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Effect of Aspiration and Evaluation of Gastric Residuals on Intestinal Inflammation, Bleeding and Gastrointestinal Peptide Level

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Abstract

Objective: To determine the effect of gastric residual aspiration and evaluation on preterm VLBW infants' gastrointestinal function, intestinal inflammation, and gastrointestinal mucosal bleeding.

Study design: This single-center randomized trial compared omission of gastric residuals vs prefeed gastric residuals in 143 infants ≤32 weeks gestation with a birthweight ≤ 1250 grams for six-weeks following birth. Serum levels of gastrin and motilin were collected between 14 and 21 days of life. Stools were collected at three and six weeks of age and analyzed for calprotectin and S100A12 levels. All stools were tested for occult blood for 6 weeks.

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Data sharing statement: Data are not available for sharing

Results: Means for gastrin (p = .999) and motilin (*P* = .694) were similar between groups and there were no statistically significant differences in adjusted means for transformed calprotectin (p = .580), and S100A12 (p = .212). Both calprotectin (p=.003) and S100A12 (p=.002) increased from week three to six. Mean percentage of stools positive for occult blood (p=.888) were similar between groups.

Conclusion: Gastrointestinal function, intestinal inflammation and gastrointestinal mucosal bleeding were similar whether aspiration and evaluation of gastric residuals were eliminated or not, suggesting routinely evaluating gastric residuals prior to every feeding may be unnecessary.

Trial registration—ClnicalTrials.gov:

Keywords

Gastric residual; Neonatal Intensive Care Unit; Very Low Birth Weight; Premature

Aspiration and evaluation of gastric residuals prior to every feeding is standard care in many neonatal intensive care units (NICU) and large gastric residuals are often considered a marker for feeding intolerance or an early symptom of necrotizing enterocolitis.(1–3) Recent evidence suggests that routinely aspirating and evaluating gastric residuals may negatively affect nutritional intake in infants born premature and when gastric residuals are omitted, infants may have improved outcomes including increased delivery of enteral nutrition, improved weight gain and decreased length of hospital stay.(4–7) However, scant information exists regarding potential physiologic risks or benefits of this routine practice including alternation in gastrointestinal function, intestinal inflammation and gastric mucosal damage.

Close contact of the feeding tube tip with the delicate gastric mucosa, as well as the negative pressure required to withdraw gastric contents, may cause gastric mucosal damage and bleeding. In addition, neonatal nurses frequently discard aspirated gastric residuals, a decision that is generally left to individual judgment rather than to NICU-specific protocols.(8) By discarding aspirated gastric residuals, important elements such as hydrochloric acid may be lost. Hydrochloric acid is essential in limiting intestinal bacterial overgrowth, thus discarding it can allow intestinal bacterial to proliferate and ultimately cause intestinal inflammation in the infant.(9, 10) Finally, because aspiration and evaluation of large gastric residuals often causes delays or discontinuation of enteral feedings, gastrointestinal peptide secretion can also be altered, thus negatively affecting the infant's gastrointestinal development and function.(4)

A paucity of information exists regarding potential physiological risks and benefits of aspirating and evaluating gastric residuals in fragile VLBW infants. Therefore, the purpose of this randomized controlled trial was to determine the effect of aspiration and evaluation of gastric residuals on gastrointestinal function, intestinal inflammation, and gastrointestinal mucosal bleeding. We hypothesized that, when gastric residual aspiration and evaluation was omitted, infants would have increased serum gastrointestinal peptide levels (gastrin and motilin), less intestinal inflammation, and fewer stools positive for occult blood during the first six weeks of life.

Methods

Subjects were enrolled in an RCT whose primary aim was to determine the effect of omission of gastric residuals on weekly enteral nutrition for six-weeks following birth (ClinicalTrials.gov:). In the parent study, infants were recruited from a Level 4 NICU between October 2013 and October 2016 and were eligible for inclusion if they were born at a gestational age of ≤ 32 weeks, had a birth weight ≤ 1,250 grams, were ≤ 72 hours old, and were receiving some enteral feeds by 72 hours of age. Infants were ineligible if they had congenital or chromosomal abnormalities or congenital anatomic gastrointestinal abnormalities. Infants were withdrawn if they developed stage II or greater necrotizing enterocolitis or spontaneous intestinal perforation (Figure 1).

Within 72 hours of life and within 24 hours of initiating enteral feeds, a member of the research team obtained informed parental consent for participation in the study. To maintain approximate balance in each treatment group, infants were randomly assigned to one of two groups using a computer-generated sequence with random length permuted blocks of sizes 4, 6, or 8. Allocation was concealed until the intervention was assigned. The parent study was approved by the University of Florida's (UF) Institutional Review Board.

Study Intervention

For the first 6 weeks of life, Group 1 underwent gastric residual aspiration and evaluation prior to every feeding, and Group 2 did not. Feeding decisions including time of initiation, rate of advancement, withholding of feeds, and human milk fortification were made according to the NICU's nutritional guidelines. Infants received only human milk (either mother's own milk [MOM] or donor human milk [DHM]).

