# Abstract

Objectives: Feeding intolerance (FI) in preterm infants is common but the etiology remains unclear. This study examined FI as a stress-related disease involving brain-gut interactions and tested the Model of Allostatic Load (AL) and Complications of Prematurity. Specific aims were: describe demographic/medical variables and biomarker levels at each time and over time for the sample; describe/compare variables and biomarker levels at each time for infants without/with FI; and compare biomarker interquartile/interpercentile distributions between infants without/with FI.

Methods: Preterm infants < 32 weeks gestation were recruited. The primary outcome was FI by day 7 defined as a feeding withheld, discontinued, or decreased because the infant was not tolerating enteral feedings. AL was operationalized using cortisol and 8-hydroxydeoxyguanosine (8-OHdG) from cord blood and from saliva and urine on days 1, 7, and 14. Descriptive statistics and comparative analyses were performed.

Results: Seven of 31 infants enrolled met criteria for FI. Infants with FI had lower median urinary cortisol on day 1 (p=0.007) and trended to have lower cortisol in the cord blood (p=0.056). Interquartile distributions were significantly different between infants without/with FI for urinary cortisol on day 1 (p=0.034) and tended for differences in 8-OHdG on day 14 (p=0.087). Interpercentile distributions were significantly different in salivary cortisol on day 14 (p=0.034) and tended for differences in 8-OHdG on day 1 (p=0.079).

Conclusions: Results support further testing of the model in a larger sample; investigation of the cellular mechanisms associated with the stress and the free-radical/anti-oxidant systems; and inclusion of prenatal factors.
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January 17, 2013

Melvin B. Heyman, MD, MPH
Editor-in-Chief
Journal of Pediatric Gastroenterology & Nutrition
Anita
RE: JPGN-NA-12-284

Dear Dr. Heyman,

Enclosed please find a revised version of our manuscript titled “Relationships between Feeding Intolerance and Stress Biomarkers in Preterm Infants” previously submitted to the Journal of Pediatric Gastroenterology and Nutrition (JPGN-NA-12-284). We believe this revised paper will be of great interest to your readers and particularly those whose research and practice are focused on the neonatal population.

The authors sincerely appreciate the comments and suggestions from the reviewers. We have carefully reviewed each comment and responded appropriately in the attached Revision Table. We have integrated almost every suggestion and believe the suggestions helped to improve the paper.

As first author, I will continue to serve as the correspondent in regard to this manuscript. I have included my mailing address and work telephone numbers. I have participated sufficiently in the conception and design of this manuscript to take public responsibility for it. All authors have reviewed the revised manuscript and declare no conflict of interest.

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Thank you for your consideration of this manuscript that supports the need to advance the knowledge for gastroenterology and nutrition in the neonatal population.

Sincerely,

Tiffany A. Moore, PhD, RN
Assistant Professor
University of Nebraska Medical Center
College of Nursing
Reviewer Comments:

<table>
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<th>Reviewer Comments</th>
<th>Moore et. al. Responses/Comments</th>
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<td>This paper provides an interesting look at the relationship between stress biomarkers and the possible development of feeding intolerance in premature infants, an important and needed area for research. However, there are certain issues with the paper that need to be addressed prior to any additional assessment.</td>
<td><strong>Revised (p.5-6):</strong> “The theoretical model for this study suggests using multiple stress biomarkers from the main allostatic body systems to define systemic physiologic dysregulation, or AL. Stress and AL were operationally defined using non-invasive stress biomarkers from the HPA axis (cortisol) and the immune system (8-hydroxydeoxyguanosine). These two specific biomarkers were chosen because they have successfully been collected for studies on preterm infants and they have been used to operationalize AL in other populations (17).”</td>
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<td>• First, the authors do not provide enough background on why the two biomarkers studied (cortisol and 8-OHdG) were chosen for investigation in relation to feeding issues in premature infants.</td>
<td>The authors revised this section to include a more in-depth discussion of the prior literature to provide further explanation on the biological plausibility of stress and feeding intolerance. <strong>Introduction (p.3-4) revised:</strong> “FI in preterm infants is believed to be a multifactorial phenomenon and the etiology remains unclear. In the adult population, brain-gut interactions have been proposed to affect enteral intolerance in critically ill patients (10). Early enteral nutrition has been used to provide trophic benefits and to decrease intestinal damage believed to be caused from increased levels of stress hormones and cytokines (10). Early enteral feeding for preterm infants is believed to provide trophic effects on the immature gastrointestinal system and decrease the intestinal damage from enteral fasting. Effects include increased permeability, decreased perfusion, intestinal villi atrophy, and decreased intestinal immunity (2, 6). Similar clinical manifestations of enteral intolerance in critically ill adults have been reported in preterm infants with suspected FI, such as increased residual, emesis, and abdominal distention (5, 10). Exploring FI in preterm infants as a stress-related condition involving brain-gut interactions is warranted. Brain-gut interactions related to intestinal motility may play an important role in FI in this population (11). The authors discuss the role of the enteric nervous system within the gastrointestinal tract in the homeostasis of gastric function. This relationship suggests the brain and stress influence intestinal motility, permeability, and immunity. Although no research studies were identified that involved the phenomenon of FI in preterm infants as a stress-related gastrointestinal complication, a theoretical model incorporating the psychobiological concepts of allostasis and allostatic load was chosen to further explore and test relationship between brain-gut interactions and the phenomenon of FI in preterm infants (12).”</td>
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<td>• The background section needs to be re-worked to include a better review of prior published literature on this topic as well as the biological plausibility of these relationships.</td>
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Moreover, in the discussion section of the review the authors only reference an adult study to explain the potential protective effects of glucocorticoids on gastrointestinal mucosa to interpret how low urinary cortisol levels at day 1 might be related to later feeding intolerance. There needs to a more thorough discussion of research findings in relation to the pediatric literature.

Limited research data was found for gastroprotection in human studies. However, the authors expanded on the hypothesized role of cortisol in intestinal maturity based on classic studies.

**References added:**


**Revised (p. 13-14):** "Cortisol is known to be involved in the maturation of intestinal enzymatic and transportation activity in the developing intestine (28, 32). Even the administration of antenatal steroids in preterm infants has been correlated with a significant decrease in intestinal permeability (33) and intestinal morbidity (34) emphasizing the importance of cortisol in intestinal maturity and function. Excessive cortisol, however, has been implicated in several gastrointestinal complications in critically ill patients (10, 18). Although limited to rodents, Filaretova and colleagues believe that moderate amounts of glucocorticoids have a gastroprotective effect from stress-induced gastric erosions (35). These protective effects from endogenous cortisol released during stress include glucose homeostasis, anti-inflammatory responses, maintenance of the intestinal membrane, and an increase in gastric mucosal perfusion and secretions. Our results suggest infants with low cortisol at birth may be at risk for FI by day 7 because of gastrointestinal immaturity from decreased cortisol in utero and a lack of gastroprotective effects from cortisol during the postnatal period. Further exploration into the origin of low cortisol at birth is necessary to determine the role of physiologic dysregulation prenatally and its inclusion in the research model. Further research also is needed to determine the homeostatic levels of cortisol in intestinal maturation and maintenance during stress.”

