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Urinary Hypoxanthine as a Measure of Increased ATP Utilization in Late Preterm Infants

Megan S. Holden, PhD, **Andrew Hopper, MD**, **Laurel Slater, BSN, RNC**, **Yayesh Asmerom, MS**, **Ijeoma Esiaba, MS**, **Danilo S. Boskovic, PhD**, and **Danilyn M. Angeles, PhD**

Departments of Basic Sciences (MSH, LS, YA, DSB, DA) and Pediatrics (AH), Loma Linda University School of Medicine; Department of Earth and Biological Sciences, Loma Linda University School of Public Health (IE), Loma Linda, California

Abstract

Objective—To examine the effect of neonatal morbidity on ATP breakdown in late preterm infants.

Study Design—Urinary hypoxanthine concentration, a marker of ATP breakdown, was measured from 82 late preterm infants on days of life (DOL) 3 to 6 using high-performance liquid chromatography. Infants were grouped according to the following diagnoses: poor nippling alone (n = 8), poor nippling plus hyperbilirubinemia (n = 21), poor nippling plus early respiratory disease (n = 26), and respiratory disease alone (n = 27).

Results—Neonates with respiratory disease alone had significantly higher urinary hypoxanthine over DOL 3 to 6 when compared with neonates with poor nippling ($P = .020$), poor nippling plus hyperbilirubinemia ($P < .001$), and poor nippling plus early respiratory disease ($P = .017$). Neonates with poor nippling who received respiratory support for 2 to 3 days had significantly higher hypoxanthine compared with infants who received respiratory support for 1 day ($P = .017$) or no days ($P = .007$).

Conclusions—These findings suggest that respiratory disorders significantly increase ATP degradation in late premature infants.

Keywords

ATP; hypoxanthine; late preterm; high-performance liquid chromatography; urine

Infants born before 37 weeks gestation are considered premature and have significantly higher morbidity and mortality rates compared with infants born between 37 and 41 weeks gestation. Much of the research involving premature infants is focused on very low birth weight (<1500 g) or infants less than 33 weeks gestation[1]; however, roughly 70% of all preterm infants are considered late preterm—infants born between $34^0/_7$ and $36^6/_7$ weeks gestation.[2–4] Furthermore, this population is increasing in number. Much of the 30%

Address correspondence to: Danilyn M. Angeles, PhD, RN, Loma Linda University School of Medicine, Loma Linda, CA 92350; dangeles@llu.edu.

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increase in premature births observed between 1981 and 2003 can be accounted for by an increase in the rate of late preterm births.[2,5] Although the rate of late preterm births declined from 9.1% in 2006 to 8.26% in 2011, it is still roughly 13% higher than it was in 1990.[4] Despite the prevalence of this population, they are still relatively understudied compared with more premature or full-term infants.

Late preterm infants tend to resemble full-term infants in size and shape; however, they are physiologically and metabolically immature compared with their term counterparts and are therefore at a higher risk for developing medical complications.[6] Late preterm infants are 4 times more likely to be diagnosed with 1 medical condition and 3.5 times more likely to be diagnosed with 2 or more medical conditions compared with their term counterparts.[7] Some of the most common morbidities experienced by late preterm infants include respiratory disease, temperature instability, hypoglycemia, hyperbilirubinemia, feeding difficulties, and infection.[3,6,8–15] Many of the morbidities associated with late preterm neonates are linked to increased oxygen need or increased metabolic demand (Figure 1).[16–21]

In addition to increased morbidity, late preterm infants have elevated resting energy expenditure compared with term infants.[22] Few studies, if any, have measured total energy expenditure in late preterm infants; however, it is known that total energy expenditure is significantly higher in moderately premature infants, 30 to 33 weeks gestation, compared with term infants.[23] Moreover, respiratory morbidity can further increase energy expenditure in premature infants.[20,24] Despite the prevalence, increased morbidity rates, and higher energy expenditure observed in the late preterm population, a thorough understanding of the metabolic consequences of morbidity in this population is lacking.