Because information regarding gastric residuals was included in the infants' medical records and monitored by clinicians, the research team and health care providers could not be blinded. However, individuals performing all laboratory analyses including testing for fecal occult blood, as well as analysis of calprotectin and S100A12 levels and serum gastrin and motilin levels, were blinded to group assignment.

Study Outcomes

Gastrointestinal function.—Serum levels of gastrin and motilin were collected from infants between 14 and 21 days of life during routine blood draws. All samples were sent to the laboratory at UF Health Shands Children's Hospital for testing. Information regarding enteral feedings including type of feeding as well as number of feeds reduced or held was obtained from the infants' medical record.

Intestinal inflammation.—Stools were collected at 3 and 6 weeks of age and immediately frozen to −80°C. Fecal calprotectin and S100A12 levels were measured using the fCal ELISA kit from BUHLMANN Laboratories AG (Schonenbuch, Switzerland) and the S100A12 ELISA kit from Cloun-Clone Corp. (Houston, TX, USA) according to manufacturers' instructions.

GI mucosal bleeding.—For the first 6 weeks, all stools were tested for occult blood by using point-of-care fecal occult test kits from Beckman Coulter (Atlanta, Georgia, USA). All tests were completed according to the manufacturer's instructions.

Statistical Analyses

Demographic characteristics of treatment groups were examined using descriptive statistics consistent with measurement level. Because they were measured at a single time-point (week three), a general linear model (GLM) approach was used to test differences in treatment group mean gastrin and motilin levels as well as number of reduced or held feeds. A General linear mixed model (GLMM) was used to analyze fecal occult blood and intestinal inflammation variables. As assessed using Bayes Information Criterion, the unstructured within-subject covariance matrix (where each variance and each covariance is estimated uniquely from the data) best fit the data. The model tested the main effects of treatment (gastric residual: No/Yes) and week (fecal occult blood: weeks 1-6; calprotectin and S100A12 levels: weeks 3 and 6), and the treatment-by-week interaction. Simple main effects, which deconstruct interacting effects, were examined for statistically significant interaction effects involving treatment.

Exploratory analyses.

Because several factors may be associated with outcomes examined in this study, and those factors may moderate response to measuring gastric residuals, we also explored models containing selected covariates relevant to the outcomes of interest. Variables that were clinically relevant to outcomes (gestational age, birth weight, weekly percent MOM consumed, race) were evaluated for inclusion as covariates within those exploratory models. Covariates were retained if they were statistically significant or if removing them reduced model fit as evaluated by the Bayes Information Criterion. The selected covariates and treatment by covariate interactions were added to the models described above. Treatment-by-covariate interactions having p-values > .05 were removed. Including relevant covariates within the randomized design could reduce error variance, increase the power to detect treatment differences, and explore heterogeneity in treatment response through evaluating interactions with treatment. Simple main effects were examined for statistically significant interaction effects involving treatment. Although the study was not powered to detect those interactions, they provided information to better understand the effect of omitting gastric residual aspiration and evaluation.

Results

From October 2013 to October 2016, 143 infants were enrolled in the study. After signed consent was obtained from their parents, 74 infants were randomized to undergo gastric residual aspiration and evaluation (GR) and 69 were randomized to have gastric residual aspiration and evaluation omitted (No GR) (Figure 1). All infants were included in the modified intent to treat analysis. The GR infants and No GR infants had mean [SD] gestational ages of 27.1 [2.4] and 27 weeks [1.2], respectively, and birth weights of 888.8 [206.6] and 915.2 [180] grams, respectively (Table 1). Examination of distribution of residuals resulted in applying a natural log transformation to raw gastrin, motilin,

calprotectin, S100A12, and number of feeds held or reduced, and an arcsin transformation to proportion of heme-positive stools in order to meet statistical model assumptions. Table 2 shows the results of the primary statistical analyses and Figure 2 indicates distribution of raw untransformed values. Table 3 (available at www.jpeds.com) presents results of the exploratory analysis.

Gastrin and Motilin

Raw (non-transformed) means for gastrin and motilin in the GR group were 80.5 pg/ml (SD=70.4; Range 16 – 490) and 228.5 pg/ml (SD=149.8; Range 59 – 753), respectively. Gastrin and motilin means for the No GR group were 92.3 pg/ml (SD=62.1; Range 10 – 310) and 225.8 pg/ml (SD=108.3; Range 65 – 485), respectively. Based on the distribution of residual values, a natural log transform was applied to both gastrin and motilin levels. Mean levels for both gastrin (p = .248) and motilin (p = .694) were similar between groups.

Exploratory analyses found both the infants' race and weekly percent of feeds consisting of MOM met criteria for inclusion as covariates and were retained in the model comparing mean gastrin levels. None of the variables examined as potential covariates met criteria for inclusion in the model comparing mean motilin levels. Least Square Mean (LSM) levels for both gastrin (p = .999) and motilin (p = .694) were similar between groups, and there was no support for moderation of the treatment effect on gastrin level by race (p=.163) or MOM (p=.833).