Another significant shortcoming of the paper is how the absence of finding any difference between 8-OHdG levels in premature infants with and without feeding intolerance is discussed by the authors. They comment that the results trend towards difference with lower levels on day 1 and higher levels on day 7 and 14. However, based on data presented in table 4 it looks like the median is lower on day 14 in infants with FI.

The AL model often uses interquartile and interpercentile ranges vs. means and medians. The authors included the means/medians for information and to demonstrate the advantage of the AL model when analyzing biomarkers. The authors revised and clarified this paragraph.

**Revised (p.15):** “The distribution for oxidative stress levels tended towards differences for infants without/with FI in our study on days 1, 7, and 14. Levels for infants with FI were distributed in lower percentile on day 1. On days 7 and 14, levels for infants with FI were distributed in the upper and lower interquartile ranges. These results also support the research model that predicted infants without/with FI would have a different distribution of stress biomarkers. Although a specific outcome of FI in studies involving oxidative stress in preterm infants was not found in the literature, Perrone et al (36) reported
that higher levels of oxidative stress biomarkers predicted the development of NEC. The lack of similar findings and significant results in our study may be related to the biomarkers used to measure oxidative stress. The biomarkers used in both studies measured different components of free radical damage caused by a non-specific oxidative stress mechanism. Both studies, however, suggest a relationship between AL and complications of prematurity because of the potential dysregulation and support the research model."

Other issues with the paper include:

1) Please discuss in more detail the limitation of the definition and measurement of FI used.

Revised (p. 16): “One limitation of the study included the definition and measurement of FI used. The phenomenon of FI involves non-specific signs and symptoms which are clinically subjective (5). The definition used in this study was an objective outcome; however, the decision to hold, discontinue, or decrease feedings was based on the subjective decision of the attending physician.”

2) There should be more discussion about the finding of a higher prevalence of FI in females in this study which is in contrast with the previously published literature.

References added:


Revised (p. 13): “However, our study focused on a brain-gut interaction as one component of idiopathic FI as opposed to NEC. McOmber, Ou, & Shulman (30) also found gender differences when observing intestinal permeability measurements in pediatric and adult subjects. The authors suggested a potential role of hormonal influences on intestinal and HPA mechanisms. Little is known about the role of gender in hormonal and intestinal relationships but in animal models of brain-gut interactions, stress in neonatal rodents caused from maternal separation has demonstrated gender-specific differences with an increase of functional intestinal diseases (i.e. Irritable Bowel Syndrome) in female rodents (31). Therefore, the higher incidence of female patients in our study further suggests a role of gender hormonal differences on intestinal and HPA mechanisms.”

3) Why are there no p values presents for means in the tables but only for medians?

The mean (SD) were provided for descriptive purposes and nonparametric tests were used due to small sample sizes. The p-values provided are for the nonparametric tests.

4) Figure 1 should also have the N in the figure in addition to the percentages.

Reviewer #2 has suggested removing Figure 1. Based on the response of both reviewers regarding Figure 1, the authors have decided to remove this figure.

5) Please provide more detail in methods about the post-hoc bonferroni correction method used.

Methods revised (p.9): “RM-ANOVA was used to compare differences over time, and if the main effect of time was significant, pair-wise comparisons were done between time points using Bonferroni correction.”

Specific mention of the bonferroni correction was removed in the results (p.11).
6) Explain why efforts were made to collect cortisol from infants at a specific time period in spite of previous studies showing no diurnal variation at this age.

Although previous studies have not shown diurnal variation, the authors wanted to minimize any potential unforeseen confounding factors.

**Revised (p. 7):** “Although neonates are not believed to display predictable cortisol patterns until a few weeks after birth (8-10), efforts were made to collect saliva between 12:00-16:00 h to limit potential circadian factors.”

**Reviewer #2**

The paper, Relationships between feeding intolerance and stress biomarkers in preterm infants presented a novel initial exploration of the relationship between two indicators of allostatic load (8-hydroxydeoxyguanosine [8-OHdG] and cortisol) and feeding intolerance. The paper was generally well written and the findings provided some evidence for further research in this area. Discussion of findings and limitations were discussed appropriately. I have only a few suggestions/questions.

- **Research purpose, aims, and hypotheses:**

  The specific aims in the Abstract and Introduction (Pg. 4) are worded differently making the meaning differ slightly. Please be consistent with the working.

  *Please note: to comply with the 250-word count, minor edits also were made to the abstract.*

  **Revised (p. 4):** “Specific aims were: describe demographic/medical variables and biomarker levels at each time and over time for the sample; describe/compare variables and biomarker levels at each time for infants without/with FI; and compare biomarker interquartile/interpercentile distributions between infants without/with FI.”

- **Methods:** Collection of cortisol is explained, but I did not see anything about the validity of cortisol levels collected in saliva or urine.

  - The same comment for 8-OHdG in collected in urine.

  **Reference added:**

  Neu M, Laudenslager ML. Cortisol: A measure of stress [abstract]. Western Institute of Nursing; 2009.

  **Revised (p.6):** “Saliva and urine specimens were used as non-invasive specimens as an alternative correlative measurement to serum cortisol in neonates (22, 23). Urinary samples are the preferred specimens for 8-OHdG levels per the 8-hydroxy-2-deoxy Guanosine EIA Kit (#589320) (Cayman Chemical, Ann Arbor, MI).”

  **What was the rationale for using both salivary**

  A secondary analysis of the data will be performed to identify the most reliable non-invasive collection
and urine cortisol in this study?

method for future research. Discussion of this rationale within the paper is believed to be beyond the scope of this manuscript.

- **Discussion**: On pg 12, last paragraph, sentence beginning; "This finding was not consistent with results with prior studies?" please cite the studies after the sentence.

  **Reference added (p. 13):**

- **Graph and Tables**: The tables are clear, but Figure 1 presents redundant information and is not needed.

  The authors appreciate the suggestion and have removed Figure 1.

**Additional Author Comments**

Please Note: Since additional references were cited, the authors removed references from the manuscript when multiple sources were cited to reduce the total number of references to 50.

The authors also made minor revisions in addition to the reviewer comments to enhance the quality and understanding of the manuscript and to decrease the word count for the manuscript. An increase in 470 words from the original manuscript was necessary to address the concerns of the reviewers. No major topic changes occurred based on these minor revisions.
Feeding Intolerance and Stress Biomarkers

Relationships between Feeding Intolerance and Stress Biomarkers in Preterm Infants

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Margaret E. Wilson
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Running Head: FEEDING INTOLERANCE AND STRESS BIOMARKERS

Word count: 5219; Tables: 4
Abstract

Objectives: Feeding intolerance (FI) in preterm infants is common but the etiology remains unclear. This study examined FI as a stress-related disease involving brain-gut interactions and tested the Model of Allostatic Load (AL) and Complications of Prematurity. Specific aims were: describe demographic/medical variables and biomarker levels at each time and over time for the sample; describe/compare variables and biomarker levels at each time for infants without/with FI; and compare biomarker interquartile/interpercentile distributions between infants without/with FI.