The aim of this study was to evaluate, noninvasively, the effects of respiratory disease, poor nippling, and hyperbilirubinemia on ATP utilization in late preterm infants. Urinary hypoxanthine was used to evaluate the effects of morbidities on ATP utilization in late preterm infants for multiple reasons. First, hypoxanthine is a major breakdown product of ATP in most human tissues, with the exception of the intestine and liver, where it is readily oxidized to uric acid by the enzyme xanthine dehydrogenase/xanthine oxidase.[25,26] Second, the normal circulatory concentration of hypoxanthine is relatively low, allowing for the detection of minor changes. Third, as described in the Methods section, urinary hypoxanthine is relatively stable under variable sampling conditions. Last, research demonstrates increased hypoxanthine under conditions of enhanced ATP breakdown like hypoxia or maximal exercise.[27–30]

Methods

Subject Enrollment and Sample Collection

Premature neonates, between $34^{0}/_{7}$ and $36^{6}/_{7}$ weeks gestation, who were admitted to Loma Linda University Children's Hospital neonatal intensive care unit (NICU) were included in this study. The Loma Linda University Institutional Review Board approved study protocol and informed consent documents. Infants with congenital anomalies, congenital heart disease, metabolic acidosis, or more than 2 major morbidities were excluded from the study. These infants were excluded from the study because they (*a*) required surgery during the

study period, (*b*) were known to have medical conditions associated with elevated hypoxanthine, or (*c*) have morbidities that may produce confounding results. Patient diagnoses were determined by a neonatologist (AH).

After parental consent was obtained, investigators collaborated with the clinical staff to obtain urine samples. Urine samples were collected by placing cotton balls over the urethral meatus. Urine-soaked cotton balls were removed from the diaper with every changing and stored at 4°C. Samples that were free of stool were combined into 24-hour aliquots over DOL 3 to 6. Validation studies performed in our laboratory show that hypoxanthine and creatinine remained stable under our sampling and processing conditions (Table 1). Urine was extracted from the cotton using pressure, centrifuged for 10 minutes at 20 000 × *g* and 4°C, filtered through a Millex syringe filter (Low Protein Binding Durapore PVD filter, 0.45 μm, 13 mm; Millipore Corp), and stored at −80°C until analysis.

Subject Classification

Neonates in the poor nippling group were those with oral motor immaturity without signs and symptoms of feeding intolerance or necrotizing enterocolitis. Neonates classified as poor nippling plus hyperbilirubinemia were those with oral motor immaturity who received phototherapy at some point over the first 6 days of life. Neonates classified as poor nippling plus initial respiratory disease were those with oral motor immaturity and evidence of apnea or ventilatory support for 3 days or less. Neonates diagnosed with respiratory disease alone are those with transient tachypnea of the newborn (TTNB) or radiographic evidence of respiratory distress syndrome (RDS) or pneumonia requiring oxygen and/or ventilatory support over at least a successive 4-day period in the first 6 days of life.

Hypoxanthine and Creatinine Quantification

Urinary hypoxanthine and creatinine concentrations were determined using an adaptation of the high-performance liquid chromatography (HPLC) method described by George et al.[31] Briefly, urine samples were thawed and sonicated before 200 μL was transferred to an Eppendorf tube containing 1×10^{-7} mol of 2-aminopurine (internal standard). The samples were then analyzed on an HPLC (Waters 996 PDA, Waters 600 controller, and 717plus autosampler; Millipore Corp) by injecting 35 μL onto a Supelcosil LC-18-S 15 cm × 4.6 mm, 5 μm column (SGE; Austin, TX), with the following isocratic conditions: 10 mM potassium dihydrogen phosphate buffer, pH 4.7, flow rate 1.0 mL/min. Creatinine, hypoxanthine, and 2-aminopurine were quantitated by obtaining peak areas at the appropriate retention times (~3.5, 8, and 13.5 minutes, respectively) and wavelengths (230, 248, and 305 nm, respectively). The area ratios of each compound to 2-aminopurine were determined and converted into concentration using standard curves. Samples were analyzed in triplicate and values with a coefficient of variation less than 10% were included in the final analysis. The limits of detection were 1.58 μM for hypoxanthine and 3.2 μM for creatinine.