Calprotectin and S100A12—In the GR group, means for raw calprotectin were 478.1 μg/g (SD=552.8; Range 12.9 – 3744.2) at week three and 618.8 μg/g (SD=665.6; Range 15.6 – 3594.3) at week 6, and means in the No GR group were 377.2 μg/g (SD=329.6; Range 32.0 – 1505.1) at week three and 537.9 μg/g (SD=553.5; Range 23.8 – 3406.4) at week six. After a natural log transformation was applied, means for transformed calprotectin were similar between groups (p = .498) and increased from week three to six (p=.004). There was no support for a differential change over time between groups (treatment by week interaction p=.692).

In the GR group, means for raw S100A12 were 202.4 ng/g (SD=321.0; Range 0 -1805.6) at week three and 183.6 ng/g (SD=335.6; Range 4.90 – 1960.0) at week six, and means in the No GR group were 200.8 ng/g (SD=350.2; Range 5.82 – 1628.9) at week three and 113.4 ng/g (SD=192.0; Range 4.28 – 1027.8) at week six. After a natural log transformation was applied, means for transformed S100A12 were similar between groups (p = .195), and were similar across weeks three to six (p=.404), with no support for a differential change over time between groups (treatment by week interaction p=. 164).

Based on results of the exploratory analyses, gestational age and weekly percent of feeds consisting of MOM were included as covariates within the transformed calprotectin model. Adjusted means for transformed calprotectin were similar between groups (p = .580) and increased from week three to six (p=.003). There was no support for moderation of treatment effect by GA (p=.681), race (p=.504), or MOM (p=429) or change over time (respective p values of .075, .120, and .766).

For transformed S100A12, gestational age and weekly percent of feeds consisting of MOM were included as covariates. Adjusted means for transformed S100A12 were similar between groups (p = .212), and increased from week three to six (p=.002), with the amount of increase dependent on gestational age (GA-by-week interaction p=.002). There was no support for moderation of the treatment effect by either GA (p=.793) or MOM (p=.474) or change over time by MOM (p=.852).

Heme Positive Stools

An arcsin transformation was applied to the proportion of stools positive for occult blood to normalize distribution of residuals given the scale of the measure. Although mean values were similar (p=.888) between GR (raw value: LSM=.25; SE=.022; $CI_{.95}$: .20, .29) and No GR groups (raw value: LSM=.25; SE=.022; $CI_{.95}$: .21, .30), there were differences over time, with an increase (p<.001) in percent positive from week 1 (raw value: LSM=.17; SE=.023; $CI_{.95}$: .13, .22) to week 3 (raw value: LSM=.31; SE=.026; $CI_{.95}$: .26, .36), and decreasing after that, with return to mean similar to baseline (p=.055) by week 6 (raw value: LSM=.20; SE=.022; $CI_{.95}$: . 16, .25). No covariates met criteria for inclusion in exploratory models.

Number of Feedings Held or Reduced

Means for raw number of feedings held or reduced were 35.28 (SD=60.96; Range 0 – 296) in the GR group, and means in the No GR group were 19.83 (SD=31.67; Range 0 – 115). After applying a natural log transformation, adjusted means for transformed feedings held or reduced were higher in the GR group than the No GR group (p=.032).

Discussion

Compared with infants born preterm with VLBW who had gastric residual aspiration and evaluation omitted, those that underwent aspiration and evaluation of gastric residuals prior to every feeding did not experience increased gastrointestinal inflammation, bleeding, or decreased gastric peptide levels.

Omitting gastric residual aspiration and evaluation prior to every feeding did not alter gastrin and motilin levels at three weeks after birth. Gastric peptides are important for structural and functional development of the neonatal gastrointestinal system.(11) Gastrin is secreted by G-cells in the antrum of the stomach, duodenum, and pancreas and is trophic to the immature gastrointestinal system, meaning it could potentially decrease the risk of feeding intolerance in VLBW infants.(12) In addition, gastrin triggers the release of hydrochloric acid, which maintains gastric acidity and is thus, an important component of the infant's immunity.(13) Secreted by endocrinocytes in the mucosa of the proximal small intestine, motilin accelerates gastric emptying and regulates gastrointestinal motility thus decreasing the risk of delayed gastric emptying and decreased intestinal motility, which contributes to feeding tolerance in VLBW infants.(14)'(15) Because gastrointestinal peptide secretion is stimulated by enteral feeding and evaluation of gastric residuals has been associated with decreased enteral intake, we hypothesized that aspirating and evaluating gastric residuals would decrease infants' gastrin and motilin levels.(4, 16, 17) Although we found no differences in

either gastrin or motilin levels between the two groups, infants in the No GR group had fewer feedings withheld or discontinued and in our previous research advanced feedings more quickly, which possibly indicates improved gastrointestinal function.(4, 7) We speculate that if gastric peptide levels had been measured more frequently than every three weeks, differences in levels between the two groups may have been more apparent. Although gastrin and motilin levels are elevated in term and late preterm infants after they are fed, little is known about gastric peptide secretion in VLBW infants.(11, 16, 17)

