milk protein digestion because of its high pre-feeding pH and low pre-feeding pepsin activity (1). Preterm infants have higher pre-feeding gastric pH than term infants (2). Pepsin, the main gastric protease, requires acidic pH for optimal activity and auto-activation from pepsinogen (Figure 1) (3, 4). The gastric pH of preterm infants may not provide for optimal pepsin activity. Indeed, pre-feeding gastric pepsin activity was lower in preterm infants than term infants at birth (5).

Gastrin production in the G cells of the stomach in response to dietary protein intake (6) is essential for gastric protein digestion as it stimulates the secretion of gastric acid (from the parietal cells of the stomach), which is required to activate gastric pepsin (Figure 1) (7). Moreover, gastrin is an important trophic factor for the gastric mucosa as it stimulates many portions of the gastrointestinal tract, including the oxyntic glands (which secrete acid in the stomach) (8). Gastrin in infants is therefore important for protein digestion and gastrointestinal tract development (9). Preterm infants produce gastrin and it has been detected in blood samples (9–11), however, no research has examined gastrin concentrations in preterm infant gastric samples. Though milk contains an array of hormones, human milk is not known to contain gastrin.

Gastrin-releasing peptide (GRP) is also essential for gastric protein digestion; its release from nerve fibers (12) stimulates the release of gastrin from gastric G cells and pepsinogen from chief cells (12–14) (Figure 1). GRP also stimulates smooth muscle contraction in the stomach to assist in digestion (12) and stimulates the release of pancreatic enzymes (15). Unlike gastrin, GRP is present in human milk (12). Within bovine milk, GRP concentrations are several hundred times higher than in the animals' plasma—suggesting either that GRP is actively transported from blood to milk or that GRP is produced within the mammary gland (12). GRP's function within milk is unknown. The extent to which GRP survives intact during gastric digestion remains unknown and has not been measured in the preterm infant stomach.

Human milk is known to contain an array of active proteases, including carboxypeptidase B2, kallikrein, plasmin, elastase, thrombin and cytosol aminopeptidase (16, 17). Our previous study revealed that human milk proteases are present in the preterm infant stomach, however, only plasmin and cathepsin D actively hydrolyzed proteins at gastric pH (16). This last study examined the concentration of each milk protease in the preterm infant stomach at a single timepoint postprandial, but did not examine proteolysis, general protease and pepsin activity across time postprandial. Therefore, we aimed to determine the capacity of preterm infant stomach to digest milk protein across time by measuring the gastric pH, proteolysis, general protease activity and the concentration of pepsin in gastric contents at 1 to 3 h postprandial. We hypothesized that the preterm infant stomach would actively degrade milk proteins with increasing breakdown across digestion time. We also aimed to determine the concentration of gastrin and GRP in the preterm infant stomach.

PATIENTS AND METHODS

Study design

This study was approved by the Institutional Review Boards of the University of California, Davis (UC Davis) and Oregon State University (OSU). Inclusion criteria were preterm infants (born <37 completed weeks of gestation) with an admission to the neonatal intensive care unit (NICU), an indwelling nasogastric or orogastric feeding tube and tolerance of full enteral feeding. Exclusion criteria were anatomic or functional gastrointestinal disorders. Samples were collected from 14 mother-infant pairs following preterm birth ranging in birth gestational age (GA) from 23 to 32 weeks during a range of postnatal age of 7–98 days (Table 1), at the UC Davis Children's Hospital NICU in Sacramento, California. The gastric samples were collected only for preterm infants that required tube feedings and had an in-place naso- or oro-gastric tube as part of their routine care. The preterm infants recruited received the standard care provided to all infants of the same degree of maturity in the NICU. Human milk samples were collected as described in our previous study (16). Briefly, human milk was collected by pumping on-site or at home with clean electric breast pumps into sterile plastic containers and stored immediately at -20°C . Nine preterm infants (overall mean $\pm$ SD = 25 ± 2.5 wk GA, 54 ± 26 days of postnatal age) were fed their mother's milk (raw/not pasteurized) fortified with Human Milk Fortifier (Similac Human Milk Fortifier Powder, Abbot Park, IL, USA) and five preterm infants (overall mean $\pm$ SD = 29 ± 4 wk GA, 27 ± 18 days of postnatal age) were fed their mother's milk (raw/not pasteurized) unfortified. The unfortified milks were only fed for a single sample collection timepoint to provide a comparison between digestion of fortified and unfortified milk—outside of the sample collection timepoints, all these infants were fed fortified human milk. The fortifier had intact bovine milk proteins, was in powder form and its protein composition was designed to match the whey:casein of human milk (60:40) using non-fat milk and whey protein concentrate. Each 25 mL of human milk was fortified with 0.25 g of bovine milk proteins (adding 10 mg protein/mL). We measured that the protein concentration was 17 ± 3 mg/mL in unfortified milk (similar to that found previously for average protein content of human milk based on the average GA and days of postnatal age of the subjects (18)) and 29 ± 4 mg/mL in fortified milk (BCA assay, Thermo Fisher Scientific, Waltham, MA, USA). The human milk feedings were delivered via the nasogastric tubes over 30 min. From the same feed, a volume (0.5–2 mL) of each preterm infant's gastric contents were collected at 1, 2 and 3 h after the initiation of feeding, in a 3-mL syringe back through the feeding tube via suction. Gastric samples were placed into sterile plastic vials and stored immediately at -20°C . Sampling for each infant was successful at all timepoints. Human milk and gastric samples were transported to OSU on dry ice and stored at -80°C .