Methods: Preterm infants < 32 weeks gestation were recruited. The primary outcome was FI by day 7 defined as a feeding withheld, discontinued, or decreased because the infant was not tolerating enteral feedings. AL was operationalized using cortisol and 8-hydroxydeoxyguanosine (8-OHdG) from cord blood and from saliva and urine on days 1, 7, and 14. Descriptive statistics and comparative analyses were performed.

Results: Seven of 31 infants enrolled met criteria for FI. Infants with FI had lower median urinary cortisol on day 1 (p=0.007) and trended to have lower cortisol in the cord blood (p=0.056). Interquartile distributions were significantly different between infants without/with FI for urinary cortisol on day 1 (p=0.034) and tended for differences in 8-OHdG on day 14 (p=0.087). Interpercentile distributions were significantly different in salivary cortisol on day 14 (p=0.034) and tended for differences in 8-OHdG on day 1 (p=0.079).

Conclusions: Results support further testing of the model in a larger sample; investigation of the cellular mechanisms associated with the stress and the free-radical/anti-oxidant systems; and inclusion of prenatal factors.

Key Words: preterm infant, feeding intolerance, allostatic load, complications of prematurity, stress
Relationships between Feeding Intolerance and Stress Biomarkers in Preterm Infants

Introduction

Feeding intolerance (FI) is a common phenomenon in the Newborn Intensive Care Unit (NICU) affecting 16 to 29% of preterm infants (1, 2). The healthcare team in the NICU responds to any suspicion of intolerance to enteral feedings because of the association between FI and necrotizing enterocolitis (NEC), a gastrointestinal emergency that remains a leading cause of morbidity and mortality in this population (3). The definition of FI varies in the literature and within clinical practice. The clinical signs of FI include abdominal distention, emesis, large gastric residual volumes and bloody stools (4, 5). Since preterm infants cannot tolerate full enteral feedings for nutrition at birth, the transition from Total Parenteral Nutrition (TPN) to full enteral feedings is gradual. Evidence to develop practice guidelines for initiating, advancing, and feeding routine (continuous vs. intermittent) remains insufficient (6, 7).

However, any clinical sign of enteral intolerance often prompts the healthcare team to withhold, discontinue, or decrease the enteral feedings. This delays advancement and prolongs the administration of TPN and intravascular access; thus increasing the risk for infection and other complications (8, 9).

FI in preterm infants is believed to be a multifactorial phenomenon and the etiology remains unclear. In the adult population, brain-gut interactions have been proposed to affect enteral intolerance in critically ill patients (10). Early enteral nutrition has been used to provide trophic benefits and to decrease intestinal damage believed to be caused from increased levels of stress hormones and cytokines (10). Early enteral feeding for preterm infants is believed to provide trophic effects on the immature gastrointestinal system and decrease the intestinal damage from enteral fasting. Effects include increased permeability, decreased perfusion, intestinal villi atrophy, and decreased intestinal immunity (2, 6).

Similar clinical manifestations of enteral intolerance in critically ill adults have been reported in preterm infants with suspected FI, such as increased residual, emesis, and abdominal distention (5, 10).

Exploring FI in preterm infants as a stress-related condition involving brain-gut interactions is warranted. Brain-gut interactions related to intestinal motility may play an important role in FI in this
population (11). The authors discuss the role of the enteric nervous system within the gastrointestinal tract in the homeostasis of gastric function. This relationship suggests the brain and stress influence intestinal motility, permeability, and immunity. Although no research studies were identified that involved the phenomenon of FI in preterm infants as a stress-related gastrointestinal complication, a theoretical model incorporating the psychobiological concepts of allostasis and allostatic load was chosen to further explore and test relationship between brain-gut interactions and the phenomenon of FI in preterm infants (12).

Allostasis describes the physiologic adaptation of body systems to maintain homeostasis in the presence of stress (13). Allostasis promotes adaptation using neural, neuroendocrine, and neuroendocrine-immune mechanisms in four body systems: autonomic nervous system, metabolic system, immune system, and the hypothalamic-pituitary-adrenal (HPA) axis. Allostatic load (AL) is the multisystem physiologic dysregulation caused by chronic states of allostasis (14). AL has furthered knowledge and understanding of various stress-related complex diseases in the adult population and is operationally defined using stress biomarkers from the four body systems associated with allostasis (15).

Exploring FI as a stress-related gastrointestinal complication due to physiological dysregulation of stress is innovative. No studies were found that incorporated the psychobiological model of AL in this population. The purposes of this study were to a) examine feeding intolerance (FI) as a stress-related condition involving brain-gut interactions in preterm infants and b) test the Model of Allostatic Load and Complications of Prematurity (12) derived from the Allostatic Load Model (16) using stress biomarkers in preterm infants; and c) examine demographic and medical variables related to these theoretical relationships. If AL is associated with FI, then infants without/with FI were predicted to display different distributions of stress biomarkers. Specific distributions of the biomarker levels for infants without/with FI are unknown. According to the Allostatic Load Model, physiologic dysregulation has been defined as an exaggerated or an inadequate response of an allostatic system.

This study included demographic variables, medical variables proposed to be related to stress and FI, and stress biomarkers of cortisol and 8-hydroxydeoxyguanosine (8-OHdG) at birth and, on days 1, 7,
and 14 in preterm infants <32 weeks gestational age (GA). The specific aims were to 1) describe
demographic variables, medical variables, and biomarker levels at each time and over time for the entire
sample; 2) describe and compare demographic variables, medical variables, and biomarker levels at each
time for preterm infants without/with FI by day 7; and 3) compare the interquartile and interpercentile
distributions of biomarker levels at each time for preterm infants without/with FI by day 7.

**Materials and Methods**

A prospective, correlational, longitudinal design was used. Approval was received from the
institutional review board. A convenience sample of preterm infants was recruited from a level III NICU
in a Midwestern US tertiary medical center. Inclusion criteria included infants <32 weeks gestational age
at birth. Exclusion criteria were major congenital anomalies. A parent of the eligible infant was
approached by research personnel within 24 hours of birth and after admission to the NICU. The infant
was enrolled after parental consent; the day of consent was regarded as day 1 of the study. Days 7 and 14
were defined as one and two weeks after the day of consent.

**Measurements**

**Feeding Intolerance**

The primary outcome was the incidence of FI by day 7. FI was defined *a priori* as a feeding
withheld, discontinued, or decreased at any time because the infant was not tolerating enteral feedings.
This defining attribute was used because it is a measurable outcome, as opposed to a subjective clinical
sign, of FI (12), and encompasses similar definitions (1, 2, 7). The incidence by day 7 was chosen based
on usual timing of advancement of trophic feedings. Data obtained from the medical record were recorded
as a categorical variable for an incidence of FI (y/n).