Stability of Hypoxanthine and Creatinine

Because urine is stored in the bladder and urine soaked cotton can remain in the diaper for up to 3 hours, we determined the stability of hypoxanthine over time and at varying

temperatures. Urine samples were collected from volunteers and hypoxanthine and creatinine were measured by HPLC in samples subjected to the following conditions: direct analysis, immediate cotton extraction and analysis, room temperature (3 hours, 24 hours, 3 days, and 1 week), 36°C (3 hours or 6 hours), 0°C (24 hours or 1 week), or −80°C (24 hours or 1 week). Hypoxanthine and creatinine as well as the ratio of hypoxanthine/creatinine were found to be stable under all conditions tested, with the mean concentration for each processing condition being within 15% of the mean for fresh urine (Table 1). When stability experiments were repeated, the direction of percent change was variable but never exceeded ±15%.

Statistics

To analyze the data, assumptions of normality and equal variance were assessed. Demographic data for categorical variables were analyzed using χ^2 test. Repeated-measures ANOVA for 1 between-subject factor (diagnosis) and 1 within-subject factor (day of life) were assessed to evaluate the effect of the morbidity on urinary hypoxanthine concentrations over time. Repeated-measures ANOVA for 1 between-subject factor (respiratory support) and 1 within-subject factor (day of life) were assessed to evaluate the effect of the mode of respiratory support on urinary hypoxanthine concentrations over time in infants diagnosed as poor nippling plus initial respiratory support. All statistical analyses were performed using SPSS Statistics for Windows Version 21. Differences were considered significant at $P < .05$.

Results

Subject Enrollment

A total of 82 infants born between $34^0/_7$ and $36^6/_7$ weeks gestation were enrolled in this study and had adequate urine collected for assay. Of the infants enrolled in this study, 8 were classified as having poor nippling alone, 21 as having poor nippling plus hyperbilirubinemia, 26 as having poor nippling plus early respiratory disease, and 27 as having respiratory disease alone.

Subject Demographics

The subjects were heterogeneous for estimated gestational age (EGA), birth weight, length of NICU stay, 5-minute APGAR, race, mode of delivery, days on total parenteral nutrition (TPN), and days until oral/enteral feeds (Tables 2 and 3). Infants who were diagnosed with poor nippling plus hyperbilirubinemia had significantly lower estimated gestational age ($P < .01$) and birth weight ($P < .01$) compared with infants diagnosed with respiratory disease alone. Infants diagnosed with respiratory disease alone had significantly longer NICU stay compared with infants diagnosed with poor nippling plus hyperbilirubinemia ($P < .01$) and infants diagnosed with poor nippling plus early respiratory disease ($P < .05$). Infants diagnosed with respiratory disease, however, had significantly lower 5-minute APGAR scores compared with infants diagnosed with poor nippling plus hyperbilirubinemia ($P < .01$) and those diagnosed with poor nippling plus early respiratory disease ($P < .01$). Additionally, infants diagnosed with respiratory disease alone had significantly longer days on TPN and days until oral/enteral feeding compared with infants diagnosed with poor nippling ($P < .01$ for days on TPN, $P = .043$ for days until oral/enteral feeding), poor

nippling plus hyperbilirubinemia ($P < .01$ for days on TPN, $P = .002$ for days until oral/enteral feeding), and infants diagnosed with poor nippling plus early respiratory disease ($P < .01$ for days on TPN, $P = .003$ for days until oral/enteral feeding).

No significant differences were observed among groups for gender, 1-minute APGAR scores, dietary additives over the course of the NICU stay, or dietary additives over the study period. We noted, however, that with the exception of poor nippling plus hyperbilirubinemia, there was a trend for more of the infants in each group to have been born via cesarean section ($P = .049$). There was a significantly higher number of white infants enrolled in the study compared with Hispanic, African American, or other ($P = .024$). Last, the nutritional intake varied significantly over every day of the study period (χ^2, $P < .001$). No infants in the study suffered from asphyxia, intraventricular hemorrhage, or patent ductus arteriosus.