General sample preparation

Samples were thawed at 4°C , pH was determined, and samples were centrifuged at $4,226 \times g$ for 10 min at 4°C . The infranate was collected, separated into aliquots and stored at -80°C . The pH of the samples were measured with an S220 SevenCompact pH/Ion meter (Mettler-Toledo) equipped with a combined sealed glass electrode.

Spectrophotometric and fluorometric assays and ELISAs

The spectrophotometric and fluorometric assays were recorded with a microplate reader (Spectramax M2, Molecular Devices, Sunnyvale, CA, USA) with two replicates of blanks, standards and samples.

Proteolysis.—The amount of proteolysis was determined by the fluorescamine assay as described previously (19) (Table S1) with an L-leucine standard curve, expressed as mM primary amines. Increases in primary amines represent increased numbers of N-terminals released via proteolysis. We also determined the primary amine concentration in a 0.02 g/mL solution of fortifier in water (in triplicate).

General protease activity.—General protease activity was determined based on the release of tyrosine and tryptophan from hemoglobin compared to a tyrosine standard curve (Table S1). The results are expressed as units per milliliter, where one unit of protease activity is the amount of enzyme that generates 1 μ mol of L-tyrosine per min. For protease activity in human milk, the assay buffer (initial at pH 1.3) was adjusted with 1 M sodium hydroxide to pH 6.4 to approximate average milk pH from this present study and our previous article (20). For protease activity in the gastric samples, the assay buffer was adjusted to pH 4.5 with 1 M sodium hydroxide to approximately match the average gastric sample pH of the present study. General protease activity was also measured in the fortifier (at 0.02 g/mL in water).

ELISAs.—All ELISAs (pepsin, gastrin and GRP) were performed according to the methods described by the manufacturers (Table S1).

Statistical methods

One-way ANOVA with repeated measures followed by Tukey's multiple comparisons test (GraphPad Prism software, version 7.03) were applied to compare human milk and gastric samples at the three times postprandial for pH, proteolysis, general protease activity and concentrations of pepsin and gastric hormones. Two-way ANOVA with repeated measures followed by Sidak's multiple comparisons test were used to determine if the measurements differed between infants fed fortified milk and those fed unfortified milk (type of meal). Linear regression models were applied to determine if the pH, proteolysis, general protease activity and concentrations of pepsin, gastrin and GRP changed across postnatal age, GA, postmenstrual age (PMA), infant body weight at birth (BW_b), infant body weight at sampling (BW_s) and feed volume. Differences were designated significant at $p < 0.05$. Pearson correlation coefficient (r) was determined when $p < 0.1$. The sample size of preterm ($N = 14$) paired milk and gastric samples was selected based on our previous study (16) and proved to be adequately powered to detect differences based on the results.