Results of the exploratory analysis found infants who consumed a greater proportion of feedings consisting of MOM had higher gastrin levels. Past research on how feeding regime affects gastrin levels in term infants varies. Although Hanekamp et al found median gastrin levels were lower in 24 critically-ill term infants fed MOM, other researchers have found no correlation between feeding regime and gastrin levels.(16, 18, 19) Once again, little is known about determinants of gastrointestinal peptide secretion in VLBW infants and it is possible that higher levels of protein and fatty acids present in the breast milk of mothers who delivered preterm infants stimulated more gastrin secretion.(20–22)

Calprotectin and S100A12 are involved in inflammatory regulation and are considered a marker for gastrointestinal inflammation including necrotizing enterocolitis.(23)’(24) We found infants who did not undergo gastric residual aspiration and evaluation prior to every feeding did not exhibit increased evidence of intestinal inflammation including elevated calprotectin or S100A12 levels at three and six weeks after birth. However, although not statistically significant, infants who underwent gastric residual evaluation had higher calprotectin and S100A12 levels which may be clinically important in infants born VLBW and at risk of feeding intolerance and intestinal inflammatory diseases such as necrotizing enterocolitis.(2)

Exploratory analysis found consumption of MOM was associated with higher fecal S100A12 levels but lower calprotectin levels. These findings are consistent with previous research that showed discrepancies in the effects of feeding regimes on intestinal inflammation in both term and preterm infants. Although higher fecal calprotectin levels have been reported in term infants exclusively breastfed,(25, 26) breastfeeding has also been correlated with both lower (27) and similar levels; (28) such discrepancies are also evident in infants born preterm and VLBW. In 75 preterm VLBW infants, Groer et al found that infants who consumed an exclusively MOM diet had significant increases in fecal calprotectin during the first six weeks of life compared with those fed either formula, DHM, or a mixed diet.(29) Conversely, Yang et al, in a small sample of 14 infants with VLBW, found no correlation between fecal calprotectin and feeding regime.(30) MOM contains microbiota and cytokines which may be responsible for elevations in fecal calprotectin and thus relatively higher levels may be indicative of healthy intestinal maturation and adaptation.(29, 31)

Infants of more advanced gestational ages had evidence of increased intestinal inflammation. However, although calprotectin levels were higher at both week three and week six in infants born more mature, only the increase in S100A12 between week three and week six correlated with gestational age. Previous research suggests that, for infants born less than

35-weeks gestation, gestational age does not affect fecal calprotectin levels.(32, 33) Although the effect of gestational age on fecal calprotectin levels in infants born preterm and VLBW varies, (30, 34) higher levels of both fecal calprotectin and S100A12 have been reported in infants born more mature.(35, 36)

Omitting gastric residual aspiration and evaluation did not affect the percentage of stools positive for occult blood. Because greater than 46% of infants' stools were heme-positive during the first six weeks following delivery, it is likely that the stools contained blood for reasons other than gastric residual aspiration, e.g., the infant swallowed blood during feeding tube insertion or routine suctioning of the nose and mouth. Overall, the percentage of stools positive for occult blood increased significantly from week one to week two and stayed higher than week one throughout the study's remaining 5 weeks. This suggests that procedures following the infants' birth may have led to their swallowing blood and subsequent heme-positive stools.

There were limitations of this study. Because information regarding gastric residual aspiration and evaluation was recorded in the infants' medical records, blinding was impossible. Thus, a change in clinician behavior due to group assignment cannot be completely excluded. In addition, because factors including feeding regime and volume may affect calprotectin and S100A12 levels, sensitivity and specificity of these biomarkers to predict intestinal inflammation may be limited. Furthermore, numerous peptides and hormones are involved in gastrointestinal processes and it is possible that inclusion of only gastrin and motilin may not be entirely reflective of gastrointestinal function. Because serum gastrin and motilin levels were drawn in conjunction with a routine blood draw, potential diurnal variation and differences based upon time since the infant was last fed as well as feeding volume may have affected results. In addition, stools were likely heme-positive for reasons other than aspiration of gastric residuals indicating their use may not have been a reliable measure of gastric mucosal damage. Finally, a type II error is always a possibility when failing to reject a hypothesis which may have affected results.

Our results suggest that omitting the common practice of aspirating and evaluating gastric residuals prior to every feeding does not decrease infants' gastrointestinal peptide secretion, increase their risk of intestinal inflammation, or increase gastrointestinal bleeding. Previous research suggests routinely evaluating gastric residuals can limit enteral nutrition and negatively impact nutritional outcomes in infants born preterm and VLBW. This study provides additional evidence to show that routinely evaluating infants' gastric residuals prior to every feeding may be unnecessary.