**Stress and Allostatic Load**

The theoretical model for this study suggests using multiple allostatic stress biomarkers to define
systemic physiologic dysregulation, or AL. Stress and AL were operationally defined using non-invasive
biomarkers from the HPA axis (cortisol) and the immune system (8-hydroxydeoxyguanosine). These two
specific biomarkers have been collected successfully for studies on preterm infants and have been used to
Feeding Intolerance and Stress Biomarkers

Operationalize AL in other populations (17). The biomarker values of preterm infants with AL may be distributed in the upper or lower percentiles and may display high/low variability between serial levels.

Specimens obtained for biomarkers were collected from the cord blood at birth and from saliva and urine on days 1, 7, and 14 to examine physiologic status over time. Cord blood levels provided information on the infant’s physiologic status at birth; day 1 measures provided baseline levels for saliva and urine and day 7 described the physiologic status at the time when feedings were expected to advance. Day 14 provided evidence of the distribution of physiologic status over time. These times also were consistent with previous biomarker studies in the population (18-21). Saliva and urine specimens were used as non-invasive specimens as an alternative correlative measurement to serum cortisol in neonates (22, 23). Urinary samples are the preferred specimens for 8-OHdG levels per the 8-hydroxy-2-deoxy Guanosine EIA Kit (#589320) (Cayman Chemical, Ann Arbor, MI).

_Cord blood_

Cord blood was obtained at birth per hospital protocol. Remaining cord blood not used for clinical purposes was processed by the Clinical Research Center (CRC). Specimens were centrifuged for 15 minutes at 1500g to retrieve the plasma and stored in a -80º C freezer labeled with the study ID.

_Saliva_

Salivary specimens were collected on each infant on days 1, 7, and 14 using a Salimetrics Infant Swab (Salimetrics LLC, State College, PA). Specimens were obtained during daytime hours (0900-2000) making efforts to avoid major stimulation 30 minutes prior to obtaining the specimen (24). The cotton swab was placed in the infant’s buccal mucosa for 5 minutes. If visible blood was found on the swab, oral care was performed prior to an additional collection attempt by gently swabbing the mouth with a moistened towelette with a limit of two attempts. Collected specimens were placed in the Salimetrics swab storage tube and labeled with the participant’s research study ID and specimen number (days 1, 7, 14) and stored in a -80º C freezer in the CRC. Although neonates are not believed to display predictable
cortisol patterns until a few weeks after birth (25), efforts were made to collect saliva between 12:00-16:00 h to limit potential circadian factors.

**Urine**

Spot urine specimens were collected on days 1, 7, and 14. Collection of urine was performed by placing a sterile cottonball onto the perineal area inside of the infant’s diaper. At the next diaper change, the cottonball was removed, placed into a 10ml syringe and squeezed into a 1.5ml microcentrifuge tube, labeled with the study ID and specimen number and stored in a -80º C freezer.

**Tests**

Cortisol was measured in the cord blood, saliva, and urine. Cord blood cortisol levels were performed by the CRC using hospital protocol. Saliva and urine specimens were analyzed using enzyme immunoassay techniques at the Endocrine Bioservices Laboratory (Omaha, NE). Each saliva specimen was assayed in duplicate using a 1:4 dilution based on the amount of saliva obtained. Each urine specimen was assayed in duplicate using a 1:80 dilution after a standard dilution test was performed using 3 separate specimens at day 1 and day 14 (each serial dilution run was associated with a displacement curve parallel to the standard curve). Standards also were assayed in duplicate and the assays sensitivity was set to 7.8 pg/50 ul. High and low interassay coefficients of variations, calculated from pooled saliva specimens assayed on each plate, were 11.99% and 9.37%, respectively. High and low intraassay coefficients of variation, also calculated from pooled saliva specimens assayed on each plate, were 7.57% and 10.85%, respectively. Creatinine levels also were obtained for each urine specimen in a 1:20 dilution based on a standard dilution test with a modified Jaffe assay (26). Urinary cortisol levels were expressed as a ratio of µg cortisol per mg creatinine.

Cord blood and urine were used for 8-hydroxydeoxyguanosine (8-OHdG) measurements. Specimens were assayed by Cayman Assay Services using the 8-hydroxy-2-deoxy Guanosine EIA Kit (#589320) (Cayman Chemical, Ann Arbor, MI). Specimens were diluted directly into EIA Buffer prior to assay. A standard curve was established by serial dilution of the 8-OHdG standard between 10.3 and
3,000 pg/ml using EIA Buffer as the matrix. The concentration of each specimen was calculated from a logistic four-parameter fit generated from the standard concentrations. Final results were expressed as a ratio of μg 8-OHdG per mg creatinine using the above creatinine results.

**Demographic and Medical Data**

Demographic and medical data were obtained from the infant’s medical record. Demographic variables included gestational age (GA) at birth, birth weight, sex, and race. Variables of the infant’s medical status were included to identify medical stability of the infant and potential factors affecting stress. These variables were Apgar score at 1- and 5-minutes, first pH, number of days requiring ventilator support by day 7, and ventilator support required by day 7 (yes/no). Three specific medical variables believed to affect cortisol levels were the administration of antenatal steroids per the infant’s admission profile (yes/no), a diagnosis of adrenal insufficiency or hypotension (yes/no), and a surgical intervention for intestinal perforation (yes/no). Infants receiving antenatal steroids or diagnosed with AI or hypotension were identified because of the conflicting literature on cortisol levels associated with these variables (18). Intestinal perforation vs. a diagnosis of necrotizing enterocolitis (NEC) was used because of the lack of a universal definition of NEC and the difficulty in distinguishing between spontaneous intestinal perforation and NEC (27). Therefore, infants in this study requiring surgical intervention for an intestinal perforation were included. Variables from the infant’s feeding plan believed to affect FI also were included: number of days NPO by day 7, day-of-life (DOL) trophic feedings began, DOL trophic feedings increased, percentage of formula/mother’s breast milk (MBM)/donor breast milk (DBM) per total feeding volume received by day 7, and number receiving formula/MBM/DBM by day 7 (yes/no).

Of note, a change in clinical practice occurred in the middle of recruitment. DBM became available after the first 13 participants were enrolled in the study. Mothers were strongly encouraged to use DBM as an option for trophic feedings until the mother was able to supply MBM. The percentage of formula/MBM/DBM per total enteral feeding volume in Table 1 and the number of infants receiving
formula/MBM/DBM by day 7 in Table 2 are not adjusted for this practice change. The percentages calculated are based on the entire sample.

**Data Analysis**

**Power Analysis**

An a priori power analysis using a two-tailed significance level of 0.05 and power >80% for a Chi-square test of the primary dependent variable (FI) was performed using G*Power version 3.1.2 (Franz Faul, Universitat Kiel, Germany, 2009). The null hypothesis was that 25% of the participants with FI would be in the lower 25th percentile for the biomarker values; 50% would be between the 25th and 75th percentiles; 25% would be in the upper 25th percentile. The alternative hypothesis was that there would be differences in distributions of biomarker values for infants without/with FI. Thirty infants were calculated to be needed to reject the null hypothesis.