RESULTS

Infant demographics and clinical conditions

Demographic details for the mother-infant pairs are presented in Table 1. Most of the enrolled infants required a feeding tube because of uncoordinated or immature capacity to

suck and swallow. Enrolled preterm infants had medical conditions typical of preterm infants, including lung immaturity (respiratory distress syndrome), bronchopulmonary dysplasia, intraventricular hemorrhage, retinopathy of prematurity, patent ductus arteriosus, and sepsis/meningitis but no overt gastrointestinal tract issues. None of the infants sampled received medications known to affect gastric pH or gastric digestion capacity, including prokinetics, H2 blockers/antagonists or proton-pump inhibitors. The enrolled infants were clinically stable at the time of sample collection.

pH

As expected, the pH values of the preterm infant gastric contents were lower than those of the paired human milk samples ($p < 0.001$, Figure 2A). Gastric pH was significantly lower in the gastric samples at 3 h compared with that at 1 and 2 h as well as lower at 2 h compared with that at 3 h ($p < 0.05$). The pH of human milk or gastric contents at 1, 2 and 3 h postprandial did not differ between preterm infants fed fortified and unfortified milk ($p > 0.05$). Milk pH decreased across postnatal age, PMA and BW_s ($p < 0.01$) but was not different across GA, BW_b or feed volume ($p > 0.05$, Table S2). Gastric pH was not different across postnatal age, PMA or BW_s in gastric contents at 1, 2 or 3 h postprandial ($p > 0.05$, Tables S2). However, gastric pH decreased with increasing GA at 1 and 2 h ($p < 0.05$, Figure 2C). The gastric pH at 1 and 2 h postprandial decreased with increasing BW_b ($p < 0.05$) and tended to decrease at 3 h ($p = 0.076$, Table S2). The pH decreased with increasing feed volume in the gastric contents at 2 h postprandial, but did not change at 1 and 3 h postprandial (Table S2).

Proteolysis

Proteolysis increased significantly from human milk to the gastric contents at 1, 2 or 3 h postprandial ($p < 0.05$, Figure 3A) (by 62%, 131% and 181%, respectively, $p < 0.001$). Proteolysis in gastric contents increased each hour from 1 to 3 h postprandial ($p < 0.05$).

Proteolysis in human milk did not differ between fortified and unfortified milk ($p = 0.99$). However, proteolysis in the gastric contents at 1 and 3 h postprandial was 95% and 54% higher, respectively, in preterm infants fed unfortified milk than those fed fortified milk ($p < 0.05$, Figure 3B). Proteolysis in the gastric contents at 2 h postprandial did not differ between preterm infants fed fortified or unfortified milk ($p = 0.68$). The observed higher mM primary amines in unfortified milk at 1 and 3 h postprandial exists despite the fact that the addition of fortifier to milk (dissolved at 0.02 g/mL to match the amount normally added to human milk) adds 0.29 mM primary amines to the fortified milk values. Proteolysis increased with increasing PMA in gastric contents at 2 h ($p = 0.023$), but did not change in human milk or in gastric contents at 1 or 3 h postprandial ($p > 0.05$). Proteolysis in human milk and gastric contents at 1, 2 or 3 h postprandial did not change with postnatal age, GA, BW_b, BW_s or feed volume ($p > 0.05$, Tables S2).

General protease activity

General protease activity increased significantly from human milk to the gastric contents at 2 h (58%, $p = 0.029$, Figure 3C). However, general protease activity did not differ between human milk and the gastric samples at 3 h ($p = 0.14$) and only tended to increase in the

gastric samples at 1 h compared with human milk (1.3-fold, $p = 0.081$). General protease activity was not significantly different among the gastric samples at 1, 2 and 3 h postprandial (overall mean = 268 ± 30 U/mL, $p > 0.05$). General protease activity in preterm infants did not differ in human milk and 1, 2 and 3 h gastric contents between fortified and unfortified milk ($p > 0.05$). The fortifier alone had no detectable protease activity. General protease activity in human milk increased with increasing postnatal age ($p = 0.006$), decreased with increasing PMA and BW_s ($p < 0.001$), but did not change with GA, BW_b or feed volume ($p > 0.05$, Tables S2). General protease activity did not change across postnatal age, GA, PMA, BW_b, BW_s or feed volume in the gastric contents at 1, 2 and 3 h postprandial ($p > 0.05$, Tables S2).

Concentration of pepsin, gastrin and gastrin-releasing peptide

As expected, pepsin was not detected in human milk. Pepsin was present in the gastric samples, and the concentration at 1, 2 and 3 h did not significantly differ (overall mean = 108 ± 18 pg/mL, $p > 0.05$, Figure 4A). Human gastrin was not detected in human milk but was present in the gastric contents (Figure 4B). Gastrin concentration remained stable in the stomach from 1 to 3 h postprandial (overall mean = 284 ± 48 pg/mL, $p > 0.05$).