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Abbreviations

NICU	Neonatal intensive care unit

MOM	Mother's own milk
GLM	General linear model
GLMM	General linear mixed model

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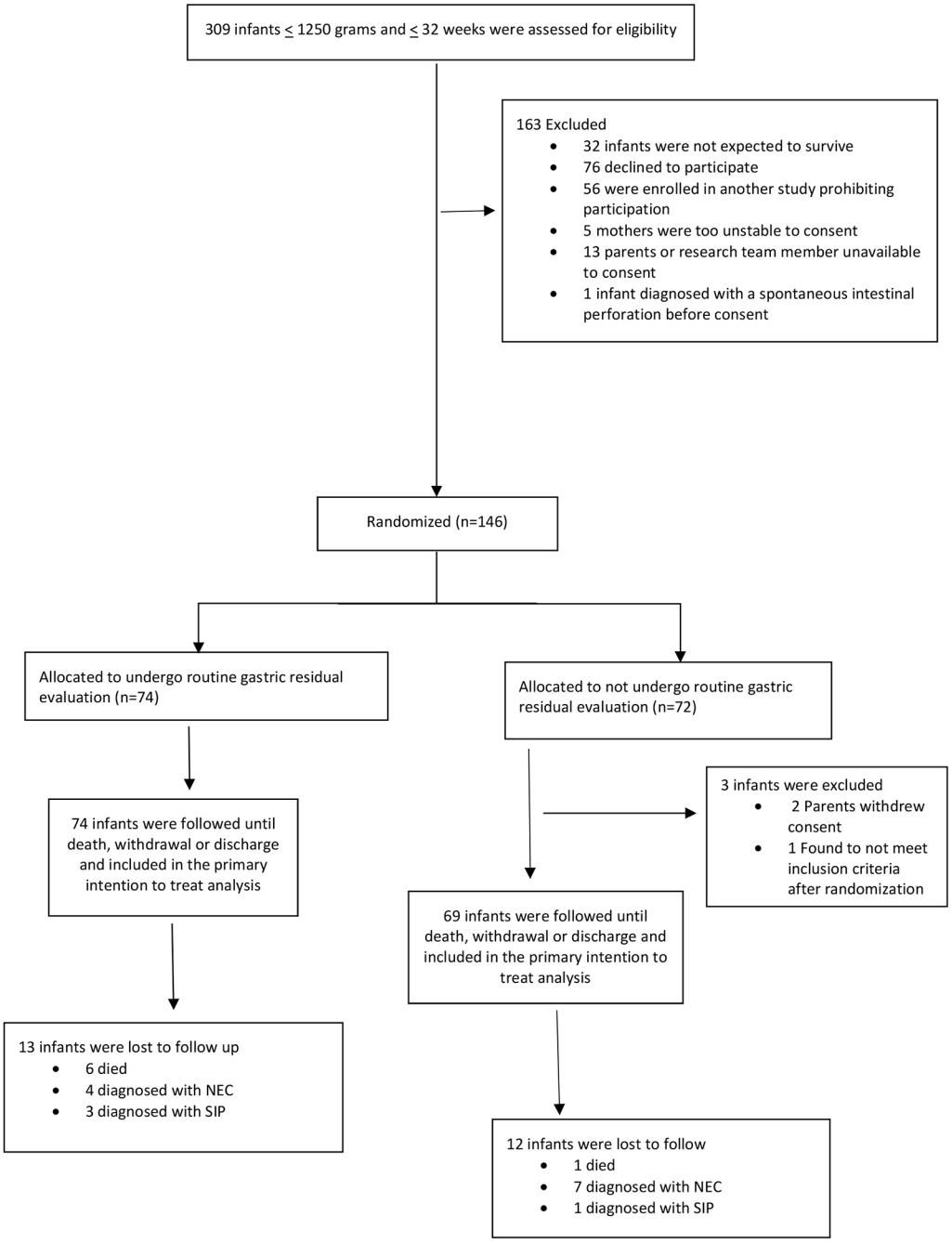
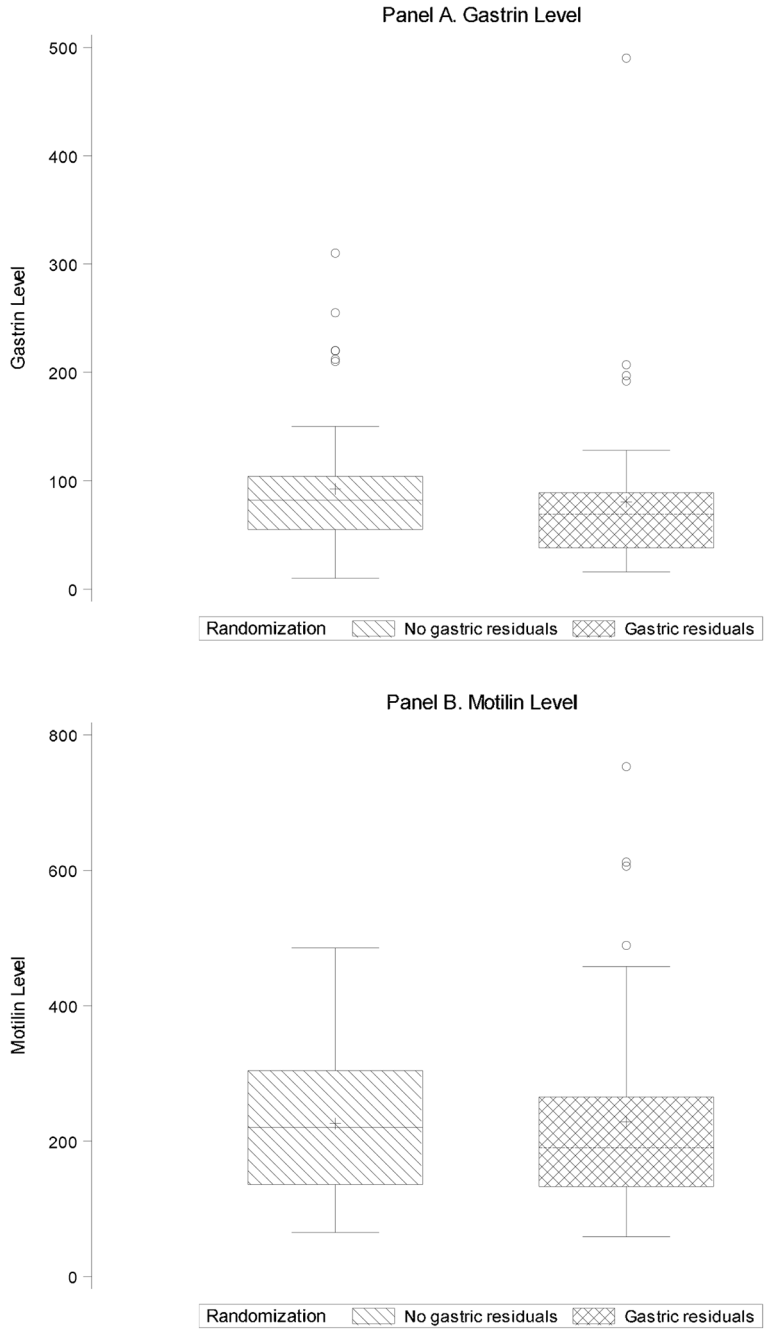
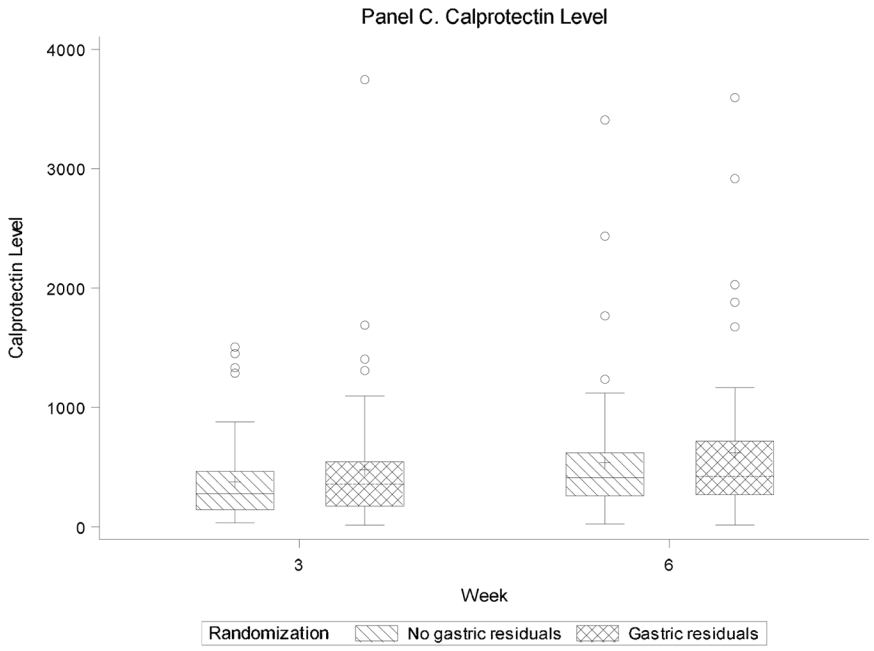
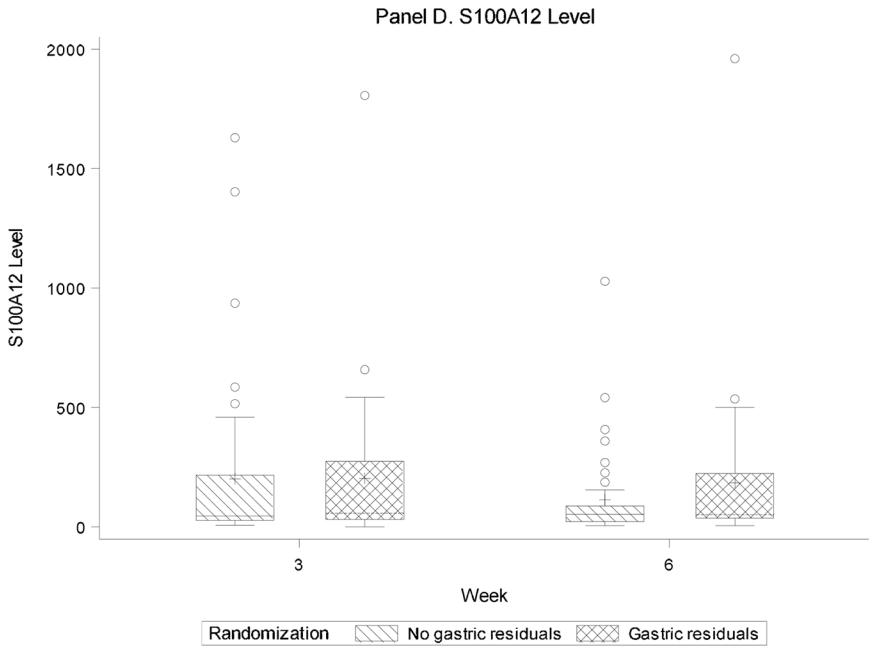


