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A Comparison of Three Vitamin D Dosing Regimens in Extremely Preterm Infants: a randomized controlled trial

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Abstract

Objective—To determine the optimal dose of vitamin D supplementation to achieve biochemical vitamin D sufficiency in extremely low gestational age newborns in a masked randomized controlled trial.

Study design—100 infants 23^{0/7} to 27^{6/7} weeks gestation were randomized to vitamin D intakes of placebo (n=36), 200 IU (n=34), and 800 IU/day (n=30) (approximating 200, 400, or 1000 IU/day, respectively, when vitamin D routinely included in parenteral or enteral nutrition is included). The primary outcomes were serum 25 (OH) vitamin D concentrations on postnatal day 28 and the number of days alive and off respiratory support in the first 28 days.

Results—At birth, 67% of infants had 25(OH) vitamin D < 20 ng/mL suggesting biochemical vitamin D deficiency. Vitamin D concentrations on day 28 were (Median [25th–75th centiles], ng/mL): Placebo: 22 [13–47], 200 IU: 39 [26–57], 800 IU: 84.5 [52–99], $p < 0.001$. There were no differences in days alive and off respiratory support (Median (25th–75th centiles), days: Placebo: 1 (0–11), 200 IU: 0 (0–8), 800 IU: 0.5 (0–22), $p=0.63$), or other respiratory outcomes among groups.

Conclusion—At birth, most extremely preterm infants have biochemical vitamin D deficiency. This biochemical deficiency is reduced on day 28 by supplementation with 200 IU/day and prevented by 800 IU/day. Larger trials are required to determine if resolution of biochemical vitamin D deficiency improves clinical outcomes. ClinicalTrials.gov: NCT01600430

Trial registration—ClinicalTrials.gov: NCT01600430

Vitamin D is a fat soluble vitamin involved in multiple physiological processes. Developmental processes regulated by vitamin D include lung development¹ and maturation of the immune system.² As vitamin D is mostly transferred to the fetus during the third trimester,³ preterm infants are born with lower vitamin D stores. Extremely low gestational

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age newborns (ELGAN) are at high risk of mortality and multiple morbidities. It is not known if vitamin D insufficiency contributes to mortality or morbidity in these infants.

The American Academy of Pediatrics (AAP) has recommended a dose of 200–400 IU of vitamin D per day for preterm infants, and the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommends 800–1000 IU. However, evidence is lacking for the magnitude of improvement in serum vitamin D concentrations or clinical outcomes in ELGAN with either target range of vitamin D supplementation. In addition, as vitamin D is critical for many essential physiological processes, it is possible that higher than normal (supra-physiological) but non-toxic concentrations of vitamin D may benefit this vulnerable population similar to vitamin A supplementation.

This was a pilot trial to identify the optimal dose of vitamin D before embarking on a large multicenter trial using the optimal dose for evaluating clinical outcomes. Our objective was to determine the optimal dose of vitamin D supplementation in ELGAN using a masked randomized controlled trial in which infants were randomized to vitamin D intakes of placebo, 200 IU, and 800 IU/day.

Methods

This multiple-dose, randomized, double-blinded, placebo-controlled trial was approved by the Institutional Review Board at the University of Alabama at Birmingham. Entry criteria were inborn infants with gestational age between 23–27 completed weeks admitted to the neonatal intensive care unit at the University of Alabama Hospital. Exclusion criteria included major congenital or chromosomal anomalies, moribund infant with low likelihood of survival as outborn infants.

Parents or guardians were approached by research staff soon after birth for informed consent. After consent, infants were randomly allocated by the research pharmacy staff using computer-generated stratified randomization codes to one of three groups: (1) placebo (intake of approximately 200 IU/day in parenteral nutrition or feeds), (2) 200 IU supplement (daily intake of approximately 400 IU due to 200 IU supplement + 200 IU in parenteral nutrition or feeds), or (3) 800 IU supplement (daily intake of approximately 1000 IU due to 800 IU supplement + 200 IU in parenteral nutrition or feeds). The 200 IU in enteral or parenteral nutrition is an estimate, based on the daily intake of feeds and TPN by infants in our NICU over the first month. As fortified human milk has approximately the same amount of vitamin D as preterm formula, and fortification is started early in our unit, no adjustments were made for daily variations in intake or for unfortified human milk.