Neither pepsin nor gastrin concentrations changed across postnatal age, GA, PMA, BW_b, BW_s or feed volume in the gastric contents at 1, 2 or 3 h postprandial (Table S2).

Human GRP was present in both human milk and the gastric contents (Figure 4C). GRP concentration remained stable from human milk to the gastric contents at 1, 2 and 3 h postprandial and did not differ between time postprandial (overall mean = 719 ± 131 pg/mL, $p > 0.05$). Specific changes in GRP concentration with postnatal age, GA, PMA, BW_b, BW_s and feed volume were minimal and inconsistent and are reported in Table S2.

Concentrations of pepsin, gastrin and GRP in preterm infants did not differ in human milk and 1, 2 and 3 h gastric contents between fortified and unfortified milk ($p > 0.05$). The fortifier alone had no detectable pepsin, gastrin or GRP. Concentrations of GRP did not correlate with concentrations of gastrin in human milk or gastric contents ($p > 0.05$). Likewise, gastrin concentrations did not correlate with pepsin concentrations in the gastric samples ($p = 0.61$).

DISCUSSION

Our previous study (20) found that cathepsin D and plasmin from human milk were still active in the preterm stomach at 2 h postprandial. Our recent research (21) also demonstrated that pepsin/cathepsin D activities and proteolysis increased from human milk to the preterm infant stomach at 2 h postprandial. However, the extent to which protease digestion changes across time postprandial in the preterm infant stomach remained unknown. This study aimed to determine the gastric capacity of preterm infants to digest human milk proteins at different time points (1, 2 and 3 h) after human milk ingestion.

Gastric pH was lower than milk pH and decreased from 1 to 3 h postprandial as preterm infants produce gastric acid. The average gastric pH measured matches that reported in the

literature for preterm infants (22–28). The decrease in pH from 1 to 3 h postprandial (5.3 to 4.2) matches that previously reported by Armand et al. (22) (a decrease from pH 5–7 at 1 h to pH 3–3.5 at 3 h postprandial for preterm infants fed human milk) and by de Oliveira et al. (28) (a decrease in gastric pH from 6.0 to 4.4 after 35 to 90 min of digestion in preterm infants). We also observed that gastric pH decreased with increasing BW_b and GA, which reflects the increased acid production capacity linked with increasing infant development. However, pepsin concentration did not change with BW_b or GA. Preterm infants have higher pre-feeding gastric pH than term infants (2), but no difference in postprandial gastric pH was found between preterm and term infants. Our recent study (21) demonstrated that gastric pH at 2 h postprandial in preterm and term infants fed with human milk did not differ, most likely due to the strong buffering effect of human milk and the higher amount of meal volume fed by term infants compared with preterm infants. As gastric hydrochloric acid can protect against pathogens (29), the higher pH in the pre-fed state could make preterm infants more susceptible to infection. However, the lack of difference in postprandial gastric pH limits this hypothesis, as pathogen intake is typically in the context of food delivery to the stomach.

We found that proteolysis increased from milk to the preterm infant stomach and increased from 1 to 3 h in the stomach postprandial. Henderson et al. (30) examined the proteolysis in the preterm infant stomach and found that up to 13.3% of human milk protein is partially hydrolyzed in the stomach of preterm infants (29.5 ± 0.6 wk GA, postnatal age not specified) by 50 min postprandial (based on acid precipitation followed by Lowry assay). The proteolysis results of Henderson et al. cannot be compared with the proteolysis results in the present study as we measured primary amine concentration and Henderson et al. measured peptide concentration.

Proteolysis in the gastric contents 1 and 3 h postprandial was higher for preterm infants fed unfortified milk than those fed fortified milk. Even though the initial pH of human milk was similar in unfortified milk and fortified milk, protein degradation appeared to be more efficient in the gastric contents 1 and 3 h postprandial when preterm infants were fed unfortified milk. However, there was no difference in proteolysis between unfortified and fortified milk at 2 h postprandial.

Our findings that proteolysis increased from milk to the stomach and across time matches with de Oliveira et al.'s (28) observations that the concentrations of major milk proteins (i.e., lactoferrin, serum albumin, α -lactalbumin and β -casein) decreased during gastric digestion of human milk in preterm infants. The loss of major milk proteins observed in the previous study is illustrated as the increase in primary amine concentration in the present study. Our results also matched with our previous study's observations (21) that the proteolysis increased from human milk to the gastric contents at 2 h postprandial in preterm infants. Moreover, the mM primary amines observed in preterm infant gastric samples in the present study from 1 to 3 h postprandial are similar to those observed previously (21) at 2 h postprandial in preterm infants and lower than those reported for term infants.