Figure 1:
Consort Diagram







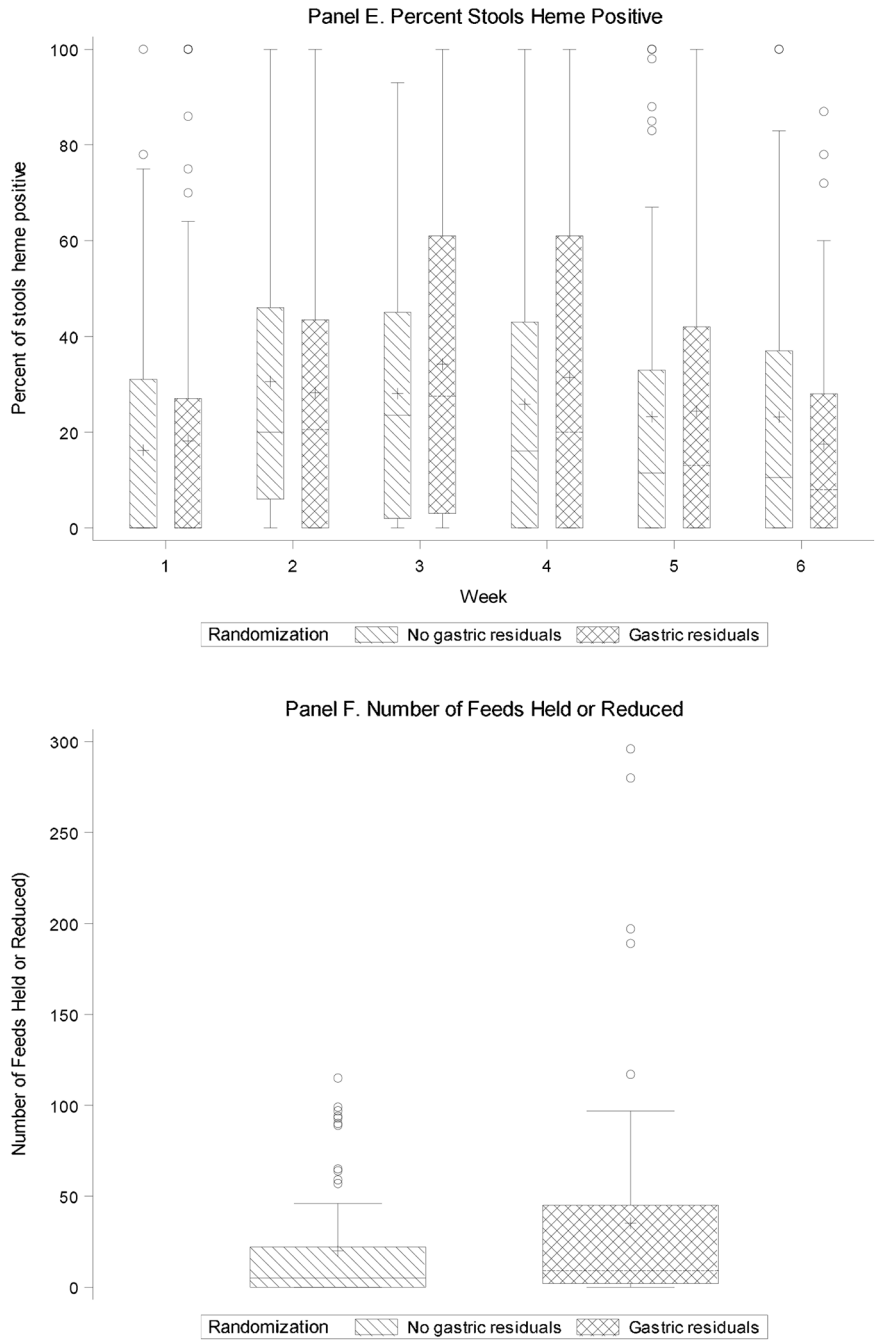


Figure 2.