**Statistical Analyses**

Statistical analyses were performed using SPSS, version 19 (SPSS Inc., Chicago, IL). Descriptive statistics for demographics, medical variables, and biomarker values were compared infants without/with FI using Mann-Whitney tests and Fisher’s exact tests. Biomarker interquartile (25%-50%-25%) and interpercentile (10%-80%-10%) distributions were compared with categorical demographic and medical variables using 2 x 3 contingency tables and Chi-square analyses. Interpercentile distributions were analyzed to explain a relationship between outliers and FI. Because the assumptions of the Chi-square analysis were not met for this study, SAS (SAS Institute Inc., Cary, NC) was used to perform Fisher’s exact test for determining statistical differences between the distributions of infants without/with FI. RM-ANOVA was used to compare differences over time, and if the main effect of time was significant, pairwise comparisons were done between time points using a Bonferroni correction.

**Results**

**Sample and Specimens**

During September 2011 to July 2012, 44 infants <32 weeks gestational age were admitted to the NICU setting. A total of ten infants were determined ineligible for the study: four had major congenital
anomalies, two were over 24 hours of age at admission, and the parents of four infants did not speak/read English. A parent of the 34 eligible infants was approached by study personnel; three parents declined to participate due to the infant’s medical status. A total of 31 infants were enrolled; 30 completed the study.

A total of 27 specimens (12.4% of 217) were missing for the entire sample. Six (19.4%) cord blood specimens were missing due to unavailability at birth. Missing saliva data on days 1, 7, and 14 (3, 6, 4 specimens, respectively) were from bloody specimens or inadequate volumes. The missing urinary cortisol data on days 1, 7, and 14 (3, 2, 1, specimens respectively) were due to procedural errors. One additional missing urine data point on day 7 in the 8-OHdG analysis was due to inadequate volume and one infant died prior to collection of day 14 specimens.

**Aim 1**

Descriptive statistics for the entire sample are presented for continuous variables on Table 1 and categorical variables on Table 2. The median DOL to begin trophic feedings was at 2 days and to increase trophic feedings was at 7 days, which is consistent with clinical practice in this NICU. MBM was received for most of the participants (n=29) and accounted for over half of the total enteral feeding volume by day 7. For the three medical variables believed to influence cortisol levels, sixteen (51.6%) of the participants received antenatal steroids, seven (25.0%) were diagnosed with AI or hypotension, and three (9.7%) required surgical intervention for intestinal perforation.

Further comparison analyses were performed on demographic and medical variables for participants who did/did not receive antenatal steroids, who were/were not diagnosed with AI or hypotension, and participants who did/did not require surgical intervention for intestinal perforation. Infants who received antenatal steroids had a lower birth weight (p=0.036), longer time to increase trophic feedings (p=0.023), and tended toward lower GA at birth (p=0.053). Infants with AI or hypotension had lower birth weight (p=0.039), required ventilation for more days by day 7 (p=0.022), more days on NPO status (p=0.006) and tended towards longer time to increase trophic feedings (p=0.053) than for those without AI or hypotension. No other significant differences were found in the demographic or medical variables between participants with these three medical variables.
Descriptive statistics for available cortisol data in the entire sample for each specimen at each time are presented in Table 3. The range, mean, standard deviation (SD), and median are listed for cord blood at birth and on days 1, 7, and 14 for salivary and urinary specimens. For the entire sample, cortisol levels were highest in the saliva and urine and had the most variability (SD and range) on day 1. Descriptive statistics for available cord blood and urinary 8-OHdG data for the entire sample are listed in Table 4. Similar to cortisol, urinary 8-OHdG was highest and had the most variability on day 1.

Salivary cortisol, urinary cortisol, and urinary 8-OHdG data for the entire sample were compared over time (days 1, 7, and 14). Since conditions were not met to assume Mauchly’s test of sphericity for any of the tests, the Greenhouse-Geisser was used. Salivary cortisol levels significantly decreased over time [F(1.039, 18.707)=9.657, p=0.005]. Post-hoc analysis indicated differences between day 1 and day 7 (p=0.016) and between day 1 and day 14 (p=0.019). Urinary cortisol and 8-OHdG levels decreased but were not statistically different over time.

Aim 2

Of the 31 participants enrolled, seven (22.6%) met the criteria for FI by day 7. Descriptive statistics and comparison analyses for infants without/with FI also are presented in Tables 1 and 2. Among the three medical variables believed to influence cortisol levels, five participants who received antenatal steroids, two with AI or hypotension, and two who required surgical intervention for intestinal perforation met the criteria for FI. A statistical difference between infants without/with FI was found for sex (p=0.037); infants without FI were more likely to be male. No other statistical differences were found for demographic and medical variables between infants without/with FI.

Descriptive statistics and comparison analyses for infants without/with FI for each specimen at each time are presented in Table 3 for cortisol and Table 4 for 8-OHdG. The range, mean, SD, and median are listed for cord blood levels at birth and for salivary and urinary specimens on days 1, 7, and 14. Infants without/with FI differed in urinary cortisol levels on day 1 (p=0.007). Medians were higher for infants without FI than for infants with FI. Cortisol in the cord blood tended (p=0.056) to be higher for
infants without FI than for infants with FI. No other significant differences were found between infants without/with FI in median cortisol levels or median 8-OHdG levels.

**Aim 3**

The interquartile distributions, interpercentile distributions, and comparison analyses using Fisher’s exact test are presented for cortisol in Table 3 and for 8-OHdG in Table 4. Salivary cortisol analyses showed a significant difference for the interpercentile distributions on day 14 (p=0.034). Infants without FI were in the lower quartiles while infants with FI were in the upper quartiles on day 14. Urinary analyses showed a significant difference in the interquartile distribution of cortisol on day 1 (p=0.034). Data from infants without FI were distributed throughout all quartiles whereas data from infants with FI fell into the lower quartiles. Urinary 8-OHdG distributions for infants without/with FI tended toward different interpercentile distributions on day 1 (p=0.079) and different interquartile distributions on day 14 (p=0.087). Data from infants without FI were in the middle and upper percentiles on day 1 and distributed throughout all quartiles on day 14. Infants with FI were in the lower percentiles on day 1 and distributed in the upper and lower quartiles on day 14.

**Discussion**

This is the first known study to examine FI in preterm infants <32 weeks GA as a stress-related condition involving brain-gut interactions and to test a model of AL in a NICU setting. Infants without/with FI were identified and compared using two stress biomarkers: cortisol and 8-OHdG. Infants with FI had lower levels of stress biomarkers in the cord blood and in urine on day 1. Infants without/with FI differed in the distribution of the biomarker levels on day 7 and 14. Despite inconsistent findings, results warrant further research into the potential role of a brain-gut interaction in FI and provide support to further test the relationship between AL and complications of prematurity. Interpretation and discussion of the data from infants with FI and the entire sample is followed by the strengths, limitations, and implications for research.