We found that the general protease activity increased from human milk to the preterm infant stomach at 2 h postprandial but remained stable at 1 and 3 h postprandial. The observed

increase in proteolysis across time despite the lack of increase in protease activity is logical as a constant protease activity (an instantaneous measurement of the enzyme activity rate) across time would continue to increase the total number of primary amine released (which was measured as a cumulative amount released across the digestion time). Our values at 1 h of general protease activity in the preterm infant stomach matched well with reports in previous work. Armand et al. (22) measured pepsin activity in the stomach of preterm infants (26–32 wk GA, 1–13 wk postnatal age) 50 min after human milk feeding. They found the pepsin activity to be 75 U/mL/kg body weight (value estimated from Figure 3A in Armand et al.) (22). Conversion of our measurements to the units used by Armand et al. (22) (by dividing our values by infant body weight in kilograms and converting to 0.1 $\mu\text{mol}/\text{min}$ tyrosine per unit) yielded similar values: 68 ± 14 , 44 ± 5 , 62 ± 12 U/mL/kg of body weight at 1, 2 and 3 h post-ingestion, respectively. Our findings that general protease did not change at 1 or 3 h matched with our previous study (21) observations that overall protease activity did not increase from human milk to the preterm infant stomach at 2 h postprandial. This recent research (21) also found that the general protease activity was lower in human milk-fed preterm infants than term infants at 2 h postprandial.

Pepsin was not detectable by ELISA in milk, but was present and remained stable in the stomach from 1 to 3 h post-ingestion. This is the first article to examine pepsin concentration in postprandial gastric samples of preterm infants.

Gastrin was not present in human milk but was detected in preterm infant stomach. The present study is the first to measure gastrin concentration in the preterm infant stomach as well as across time post-ingestion (overall mean of gastric contents = 284 ± 48 pg/mL). After conversion of our measurements to moles per liter using the molecular weight of gastrin (2,098.22 g/mol), the gastrin concentration in the gastric contents overall mean was $136 \text{ pM} \pm 22 \text{ pM}$. Previously, for infants, gastrin has only been measured in the blood and not in the gastric lumen. Lucas et al. (9) found that gastrin concentrations in cord blood of term (42 pM) and preterm infants (38 pM, 33 wk GA, 1–24 days postnatal age) were similar, but that both were higher than those in fasting adult blood (7 pM). They also found that gastrin concentration in plasma was 2.8 times higher in preterm infants in the fed state (approximately 60 pM) compared with the unfed state (21 pM). Lucas et al. (31) found similar results of gastrin in the plasma of preterm infants in the fed and unfed states. The three fold higher concentration in gastric contents compared to the historical measures in the plasma suggest a possible biological role for gastrin secreted into the gastric lumen.

We detected GRP both in human milk and in the gastric contents from 1 to 3 h post-ingestion. GRP is known to be present in human milk (32). After conversion of our measurements to moles per liter using the molecular weight of GRP (2,859.38 g/mol), the GRP concentration in human milk (overall mean = 181 ± 22 pM) matched the previous study's finding of 100 pM of GRP in human milk (12). GRP was also found in bovine milk (33), and the concentration decreased across lactation time from 2 to 7 months (2.1 nM to 0.35 nM). We did not detect GRP in the fortifier alone or a difference in GRP concentration in fortified milk compared with unfortified milk, which confirms that fortifier did not contribute to our GRP results. GRP had not previously been measured in either the term or preterm infant stomach. We were not able to determine how much GRP was produced by the preterm

infants themselves and how much was derived from milk, however the lack of a difference in milk GRP content across GA suggests the observed difference at one hour is related to infant production. We also observed that GRP decreased with increasing GA and BW_p in the gastric contents at 1 h postprandial. GRP may play a more important role in preterm infants than in term infants, however, more research is needed to clarify its role in infant digestion.