Box plot panels for values for raw untransformed gastrin, motilin, calprotectin, S100A12, stools positive for occult blood and number of feeds held or reduced

NOTE: Bottom and top edges of the box are 25th and 75th percentile. Plus inside box indicates mean; line inside box indicates median. Whiskers indicate range of values outside intra-quartile range (IQR). Circles are outliers (greater than 1.5*IQR).

Table 1:

Baseline Characteristics of the Infants

Characteristic	GR Evaluation (n=74)	No GR Evaluation (n=69)
Gestational age (weeks)	27.13 ± 2.4	26.98 ± 1.171
Birth weight (grams)	888.8 ± 206.6	915.2 ± 180
Race		
Caucasian	49 (66.2%)	28 (40.58%)
African American	22 (29.7%)	39 (56.5%)
Asian	0 (0%)	1 (1.45%)
Other	3 (4.1%)	1 (1.45%)
Ethnicity		
Hispanic	10 (13.51%)	6 (8.70%)
Gender		
Male	37 (50%)	36 (52.17%)
Female	37 (50%)	33 (44.14%)
Mode of delivery		
Cesarean section	55 (74.32%)	54 (78.26%)
Vaginal	19 (25.68%)	23 (33.33%)
Multiple births	18 (24.32%)	15 (21.75%)
Received antenatal steroids	68 (91.89%)	56 (81.16%)
5-minute Apgar score [median (IQr)]	7 (6,8)	7 (5,8)
SNAP-II score	20.58 ± 12.58	21.25 ± 12.51
Type of feeding		
% Mother's own milk	55.04% ± 39.9	49.87% ± 38.52

Table 2:

Results for outcomes analyzed using general linear mixed model

Model	p	Estimate[a] (CI_{95}): Residual	Estimate[a] (CI_{95}): No Residual
Log_e Gastrin (pg/ml)			
Treatment	.248	4.17 (3.99, 4.35)	4.32 (4.14, 4.50)
		80.50 (62.59, 98.41)[b]	92.29 (74.21, 110.37)[b]
Log_e Motilin (pg/ml)			
Treatment	.694	5.25 (5.09, 5.41)	5.30 (5.14, 5.45)
		228.5 (191.8, 265.1)[b]	225.8 (189.6, 262.1)[b]
Log_e calprotectin (μg/g)			
Week	.004		
Treatment	.498	5.86 (5.67, 6.06)	5.77 (5.57, 5.96)
		557.8 (454.4, 661.1)[b]	456.1 (353.7, 558.5)[b]
Log_e S100A12 (ng/g)			
Week	.404		
Treatment	.195	4.35 (4.01, 4.70)	4.04 (3.70, 4.37)
		195.2 (119.3, 271.0)[b]	144.4 (68.9, 220.0)[b]
ARCSIN Percent positive fecal occult blood			
Week	<.001		
Treatment	.888	.428 (.365, .492)	.435 (.371, .499)
		.247 (.203, .290)[b]	.254 (.210, .298)[b]
Log_e Number Reduced or Held Feeds			
Treatment	.032	2.38 (1.97, 2.80)	1.74 (1.33, 2.16)
		35.3 (23.4, 47.2)[b]	19.8 (7.9, 31.8)[b]

[a] All estimates are least square means

[b] Untransformed values - provided for clinical reference only.

Table 3:

Results of exploratory outcomes analyzed using general linear mixed model

Model	p	Estimate[a] (CI_{95}): Residual	Estimate[a] (CI_{95}): No Residual
Log_e Gastrin			
Race[e]	<.001		
Weekly % MOM[b] Median	.044		
Treatment	.999	4.26 (4.11, 4.44)	4.28 (4.11, 4.44)
		91.2 (74.3, 108.2)[d]	88.0 (71.4, 104.6)[d]
Log_e calprotectin			
Week	.003		
GA[c]	.011		
Weekly % MOM[b] Median	.007		
Treatment	.580	5.85 (5.66, 6.04)	5.78 (5.59, 5.96)
		556.6 (453.8, 659.4)[d]	456.3 (354.4, 558.2)[d]
Log_e S100A12			
Week	.002		
GA[c]	.935		
GA[c]*Week	.002		
Weekly % MOM[b] Mean	.043		
Treatment	.212	4.34 (4.00, 4.68)	4.04 (3.70, 4.37)
		193.4 (117.4, 269.3)[d]	146.5 (70.9, 222.1)[d]
ARCSIN Percent positive fecal occult blood			
Week	<.001		
Treatment	.888	.428 (.365, .492)	.435 (.371, .499)
		.247 (.203, .290)[d]	.254 (.210, .298)[d]
Log_e Number Reduced or Held Feeds			
Birth Weight	<.001		
Weekly % MOM[b] Median	.080		
Treatment	.034		
		2.35 (1.98, 2.73)	1.77 (1.40, 2.15)
		34.1 (23.5, 44.7)[d]	21.0 (10.4, 31.6)[d]

[a]All estimates are least square means

[b]Mother's own milk

[c]Gestational age

[d] Untransformed values – provided for clinical reference only.

[e] Race dichotomized into African-American and Non-African-American