Infants received 0.25 mL of study drug (either vitamin D (Enfamil D-Vi-Sol™) or normal saline (placebo)) every 6 hours through the orogastric tube just before feeds. The medication was dispensed by a research pharmacist in an amber syringe to mask the caregivers. Infants in the placebo group received only saline, and infants in the 200 IU/day group received one dose of vitamin D and three doses of saline and infants in the 800 IU/day group received four doses of vitamin D and none of saline. Each infant was randomized during the first

seven days after birth and within 72 hours after initiating enteral feeds. Infants were monitored before every feed for feeding intolerance (abdominal distension or increased gastric residuals). Serum electrolytes, blood gases, and ionized calcium were measured every day while on parenteral nutrition and twice weekly while on enteral feeds during the first month. The intervention was continued until postnatal day 28. Infants were discontinued from study drug administration if they developed necrotizing enterocolitis Bell's stage II or greater, spontaneous intestinal perforation, or if feeds were stopped for more than 24 hrs by the clinical team. Clinical data were collected by a trained research coordinator.

For the estimation of serum 25(OH) vitamin D concentration, whole blood (0.2 mL to 0.25 mL) was collected at birth (cord blood) and at the time of routine sampling on day 14 ($\pm$ 2 days), and day 28 of life ($\pm$ 3 days) and centrifuged to collect serum. The samples were stored in -80°C for later analysis. Analysis of the serum 25(OH) vitamin D concentrations was with an enzyme-linked immunosorbent assay (ELISA; Eagle Biosciences, Inc, Nashua, NH) with a dynamic range of 4–120 ng/mL, 100% cross-reactivity between 25(OH)-vitamin D3 and D2, $<10\%$ CV for intra-assay and inter-assay variation, and normal range of 30–70 ng/mL for both adults and older children.

Primary Outcomes

The pharmacological outcome was the serum concentration of 25(OH) vitamin D at postnatal day 28, and the primary clinical outcome was the total number of days alive and off respiratory support in the first 28 days. Respiratory support was defined as the need for supplemental oxygen and/or positive pressure ventilation to maintain normal target oxygen saturations (88 – 95%). The number of days on supplemental oxygen is a surrogate of BPD and is directly related with chronic lung disease in preterm infants.

Secondary Outcomes

The secondary outcomes include other respiratory clinical outcomes including retinopathy of prematurity (stages I–III, plus disease, or disease requiring treatment), proven necrotizing enterocolitis (Bell's Stage II and III), and intraventricular hemorrhage (Grade I–IV), confirmed sepsis and/or meningitis, nutrition, hospitalization, and death. These clinical outcomes were evaluated during the entire initial hospitalization, and the feeding outcomes were cross-sectional at day 14 and day 28 (as most infants are on full feeds by three weeks of age).

Sample Size

A planned sample size of 31 patients per group was estimated to be sufficient to detect a 40% change in serum 25(OH) vitamin D concentration on study day 28 (the primary outcome) with 80% power at an alpha of 0.05 by ANOVA, with the assumption that the standard deviation would be 50% of the mean. A sample size of 20 per group would be sufficient to determine if a difference of 50% in vitamin D concentrations on postnatal day 28 was significant. The total sample size was increased to 100 to account for some attrition in sample size due to death or inadequate sample for analysis. The trial was not powered for clinical outcomes.

A one-way analysis of variance (ANOVA) was used for calculating statistical significance of continuous variables, with the Tukey multiple-comparison test used if $p < 0.05$ by ANOVA. Analysis was by “intention-to-treat”. The chi-square test was used for categorical variables. SigmaPlot Version 12 (Systat Software Inc., San Jose, CA) was used for analysis.