Urinary Hypoxanthine and Subject Diagnoses

Repeated-measures analysis of urinary hypoxanthine over DOL 3 to 6 revealed significantly higher hypoxanthine over time for infants diagnosed as having respiratory disease alone compared with infants diagnosed with poor nippling ($P = .020$), poor nippling plus hyperbilirubinemia ($P < .001$), and poor nippling plus early respiratory disease ($P = .017$; Figure 2). There was a trend, although not significant, for infants with poor nippling plus early respiratory disease to have higher urinary hypoxanthine on DOL 3 to 5 when compared with infants diagnosed with poor nippling alone and those diagnosed with poor nippling plus hyperbilirubinemia. However, we found that hypoxanthine concentration on DOL 3 to 6 in neonates with poor nippling plus early respiratory disease was significantly higher for infants who required respiratory support for the first 2 to 3 days of life when compared with infants who received no respiratory support ($P = .007$) or were on respiratory support for only 1 day ($P = .017$; Figure 3A). When we restricted our analysis to only nasal respiratory support, infants who received respiratory support for more than 2 days had significantly higher hypoxanthine on DOL 3 to 6 compared with infants who were on room air ($P = .014$; Figure 3B).

Discussion

Our preliminary work shows that hypoxanthine and creatinine are stable in urine and can be used to evaluate the effect of morbidity on ATP metabolism in late preterm infants. More important, we found that late preterm infants diagnosed with respiratory disease alone had significantly higher urinary hypoxanthine concentrations over time when compared with infants diagnosed with poor nippling alone, poor nippling plus hyperbilirubinemia, or poor nippling plus early respiratory disease. This indicates that, within this late preterm population, specific disorders can change ATP metabolism and induce enhanced purine breakdown.

We further observed a trend for infants diagnosed with poor nippling plus early respiratory disease to have higher urinary hypoxanthine compared with infants diagnosed with poor nippling alone or poor nippling plus hyperbilirubinemia over day 3 through 5 of life. Although the difference was not significant, we hypothesized that the higher urinary

hypoxanthine was most likely a reflection of early respiratory support in this population. On investigating the effects of respiratory support within the poor nippling plus early respiratory disease population, we found that infants who received 2 to 3 days of respiratory support had significantly higher urinary hypoxanthine compared with infants who received only 1 day of respiratory support or were on room air. This further indicates enhanced ATP breakdown as a result of respiratory issues, even within this subpopulation.

It is well documented that respiratory issues are common in the late preterm infants.[3,32,33] This population is known to have a higher incidence of transient tachypnea of the newborn, respiratory distress syndrome, persistent pulmonary hypertension of the newborn, apnea, and respiratory failure.[13,34–37] Khashu et al report that late preterm infants have 4.4 times the relative risk of respiratory morbidity than term infants.[38] In addition, due to their prematurity, late preterm infants have lower energy stores and higher energy needs at birth compared with term infants.[14,22,23,39] The available energy stores can be further decreased when the newborn is challenged by additional stressors, such as respiratory disease.[18,20,24,40] Due to the increased energy needs in this population, particularly when combined with respiratory disease, it may be prudent to adjust the diet of late preterm infants so these energy demands are more adequately met.

Studies involving very low birth weight infants indicate that early and aggressive introduction of total parenteral nutrition and enteral feeding can result in better growth, reduced nutritional deficits, enhanced achievement of full enteral feedings, decreased morbidity, and improved neurodevelopment.[41–43] Furthermore, it was reported that simultaneous low-dose amino acid infusion and breast milk feeding reduces the time of mechanical ventilation in premature infants with respiratory distress syndrome, possibly by ensuring improved energy support to aid respiratory muscle strength.[44,45] These studies highlight the advantages of optimizing nutritional support in premature neonates through the implementation of dietary additives. More important, these data suggest that adjusting the diet of late preterm infants to resemble the higher calorie, lipid, and protein diet administered to more premature infants may provide better nutritional support for this population to combat respiratory challenges. Additionally, the utilization of dietary additives such as human milk fortifier, medium-chain triglyceride oil, or whey protein may help supplement the energy needs of this population. Further research is needed to determine if optimizing daily energy and protein intake can reduce ATP breakdown in late preterm infants.