The incidence of FI in our study was consistent with previous reports using similar criteria and sample demographics (1, 2). The incidence of FI was more common in female patients, a finding that is
not consistent with the literature (29). However, our study focused on a brain-gut interaction as one component of idiopathic FI as opposed to NEC. McOmber, Ou, & Shulman (30) also found gender differences when observing intestinal permeability measurements in pediatric and adult subjects. The authors suggested a potential role of hormonal influences on intestinal and HPA mechanisms. Little is known about the role of gender in hormonal and intestinal relationships but in animal models of brain-gut interactions, stress in neonatal rodents caused from maternal separation has demonstrated gender-specific differences with an increase of functional intestinal diseases (i.e. Irritable Bowel Syndrome) in female rodents (31). Therefore, the higher incidence of female patients in our study further suggests a role of gender hormonal differences on intestinal and HPA mechanisms. No other differences in demographic and medical variables were found between infants without/with FI in our study.

Infants with FI had lower cortisol levels in the cord blood and significantly lower cortisol in the urine on day 1. This finding was not consistent with results from prior studies but supports the research model that predicted infants without/with FI would have different distributions of stress biomarkers. Although the etiology of FI is multifactorial, low cortisol levels at birth may increase the risk for FI during the first week of life. Cortisol is known to be involved in the maturation of intestinal enzymatic and transportation activity in the developing intestine (28, 32). Even the administration of antenatal steroids in preterm infants has been correlated with a significant decrease in intestinal permeability (33) and intestinal morbidity (34) emphasizing the importance of cortisol in intestinal maturity and function. Excessive cortisol, however, has been implicated in several gastrointestinal complications in critically ill patients (10, 18). Although limited to rodents, Filaretova and colleagues believe that moderate amounts of glucocorticoids have a gastroprotective effect from stress-induced gastric erosions (35). These protective effects from endogenous cortisol released during stress include glucose homeostasis, anti-inflammatory responses, maintenance of the intestinal membrane, and an increase in gastric mucosal perfusion and secretions. Our results suggest infants with low cortisol at birth may be at risk for FI by day 7 because of gastrointestinal immaturity from decreased cortisol in utero and a lack of gastroprotective effects from
cortisol during the postnatal period. Further exploration into the origin of low cortisol at birth is necessary to determine the role of physiologic dysregulation prenatally and its inclusion in the research model. Further research also is needed to determine the homeostatic levels of cortisol in intestinal maturation and maintenance during stress.

Aucott and colleagues (18) found higher serum cortisol levels on DOL 1 were related to intestinal perforation; however, they did not report cord blood or urinary values. These differences suggest the need to further understand the role of stress-induced glucocorticoid exposure in utero and the infant’s ability to transition to extrauterine life. The varying results based on specimen source may provide insight into the timing of excessive or inadequate glucocorticoid damage, the cellular implications of excessive or inadequate glucocorticoids, and the difference between overall endocrine status vs. an acute HPA stress response. For example, low cortisol in the cord blood and urine on day 1 may be more reflective of prenatal predictors and general endocrine status while high serum or salivary cortisol on day 1 suggests a more exaggerated response during transition to extrauterine life which emphasizes the negative effects of excessive cortisol. The results of both studies, however, lend support for brain-gut interactions, physiologic dysregulation, and AL in preterm infants.

The statistical difference in salivary cortisol distributions on day 14 for infants without/with FI also may support the research model used in this study. Although only four salivary specimens were available for infants with FI on day 14, the infants within the upper percentile were not receiving hydrocortisone nor had an intestinal perforation requiring surgical intervention which would be confounding factors for higher levels of cortisol. A possible explanation for this finding could be related to allostasis and AL. Similar to day 1, urinary cortisol levels on day 14 were not consistent with the salivary levels on day 14. Interpretation of the data using the Model of Allostatic Load and Complications of Prematurity (12) suggests that these infants may have maladaptive response patterns to acute stressors and were at increased risk for AL and complications of prematurity. Since infants with FI had lower levels of cortisol at birth, physiologic dysregulation could have occurred in utero. On the other hand,
these infants may be displaying an exaggerated response during the transition to extrauterine life to compensate for inadequate cortisol levels in utero.

The distribution for oxidative stress levels tended towards differences for infants without/with FI in our study on days 1, 7, and 14. Levels for infants with FI were distributed in the lower percentile on day 1 and in the upper and lower interquartile ranges on days 7 and 14. These results also support the research model that predicted infants without/with FI would have a different distribution of stress biomarkers. Although a specific outcome of FI in studies involving oxidative stress in preterm infants was not found in the literature, Perrone et al (36) reported that higher levels of oxidative stress biomarkers predicted the development of NEC. The lack of similar findings and significant results in our study may be related to the biomarkers used to measure oxidative stress. The biomarkers used in both studies measured different components of free radical damage caused by a non-specific oxidative stress mechanism. Both studies, however, suggest a relationship between AL and complications of prematurity because of the potential dysregulation and support the research model.

Further analyses into confounding factors for oxidative stress are necessary to interpret these results sufficiently. The free-radical/antioxidant imbalance is complicated by the excessive oxygen-free radicals associated with neonatal diseases (37, 38), an immature anti-oxidant system (21, 38-41), blood transfusions (42), pain (43) as well as nutritional support such as MBM and formula (40, 44) and parenteral nutrition (33, 45). The variation of non-specific oxidative stress measurements used in prior studies is a major limitation in efforts to fully understand the complexity of the free-radical/antioxidant imbalance. These mixed results emphasize the need to further explore the cellular mechanisms affecting the excess and/or inadequate specific free-radicals and antioxidants.

The demographics for our entire sample were comparable to the studies mentioned above. Most performed comparison analyses between groups based on interventions received or medical variables. Therefore, we included comparison analyses for three specific medical variables which, according to previous findings, may have confounded our results. The significant differences found in medical
variables for infants receiving antenatal steroids were expected because of the clinical practice of antenatal steroids in preterm labor (46). The lower birth weight and GA at birth may have influenced a more conservative feeding plan. The differences in medical variables for infants with AI or hypotension were expected since these diagnoses are common in extremely low birth weight infants (47). Furthermore, infants requiring blood pressure support often have a more conservative feeding plan (48).

The salivary and urinary cortisol levels in the entire sample were consistent with previous studies (18, 20). The wider variation of levels on day 1 may be explained by the collection window in this study (0-24 hours after birth) and Aucott’s study (12-48 hours after birth) since a surge of cortisol is thought to be normal at birth (28, 47, 49). Cortisol levels may have been confounded by the timing of specimen collection after birth. Similar to cortisol, our 8-OHdG data for the entire sample validates the free-radical/antioxidant imbalance at birth and is consistent with other studies reporting high levels of oxidative stress in preterm infants (19, 21, 38, 44). Our 8-OHdG levels decreased over time which is consistent with the findings of NASSI (38) and Longini (19). In contrast, Vento (21) and Friel (44) reported an increase in oxidative stress levels over time. These mixed findings may be explained by changes in clinical practice and varying measurements. The research conducted by the experts listed above has changed neonatal resuscitation guidelines recommended by the American Academy of Pediatrics (50). Therefore modifications in clinical practice such as using low oxygen concentrations for resuscitation, allowing lower saturations during transition to extrauterine life, and lowering target ranges for saturation limits may be confounding factors for comparison analyses.