Strengths and limitations

A strength of this study is the sampling of preterm infant gastric contents at multiple timepoints across digestion to better understand how digestion progresses with time. Our study is limited by the small number of infants enrolled. Another study with more infants in each group needs to be performed to better understand and compare the digestibility of fortified and unfortified milks. Another potential limitation is that the freezing and thawing of samples prior to analysis could affect the measured protease activity. However, as all samples were frozen and thawed, the relative comparisons of protease activity are most likely unaffected. Another limitation is that we compared relative amounts of degradation for proteolysis, but did not determine the percent of proteolysis these amounts represented in comparison to a complete hydrolysis of the sample.

CONCLUSION

Our study is the first to examine how gastric proteases and hormones change across time post-ingestion of human milk in preterm infants. Gastric pH decreases and total proteolysis increases over time after feeding. Protease activity increased from human milk to the preterm infant stomach at 2 h postprandial, but was stable at 1 and 2 h postprandial. Pepsin, GRP, and gastrin were present in the preterm stomach but concentrations were not correlated. Elevated levels of gastrin in the stomach compared to plasma levels suggest a potential intraluminal role for this hormone in the preterm infant. Given the challenges of achieving adequate growth in preterm infants, further study is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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VDM conducted the research and analyzed the data. VDM and YQ conducted the statistical analyses. MAU provided the milk and gastric samples. VDM and DCD designed the study and drafted the manuscript. VDM and DCD have primary responsibility for the final content. All authors read and approved the final manuscript.

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ABBREVIATIONS

GA	birth gestational age
GRP	gastrin-releasing peptide

PMA	postmenstrual age
BW_b	infant body weight at birth
BW_s	infant body weight at sampling.

REFERENCES

1. Dallas DC, Underwood MA, Zivkovic AM, German JB. Digestion of protein in premature and term infants. *J Nutr Disord Ther* 2012; 2 3:112–21. [PubMed: 24744976]
2. Hamosh M, Scanlon JW, Ganot D, Likel M, Scanlon KB, Hamosh P. Fat digestion in the newborn: characterization of lipase in gastric aspirates of premature and term infants. *J Clin Invest* 1981; 67 3:838–46. [PubMed: 7204558]
3. Wolfe MM, Soll AH. The physiology of gastric acid secretion. *N Engl J Med* 1988; 319 26:1707–15. [PubMed: 3060722]
4. Henschel M, Newport M, Parmar V. Gastric proteases in the human infant. *Neonatology* 1987; 52 5:268–72.
5. Adamson I, Esangbedo A, Okolo A, Omene J. Pepsin and its multiple forms in early life. *Neonatology* 1988; 53 5:267–73.
6. Uvnas-Moberg K, Widstrom A, Marchini G, Winberg J. Release of GI hormones in mother and infant by sensory stimulation. *Acta Paediatr* 1987; 76 6:851–60.
7. Freeman HJ, Kim Y. Digestion and absorption of protein. *Annu Rev Med* 1978; 29 1:99–116. [PubMed: 348054]
8. Johnson LR. New aspects of the trophic action of gastrointestinal hormones. *Gastroenterology* 1977; 72 4 Pt.2:788–92. [PubMed: 838237]
9. Lucas A, Adrian TE, Christofides N, Bloom SR, Aynsley-Green A. Plasma motilin, gastrin, and enteroglucagon and feeding in the human newborn. *Arch Dis Child* 1980; 55 9:673–7. [PubMed: 7436530]
10. Hyman PE, Clarke DD, Everett SL, Sonne B, Stewart D, Harada T, et al. Gastric acid secretory function in preterm infants. *J Pediatr* 1985; 106 3:467–71. [PubMed: 3919168]
11. Lucas A, Bloom SR, Aynsley-Green A. Postnatal surges in plasma gut hormones in term and preterm infants. *Biol Neonate* 1982; 41 1–2:63–7. [PubMed: 6802191]
12. Takeyama M, Kondo K, Takayama F, Kondo R, Murata H, Miyakawa I. High concentration of a gastrin releasing peptide-like immunoreactive substance in pregnant human milk. *Mol Cell Biol Res Commun* 1991; 176 2:931–7.
13. Hildebrand P, Lehmann F, Ketterer S, Christ A, Stingelin T, Beltinger J, et al. Regulation of gastric function by endogenous gastrin releasing peptide in humans: studies with a specific gastrin releasing peptide receptor antagonist. *Gut* 2001; 49 1:23–8. [PubMed: 11413106]
14. Chew C. Peptidergic regulation of gastric acid secretion. *Gastrointestinal regulatory peptides*: Springer, 1993.
15. Horstmann O, Nustede R, Schmidt W, Stöckmann F, Becker H. On the role of gastrin-releasing peptide in meal-stimulated exocrine pancreatic secretion. *Pancreas* 1999; 19 2:126–32. [PubMed: 10438158]
16. Demers-Mathieu V, Nielsen SD, Underwood MA, Borghese R, Dallas DC. Analysis of milk from mothers who delivered prematurely reveals few changes in proteases and protease inhibitors across gestational age at birth and infant postnatal age. *J Nutr* 2017; 147 6:1152–9. [PubMed: 28424255]
17. Dallas DC, Murray NM, Gan J. Proteolytic systems in milk: perspectives on the evolutionary function within the mammary gland and the infant. *J Mammary Gland Biol Neoplasia* 2015; 20 3–4:133–47. [PubMed: 26179272]
18. Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. *Clin Nutr* 2011; 30 2:215–20.
19. Larsen LB, Rasmussen MD, Bjerring M, Nielsen JH. Proteases and protein degradation in milk from cows infected with *Streptococcus uberis*. *Int Dairy J* 2004; 14 10:899–907.