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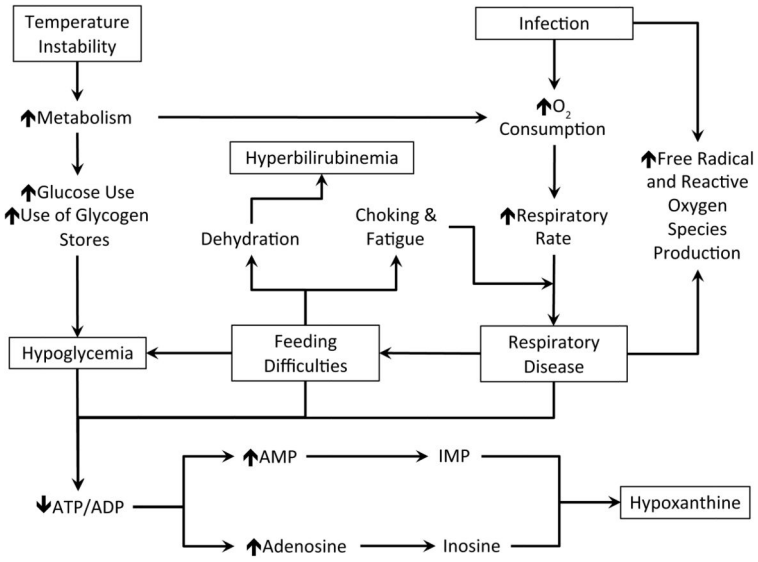
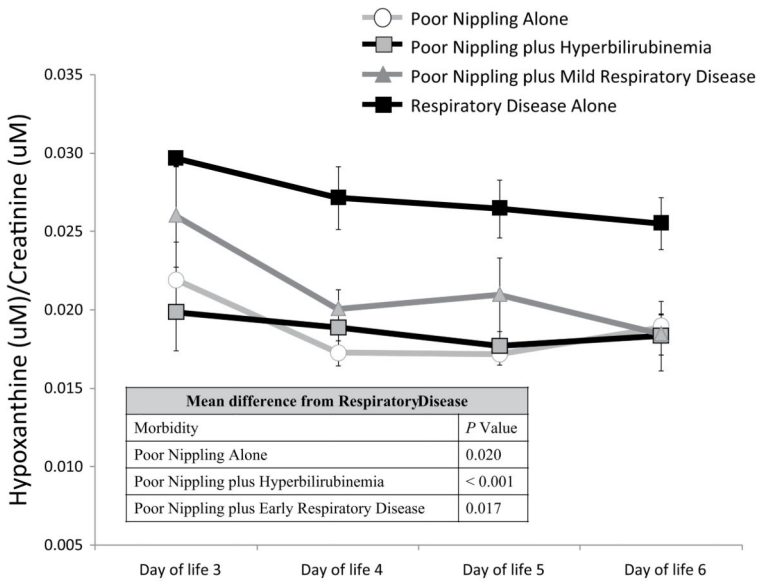


Figure 1.

Pathway Depicting the Interrelationship Between Common Late Preterm Morbidities and Hypoxanthine, a Marker of ATP Breakdown.

Abbreviations: O_2, oxygen; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate.



Mean difference from RespiratoryDisease	
Morbidity	*P* Value
Poor Nippling Alone	0.020
Poor Nippling plus Hyperbilirubinemia	< 0.001
Poor Nippling plus Early Respiratory Disease	0.017

Figure 2.

Difference Between Days of Life 3 to 6 Urinary Hypoxanthine for Late Preterm Infants Diagnosed With Poor Nippling Alone (n = 8), Poor Nippling Plus Hyperbilirubinemia (n = 21), Poor Nippling Plus Early Respiratory Disease (n = 26), or Respiratory Disease Alone (n = 27).

P values represent significant differences between the indicated group and infants with respiratory disease over time.