Strengths of the study were the longitudinal design, use of non-invasive biomarkers, an innovative approach for explaining FI using brain-gut interactions, and testing a model of AL in the preterm infant population. One limitation of the study included the definition and measurement of FI used. The phenomenon of FI involves non-specific signs and symptoms which are clinically subjective (5). The definition used in this study was an objective outcome; however, the decision to hold, discontinue, or decrease feedings was based on the subjective decision of the attending physician. Other
limitations included small sample size, missing specimen data, feeding practice change occurring in the middle of recruitment, and methods of specimen collection. Implications for future research include modifying the Model of Allostatic Load and Complications of Prematurity (12) to include prenatal predictors and testing the research model on other complications of prematurity using a larger sample and more biomarkers. Further scientific investigation is needed to understand the cellular mechanisms associated with the stress response and the free-radical/anti-oxidant systems. The differences in cord blood cortisol between infants without/with FI also suggests a critical role of prenatal predictors and fetal programming as contributing factors to neonatal outcomes. Thus, additional prenatal data are indicated.

In summary, this study tested whether a brain-gut interaction affecting physiologic dysregulation was associated with FI in preterm infants. Differences were found in the distribution of some of the stress biomarkers at some of the times for infants without/with FI within the first two weeks of life as predicted by the research model. Although the data from this study remains inconclusive, our results warrant further research into relationships between physiologic dysregulation, AL, and complications of prematurity. Identifying infants at-risk for physiologic dysregulation based on multiple biomarker levels during the prenatal period and the first week of life will allow for preventative strategies to be developed and tested. This study is an initial investigation into the etiology of complications of prematurity using psychobiological mechanisms and physiologic dysregulation.
References


49. Liggins GC. The role of cortisol in preparing the fetus for birth. Reprod Fertil Dev. 1994;6(2):141-50

Table 1. Descriptive Statistics for Demographic and Medical Variables for Entire Sample (n=27), Infants Without/With Feeding Intolerance (FI), and Mann-Whitney U results for Infants Without/With FI

<table>
<thead>
<tr>
<th>Variable (units)</th>
<th>Sample n=31</th>
<th>Without FI n=24</th>
<th>With FI n=7</th>
<th>p-value,+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean/Median (SD)</td>
<td>Mean/Median (SD)</td>
<td>Mean/Median (SD)</td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>29.0/29.3 (1.9)</td>
<td>28.8/29.2 (2.0)</td>
<td>29.7/30.4 (1.7)</td>
<td>0.309</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>1192/1130 (376)</td>
<td>1196/1046 (419)</td>
<td>1180/1184.0 (184)</td>
<td>0.741</td>
</tr>
<tr>
<td>Apgar score (minutes)</td>
<td>4.7/5.0 (2.3)</td>
<td>4.5/4.5 (2.4)</td>
<td>5.3/5.0 (1.8)</td>
<td>0.366</td>
</tr>
<tr>
<td>1-minute</td>
<td>6.9/7.0 (1.4)</td>
<td>6.9/7.0 (1.4)</td>
<td>6.9/6.0 (1.5)</td>
<td>0.942</td>
</tr>
<tr>
<td>5-minutes</td>
<td>4.7/5.0 (2.3)</td>
<td>4.5/4.5 (2.4)</td>
<td>5.3/5.0 (1.8)</td>
<td>0.366</td>
</tr>
<tr>
<td>First pH‡</td>
<td>7.31/7.32 (0.07)</td>
<td>7.31/7.32 (.07)</td>
<td>7.30/7.32 (.07)</td>
<td>0.941</td>
</tr>
<tr>
<td>Ventilator support by Day 7 (days)</td>
<td>3.0/2.0 (3.0)</td>
<td>3.4/2.5 (3.3)</td>
<td>1.6/2.0 (0.5)</td>
<td>0.499</td>
</tr>
<tr>
<td>NPO by Day 7 (days)</td>
<td>2/1.0 (1.8)</td>
<td>1.8/1.0 (1.8)</td>
<td>2.6/2.0 (1.8)</td>
<td>0.107</td>
</tr>
<tr>
<td>Day of life trophic feedings began</td>
<td>2.2/2.0 (1.3)</td>
<td>2.3/2.0 (1.5)</td>
<td>2.0/2.0 (0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Day of life trophic feedings increased</td>
<td>7.0/6.0 (3.6)</td>
<td>6.4/6.0 (3.0)</td>
<td>9.1/10.0 (4.9)</td>
<td>0.173</td>
</tr>
<tr>
<td>% Formula</td>
<td></td>
<td>27.7/1.0 (34.4)</td>
<td>24.5/1.0 (33.7)</td>
<td>38.9/51.0 (37.4)</td>
</tr>
<tr>
<td>% Mother’s Breast Milk</td>
<td></td>
<td>55.9/57.0(36.5)</td>
<td>57.9/68.0 (39.0)</td>
<td>49.0/44.0 (27.2)</td>
</tr>
<tr>
<td>% Donor Breast Milk</td>
<td></td>
<td>13.1/0.0 (27.2)</td>
<td>13.5/0.0 (29.3)</td>
<td>11.9/0.0 (20.5)</td>
</tr>
</tbody>
</table>

* FI was defined a priori as a feeding withheld, discontinued, or decreased at any time because the infant was not tolerating enteral feedings by day 7.
† Independent Samples Mann-Whitney U test for infants without/with FI
‡ First recorded pH in medical record excluding cord pH; venous, capillary, and arterial samples included.
§ One infant without FI did not have a pH recorded in the medical record.
|| Mean percentage of the total volume of enteral feedings received by day 7
Table 2. Descriptive statistics for demographic and medical variables for entire sample (n=27), infants without/with feeding intolerance (FI), and Fisher’s exact test for infants without/with FI