20. Demers-Mathieu V, Nielsen SD, Underwood MA, Borghese R, Dallas DC. Changes in proteases, antiproteases and bioactive proteins from mother's breast milk to the premature infant stomach. *J Pediatr Gastroenterol Nutr* 2017; DOI: 10.1097/MPG.0000000000001719.
21. Demers-Mathieu V, Qu Y, Underwood M, Borghese R, Dallas D. Premature infants have lower gastric digestion capacity for human Milk proteins than term infants. *J Pediatr Gastroenterol Nutr* 2017; DOI: 10.1097/MPG.0000000000001835
22. Armand M, Hamosh M, Mehta NR, Angelus PA, Philpott JR, Henderson TR, et al. Effect of human milk or formula on gastric function and fat digestion in the premature infant. *Pediatr Res* 1996; 40 3:429–37. [PubMed: 8865280]
23. Hamosh M, Sivasubramanian KN, Salzman-Mann C, Hamosh P. Fat digestion in the stomach of premature infants. I. Characteristics of lipase activity. *J Pediatr* 1978; 93 4:674–9. [PubMed: 29953]
24. Weisselberg B, Yahav J, Reichman B, Jonas A. Basal and meal-stimulated pepsinogen secretion in preterm infants: a longitudinal study. *J Pediatr Gastroenterol Nutr* 1992; 15 1:58–62. [PubMed: 1403451]
25. Roman C, Carriere F, Villeneuve P, Pina M, Millet V, Simeoni U, et al. Quantitative and qualitative study of gastric lipolysis in premature infants: do MCT-enriched infant formulas improve fat digestion? *Pediatr Res* 2007; 61 1:83–8. [PubMed: 17211146]
26. Yahav J, Carrion V, Lee PC, Lebenthal E. Meal-stimulated pepsinogen secretion in premature infants. *J Pediatr* 1987; 110 6:949–51. [PubMed: 3108476]
27. Harries JT, Fraser AJ. The acidity of the gastric contents of premature babies during the first fourteen days of life. *Neonatology* 1968; 12 3–4:186–93.
28. de Oliveira SC, Bellanger A, Menard O, Pladys P, Le Gouar Y, Dirson E, et al. Impact of human milk pasteurization on gastric digestion in preterm infants: a randomized controlled trial. *Am J Clin Nutr* 2017; 105 2:379–90. [PubMed: 28052887]
29. Smith JL. The role of gastric acid in preventing foodborne disease and how bacteria overcome acid conditions. *J Food Prot* 2003; 66 7:1292–303. [PubMed: 12870767]
30. Henderson TR, Hamosh M, Armand M, Mehta NR, Hamosh P. Gastric proteolysis in the preterm infant: protein digestion is limited and is not affected by diet, human milk or formula. *Pediatr Fles* 1998; 43:101–.
31. Lucas A, Bloom SR, Aynsley-Green A. Metabolic and endocrine consequences of depriving preterm infants of enteral nutrition. *Acta Paediatr Scand* 1983; 72 2:245–9. [PubMed: 6837298]
32. Takeyama M, Kondo K, Hayashi Y, Yajima H. Enzyme immunoassay of gastrin releasing peptide (GRP)-like immunoreactivity in milk. *Chem Biol Drug Des* 1989; 34 1:70–4.
33. Frank-Peterside N, Others. Gastrin-releasing peptide immunoreactivity in milk and milk products. *Global J Pure Appl Sci* 2000; 6 2:221–5.