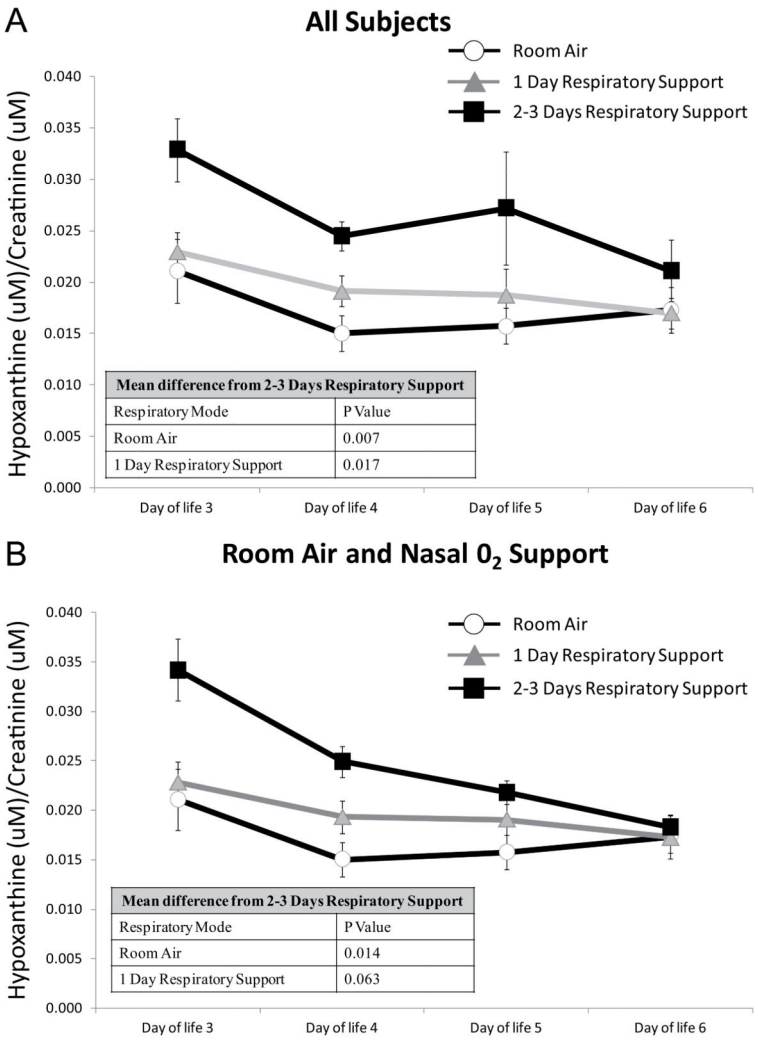


Figure 3.

Effect of Number of Days of Respiratory Support on Urinary Hypoxanthine in Infants Diagnosed as Poor Nippling Plus Early Respiratory Disease.

(A) Infants on room air, receiving nasal oxygen support (high flow nasal cannula, nasal continuous positive airway pressure, or nasal intermittent positive pressure ventilation) or synchronized intermittent mandatory ventilation. (B) Infants on room air or receiving nasal oxygen support (high flow nasal cannula, nasal continuous positive airway pressure, or nasal intermittent positive pressure ventilation) only. *P* values represent significant differences between the indicated group and infants on 2 to 3 days respiratory support over time.

Table 1

Representative Time and Temperature Stability of Hypoxanthine and Creatinine.

	Hypoxanthine	Creatinine	Hypoxanthine/Creatinine
Immediately after collection			
Straight urine	113.52 (0.00)	3190.16 (0.00)	0.0356 (0.00)
Extracted from cotton	115.42 (1.67)	3165.66 (−0.78)	0.0365 (2.47)
Room temperature			
3 hours	118.11 (4.04)	3167.73 (−0.70)	0.0373 (4.48)
24 hours	115.41 (1.66)	3051.40 (−4.35)	0.0374 (4.99)
3 days	113.90 (0.33)	3270.32 (2.51)	0.0328 (−2.13)
1 week	105.76 (−6.84)	3173.32 (−0.53)	0.0333 (−6.34)
37°C			
3 hours	114.29 (0.67)	3189.22 (−0.03)	0.0358 (0.70)
6 hours	115.75 (1.96)	3136.34 (−1.69)	0.0369 (3.71)
0°C			
24 hours	118.24 (3.98)	3097.36 (−2.91)	0.0381 (7.10)
1 week	102.24 (−9.94)	3161.66 (−0.89)	0.0323 (−9.12)
−80°C			
24 hours	117.28 (3.31)	3098.28 (−2.88)	0.0379 (6.38)
1 week	102.08 (−10.08)	3126.06 (−2.01)	0.0327 (−8.24)

[a]Data are mean (% change from fresh urine). Data are representative of a single urine sample. The experiment was performed on 3 separate urine samples with similar results.