<table>
<thead>
<tr>
<th></th>
<th>Sample Size (n=31)</th>
<th>Without FI (n=24)</th>
<th>With FI (n=7)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (64.5%)</td>
<td>18 (75.0%)</td>
<td>2 (28.6%)</td>
<td>0.037</td>
</tr>
<tr>
<td>Female</td>
<td>11 (35.5%)</td>
<td>6 (25.0%)</td>
<td>5 (71.4%)</td>
<td></td>
</tr>
<tr>
<td><strong>Race‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>26 (83.9%)</td>
<td>19 (79.2%)</td>
<td>7 (100%)</td>
<td>0.737</td>
</tr>
<tr>
<td>Black</td>
<td>3 (9.7%)</td>
<td>3 (12.5%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2 (6.5%)</td>
<td>2 (8.3%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Ventilation required by Day 7§</strong></td>
<td>26 (83.9%)</td>
<td>19 (79.2%)</td>
<td>7 (100%)</td>
<td>0.250</td>
</tr>
<tr>
<td><strong>Antenatal Steroids§</strong></td>
<td>16 (51.6%)</td>
<td>11 (45.8%)</td>
<td>5 (71.4%)</td>
<td>0.224</td>
</tr>
<tr>
<td><strong>Adrenal Insufficiency or hypotension§</strong></td>
<td>7 (25.0%)</td>
<td>5 (23.8%)</td>
<td>2 (28.6%)</td>
<td>0.581</td>
</tr>
<tr>
<td><strong>Intestinal perforation§</strong></td>
<td>3 (9.7%)</td>
<td>1 (4.2%)</td>
<td>2 (28.6%)</td>
<td>0.120</td>
</tr>
<tr>
<td><strong>Formula§</strong></td>
<td>17 (54.8%)</td>
<td>13 (54.2%)</td>
<td>4 (57.1%)</td>
<td>0.617</td>
</tr>
<tr>
<td><strong>MBM§</strong></td>
<td>29 (93.5%)</td>
<td>22 (91.7%)</td>
<td>7 (100%)</td>
<td>0.594</td>
</tr>
<tr>
<td><strong>DBM§ (n=18)‖</strong></td>
<td>10 (32.3%)</td>
<td>7 (22.6%)</td>
<td>3 (42.9%)</td>
<td>0.401</td>
</tr>
</tbody>
</table>

* FI was defined *a priori* as a feeding withheld, discontinued, or decreased at any time because the infant was not tolerating enteral feedings by day 7.
† p-value for Fisher’s exact test between infants without/with FI
‡ database did not contain information indicating Hispanic/non-Hispanic status
§ number of infants per medical record (y/n)
‖ a change in clinical practiced occurred for the option for infants to receive DBM after the first 13 patients were recruited.
<table>
<thead>
<tr>
<th></th>
<th>Cord blood (ug/dL)</th>
<th>Saliva (ug/dL)</th>
<th>Urine (ug/mg Creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Birth (n=19/6)†</td>
<td>Day 1 (n=21/7)†</td>
<td>Day 7 (n=20/5)†</td>
</tr>
<tr>
<td>Range‡</td>
<td>0.4 – 10.4</td>
<td>1.4 – 110.0</td>
<td>1.7 – 15.5</td>
</tr>
<tr>
<td>Mean (SD§)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire Sample</td>
<td>2.9 (1.9)</td>
<td>26.8 (32.1)</td>
<td>5.8 (3.1)</td>
</tr>
<tr>
<td>Without FI</td>
<td>3.3 (2.0)</td>
<td>29.0 (32.4)</td>
<td>5.9 (3.4)</td>
</tr>
<tr>
<td>With FI</td>
<td>1.7 (1.3)</td>
<td>20.1 (32.9)</td>
<td>5.2 (1.3)</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire Sample</td>
<td>2.6</td>
<td>13.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Without FI</td>
<td>2.9</td>
<td>14.7</td>
<td>5.1</td>
</tr>
<tr>
<td>With FI</td>
<td>1.5</td>
<td>6.2</td>
<td>5.3</td>
</tr>
<tr>
<td>p-value¶</td>
<td>0.056</td>
<td>0.301</td>
<td>0.946</td>
</tr>
<tr>
<td>Interquartiles (25%-50%-25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without FI</td>
<td>4-10-5</td>
<td>5-10-6</td>
<td>5-9-6</td>
</tr>
<tr>
<td>With FI</td>
<td>4-1-1</td>
<td>2-4-1</td>
<td>1-4-0</td>
</tr>
<tr>
<td>p-value¶</td>
<td>0.139</td>
<td>0.864</td>
<td>0.542</td>
</tr>
<tr>
<td>Interpercentiles (10%-80%-10%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without FI</td>
<td>0-17-2</td>
<td>1-18-2</td>
<td>2-16-2</td>
</tr>
<tr>
<td>With FI</td>
<td>2-4-0</td>
<td>1-6-0</td>
<td>0-5-0</td>
</tr>
<tr>
<td>p-value¶</td>
<td>0.099</td>
<td>0.708</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* FI was defined *a priori* as a feeding withheld, discontinued, or decreased at any time because the infant was not tolerating enteral feedings by day 7.
† Specimens available for infants without/with FI
‡ Minimum-maximum values
§ Standard Deviation (SD)
¶ Independent Samples Mann-Whitney U test for infants without/with FI, cases excluded test-by-test
Fisher's Exact Test for infants without/with FI
Table 4. Descriptive Statistics and Interquartile (25%-50%-25%) and Interpercentile (10%-80%-10%) Distributions for 8-hydroxydeoxyguanasine (8-OHdG) for Entire Sample, Infants Without/With Feeding Intolerance (FI) for Each Specimen at Each Time, and Comparison Analyses for Infants Without/With FI.

<table>
<thead>
<tr>
<th></th>
<th>Cord blood (ng/ml)</th>
<th>Urine 8-OHdG (ug/mg Creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Birth (n=19/6)†</td>
<td>Day 1 (n=22/6)†</td>
</tr>
<tr>
<td>Range‡</td>
<td>3.1-17.2</td>
<td>0.42-19.03</td>
</tr>
<tr>
<td>Mean (SD§)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire Sample</td>
<td>8.1 (4.2)</td>
<td>2.11 (4.35)</td>
</tr>
<tr>
<td>Without FI</td>
<td>8.6 (4.7)</td>
<td>2.47 (4.86)</td>
</tr>
<tr>
<td>With FI</td>
<td>6.7 (1.7)</td>
<td>0.79 (0.30)</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire Sample</td>
<td>7.5</td>
<td>0.95</td>
</tr>
<tr>
<td>Without FI</td>
<td>7.6</td>
<td>0.96</td>
</tr>
<tr>
<td>With FI</td>
<td>7.4</td>
<td>0.81</td>
</tr>
<tr>
<td>p-value¶</td>
<td>0.426</td>
<td>0.117</td>
</tr>
</tbody>
</table>

Interquartile Ranges (25%-50%-25%)

|                  |                    |                                 |                 |                 |
| Without FI       | 5-8-6              | 5-11-6                         | 5-13-4          | 4-15-5          |
| With FI          | 1-5-0              | 2-3-1                          | 2-1-3           | 3-1-2           |
| p-value¶         | 0.238              | 1.0                            | 0.107           | 0.087           |

Interpercentile Ranges (10%-80%-10%)

|                  |                    |                                 |                 |                 |
| Without FI       | 2-15-2             | 0-20-2                         | 1-20-1          | 2-20-2          |
| With FI          | 0-6-0              | 2-4-0                          | 1-4-1           | 1-4-1           |
| p-value¶         | 1.0                | 0.079                          | 0.192           | 0.269           |

* FI was defined a priori as a feeding withheld, discontinued, or decreased at any time because the infant was not tolerating enteral feedings by day 7.
† Specimens available for infants without/with FI
‡ minimum-maximum values
§ Standard Deviation (SD)
¶ Independent Samples Mann-Whitney U test for infants without/with FI, cases excluded test-by-test
¶ Fisher’s Exact Test for infants without/with FI
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