Key Notes:

- This study examined the gastric digestion capacity of 14 preterm infants for human milk protein at 1, 2 and 3 h postprandial.
- The preterm infant stomach actively degraded milk proteins with increasing breakdown across digestion time.
- Pepsin, gastrin-releasing peptide and gastrin were present in the preterm stomach and stable across digestion time.

Figure 1. Schematic of the stimulation and release of GRP, gastrin and pepsinogen in the stomach. (A) GRP from nerve fibers stimulates the release of gastrin by G cells in the stomach. (B) Gastrin stimulates the secretion of HCl from parietal cells in the stomach. (C) Gastrin also stimulates chief cells to produce pepsinogen in the stomach. (D) HCl activates gastric pepsinogen to pepsin.

Figure 2.

(A) Change of pH in human milk (blue boxplot) to infant gastric contents at 1 (G-1h, pink boxplot), 2 (G-2h, red boxplot) and 3 h (G-3h, orange boxplot) after the beginning of feeding. Paired milk and gastric samples were collected in preterm infants (23–32 wk of birth gestational age, 7–98 days of postnatal age). Values are min, median and max, $n = 14$ for milk, G-1h, G-2h and G-3h. Letters a, b, c and d show statistically significant differences between groups ($p < 0.05$) using one-way ANOVA with repeated measures followed with Tukey's multiple comparisons test. (B) Change of pH in human milk across postnatal age (23–32 wk) of preterm infants. (C) Change of pH in gastric contents at 2 h postprandial across gestational age (23–32 wk) of preterm infants. Values are p-values for $n = 14$ for postnatal age (B) and gestational age (C) via regression linear models. r , Pearson correlation coefficient.

Figure 3.

(A) Proteolysis and (C) general protease activity in human milk (blue bars) and infant gastric samples at 1 (G-1h, pink), 2 (G-2h, red) and 3 h (G-3h, orange) after the beginning of feeding. Paired milk and gastric samples were collected in preterm infants (23–32 wk of birth gestational age, 7–98 days of postnatal age). Values are min, median and max, n = 14 for milk, G-1h, G-2h and G-3h. Letters a, b, c and d show statistically significant differences between groups (p < 0.05) using one-way ANOVA with repeated measures followed with Tukey's multiple comparisons test. (B) Proteolysis in fortified milk (blue boxplot) and unfortified milk (red boxplot) and gastric contents at 1, 2 and 3 h postprandial. Asterisks show statistically significant differences between fortified milk and unfortified milk using two-way ANOVA with repeated measures followed with Sidak's multiple comparisons test (**: p < 0.01; * p < 0.05). Values are min, median and max, n = 9 for fortified and n = 5 for unfortified for milk, G-1h, G-2h and G-3 h.

Figure 4.

Concentration of (A) pepsin, (B) gastrin and (C) gastrin-released peptide (GRP) in human milk (blue boxplot) and infant gastric contents at 1 (G-1h, pink boxplot), 2 (G-2h, red boxplot) and 3 h (G-3h, orange boxplot) after the beginning of feeding. Paired milk and gastric samples were collected in preterm infants (23–32 wk of birth gestational age, 7–98 days of postnatal age). Values are min, median and max, $n = 14$ for milk, G-1h, G-2h and G-3 h. No statistically significant difference between groups ($p > 0.05$) using one-way ANOVA with repeated measures.

Table 1:

Demographics of preterm-delivering mother-infant pairs sampled for milk and gastric contents.¹

	Preterm-delivering mother-infant pairs
GA, weeks	26.6 ± 3.4 (23–32)
Postnatal age, days	40.0 ± 29.2 (7–98)
Postmenstrual age, weeks	32.4 ± 2.4 (30–37)
Birth weight, kg	1.0 ± 0.4 (0.5–1.6)
Weight at collection, kg	1.6 ± 0.5 (1.0–2.3)
Meal volume, mL	17 ± 12 (4 – 40)
Meal frequency	8 times per day (3 hours intervals)
Infant sex	13 females, 1 male
Mother's age, years	35.1 ± 3.3 (32–39)
HMF or HM feeding, n	10 HMF; 5 HM

¹ All values are mean ± SD (range), n = 14 for number of paired milk and gastric samples. GA = birth gestational age, HMF = human milk fortified; HM = human milk without fortification.