Table 2

Subject Demographics.[a]

	Poor Nippling (n = 8)	Poor Nippling Plus Hyperbilirubinemia (n = 21)	Poor Nippling Plus Early Respiratory Disease (n = 26)	Respiratory Disease (n = 27)	*P* Value
EGA (weeks)	35.0 ± 0.6	34.4 ± 0.4	34.9 ± 0.7	35.1 ± 0.7	.001[b]
Birth weight (g)	2306.1 ± 854.2	2117.8 ± 460.5	2322.5 ± 428.5	2650.5 ± 489.0	.005[b]
Length of stay (days)	16.4 ± 5.8	15.1 ± 5.7	17.4 ± 7.8	28.7 ± 21.9	.004[b,c]
APGAR, 1 minute	7 ± 2	7 ± 2	7 ± 2	6 ± 3	.363
APGAR, 5 minute	8 ± 1	9 ± 1	8 ± 1	7 ±2	.000[b,c]
Gender, n (%)					.596[d]
Male	6 (75%)	12 (57.1%0	15(57.7%)	13 (48.1%)	
Female	2 (625%)	9 (42.9%)	11 (42.3%)	14 (51.9%)	
Race, n (%)					.024[d]
White	2 (25.0%)	18 (85.7%)	16 (61.5%)	19 (70.4%)	
Hispanic	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (7.4%)	
African American	0 (0.0%)	2 (9.5%)	3 (11.5%)	2 (7.4%)	
Other	6 (75.0%)	1 (4.8%)	7 (27.0%)	4 (14.8%)	
Mode of delivery, n (%)					.049[d]
Vaginal	2 (25%)	14 (66.7%)	12 (46.2%)	8 (29.6%)	
C/S	6 (75%)	7 (33.3%)	14 (53.8%)	19 (70.4%)	

Abbreviations: EGA, estimated gestational age; C/S, Cesarean section.

[a] Data are mean ± standard deviation.

[b] Poor nippling plus Hyperbilirubinemia significantly different than Respiratory Disease.

[c] Poor nippling plus Early Respiratory Disease significantly different than Respiratory Disease (one-way ANOVA).

[d] Chi-square test.

Table 3

Nutritional Intake and Dietary Additives.

	Poor Nippling (n = 8)	Poor Nippling Plus Hyperbilirubinemia (n = 21)	Poor Nippling Plus Early Respiratory Disease (n = 26)	Respiratory Disease (n = 27)	*P* Value
Days on TPN	2.4 ± 1.5	3.0 ± 2.4	3.5 ± 1.8	15.0 ± 13.5	<.001[a]
Days until oral/enteral feeds	0.1 ± 0.3	0.3 ± 0.5	1.0 ± 0.7	8.2 ± 12.5	<.001[a]
Additives received during NICU stay					.280[b]
None	3 (37.5%)	10 (47.6%)	14 (53.8%)	12 (44.4%)	
ECP	3 (37.5%)	2 (9.5%)	5 (19.2%)	6 (22.2%)	
HMF	1 (12.5%)	1 (4.8%)	1 (3.8%)	1 (3.7%)	
ECP & Prosobee lipil with Fe	1 (12.5%)				
HMF & ECP		2 (9.5%)	3 (11.5%)	4 (14.8%)	
HMF, ECP, & LHMF		3 (14.3%)	2 (7.7%)	1 (3.7%)	
HMF & LHMF		3 (14.3%)			
HMF & Bene protein			1 (3.8%)		
HMF, ECP, MCT oil and pregestemil				1 (3.7%)	
Pregestemil				1 (3.7%)	
ECP & Enfamil AR				1 (3.7%)	
Additives received during study period					.277[b]
None	6 (75.0%)	16 (76.2%)	24 (92.3%)	27 (100.0%)	
ECP	1 (12.5%)	1 (4.8%)	2 (7.7%)	0 (0.0%)	
HMF	1 (12.5%)	1 (4.8%)	0 (0.0%)	0 (0.0%)	
LHMF	0 (0.0%)	1 (4.8%)	0 (0.0%)	0 (0.0%)	
HMF & ECP	0 (0.0%)	1 (4.8%)	0 (0.0%)	0 (0.0%)	
LHMF & HMF	0 (0.0%)	1 (4.8%)	0 (0.0%)	0 (0.0%)	
Day 3 nutritional intake, n (%)					<.001[a]
TPN	0 (0.0%)	0 (0.0%)	1 (3.8%)	21 (77.8%)	
Formula (F)	1 (12.5%)	3 (14.3%)	2 (7.7%)	0 (0.0%)	
Breast milk (BM)	0 (0.0%)	2 (9.5%)	1 (3.8%)	0 (0.0%)	
BM/F	4 (50.0%)	3 (14.3%)	4 (15.4%)	1 (3.7%)	

	Poor Nippling (n = 8)	Poor Nippling Plus Hyperbilirubinemia (n = 21)	Poor Nippling Plus Early Respiratory Disease (n = 26)	Respiratory Disease (n = 27)	*P* Value
TPN/F	3 (37.5%)	2 (9.5%)	11 (42.3%)	3 (11.1%)	
TPN/BM	0 (0.0%)	7 (33.3%)	4 (42.3%)	0 (0.0%)	
TPN/BM/F	0 (0.0%)	4 (10.9%)	3 (11.5%)	2 (7.4%)	
Day 4 nutritional intake, n (%)					<.001[b]
TPN	0 (0.0%)	0 (0.0%)	0 (0.0%)	14 (51.9%)	
Formula (F)	2 (25.0%)	3 (14.3%)	5 (19.2%)	0 (0.0%)	
Breast milk (BM)	0 (0.0%)	5 (23.8%)	2 (7.7%)	1 (3.7%)	
BM/F	3 (37.5%)	5 (23.8%)	6 (23.1%)	0 (0.0%)	
TPN/F	1 (12.5%)	1 (4.8%)	3 (11.5%)	3 (11.1%)	
TPN/BM	0 (0.0%)	2 (9.5%)	5 (19.2%)	3 (11.1%)	
TPN/BM/F	2 (25.0%)	5 (23.8%)	5 (19.2%)	6 (22.2%)	
Day 5 Nutritional Intake, n (%)					<.001[b]
TPN	0 (0.0%)	0 (0.0%)	0 (0.0%)	13 (48.1%)	
Formula (F)	1 (12.5%)	3 (14.3%)	5 (19.2%)	0 (0.0%)	
Breast milk (BM)	0 (0.0%)	6 (28.6%)	4 (15.4%)	1 (3.7%)	
BM/F	7 (87.5%)	6 (28.6%)	11 (42.3%)	1 (3.7%)	
TPN/F	0 (0.0%)	1 (4.8%)	2 (7.7%)	3 (11.1%)	
TPN/BM	0 (0.0%)	4 (19.0%)	2 (7.7%)	3 (11.1%)	
TPN/BM/F	0 (0.0%)	1 (4.8%)	2 (7.7%)	6 (22.2%)	
Day 6 nutritional intake, n (%)					<.001[b]
TPN	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (40.7%)	
Formula (F)	1 (12.5%)	2 (9.5%)	7 (26.9%)	1 (3.7%)	
Breast milk (BM)	0 (0.0%)	8 (38.1%)	6 (23.1%)	1 (3.7%)	
BM/F	7 (87.5%)	8 (38.1%)	9 (34.6%)	1 (3.7%)	
TPN/F	0 (0.0%)	1 (4.8%)	1 (3.8%)	2 (7.4%)	
TPN/BM	0 (0.0%)	1 (4.8%)	0 (0.0%)	6 (22.2%)	
TPN/BM/F	0 (0.0%)	1 (4.8%)	3 (11.5%)	5 (18.5%)	

Abbreviations: ECP, Enfacare powder; HMF, human milk fortifier; LHMF, liquid human milk fortifier; MCT oil, medium-chain triglyceride oil; EMAR, Enfamil AR; TPN, total parenteral nutrition; F, formula; BM, breast milk.

[a] Significantly different from Respiratory Disease; one-way ANOVA with Bonferroni correction.

[b] Chi-square test.