Results

Between June 2012 and October 2014, 100 infants with birth weights ranging from 360 g to 1290 g (mean 770 ± 215 g) were randomized to three vitamin D daily intake groups: 36 infants to the placebo group, 34 to the 200 IU/day, and 30 to the 800 IU/day. Of the 100 infants, 37 did not complete the study: 15 due to death, 12 developed necrotizing enterocolitis or spontaneous intestinal perforation, and 12 were not fed for more than 24 hours (Figure 1; available at www.jpeds.com). Serum 25(OH) vitamin D concentrations were not available for 15, 22 (7 due to death), and 30 (2 due to death) infants at birth, day 14, and 28 respectively. Maternal characteristics did not differ among groups and infant birth weights were not statistically different (Table I). There was a trend towards lower Apgar scores (< 3 at 5 minutes) in the 200 IU/day group. The median age at commencement of feeds was 3 days in this population, and vitamin D supplementation was started on median day 5 in all groups.

At birth, 67% (57/85) of the infants had 25(OH) vitamin D concentrations < 20 ng/mL, with an average concentration of 17.5 ng/mL (SD 11; 25th–75th centiles: 10–21; Range 3–59) (Figure 2). At postnatal day 14, the placebo and 200 IU groups had serum 25(OH) vitamin D concentrations that were less than that in the 800 IU group (ng/mL; Median (25th–75th centiles): placebo group: 23 (16–30) and 200 IU group: 29 (23–39) vs. 800 IU group: 52 (41–64), $p < 0.05$). At postnatal day 28, the 800 IU/day group had the highest average serum 25(OH) vitamin D concentration (ng/mL; Median (25th–75th centiles): 85 (52–99) vs. placebo group: 22 (13–47) and 200 IU group: 39 (26–57), $p < 0.05$), and the majority of infants in the 800 IU/day group had 25 (OH) vitamin D above the desired range of 20–60 ng/mL. At this time point, the percentage of infants in each group with biochemical vitamin D deficiency (< 20 ng/mL) was 41% in the placebo group, 16% in the 200 IU group, and 0% in the 800 IU group ($p = 0.2$). Calcium and phosphate levels were from laboratory studies for clinical purposes closest to postnatal day 28 in 69 and 57 infants, respectively. The mean calcium level was 9.8 mg/dL (SD 0.9) for all infants (n; mean (SD): 200 IU: 9.6 (0.9); 400 IU: 10 (1.2); 1000 IU: 9.9 (0.8)) with a mean phosphate level of 4.5 (SD 1.7) overall (n; mean (SD): 200 IU: 4.4 (1.9); 400 IU: 4.9 (1.8); 1000 IU: 4.5 (1.3)).

There were no differences in days alive and off respiratory support as days alive and off mechanical ventilation in the first 28 days. The rates of early-onset sepsis among the three groups were similar (Table II). The rate of late-onset sepsis in the 800 IU/day vitamin D group was not statistically different compared with the other two groups. Death at 36w PMA was increased and hospitalization at 36 w PMA was decreased in the 200 IU/day group, although mortality or bronchopulmonary dysplasia (BPD) by 36 weeks PMA did not differ among the groups. There were fifteen deaths overall, with two, nine, and four occurring in the placebo, 200 IU/day, and 800 IU/day groups, respectively. Of these, seven deaths occurred during the first 14 days, two between day 15 and 28, and the remaining six

after 28 days. The most common cause of death was respiratory distress syndrome (7), four with associated pulmonary hemorrhage and one with severe intraventricular hemorrhage. Bronchopulmonary dysplasia was the cause of death in five infants. The cause of death in the other four infants was necrotizing enterocolitis, herpes simplex viral meningitis, pulmonary vein stenosis, and sepsis. Other clinical outcomes during the entire hospitalization, including retinopathy of prematurity (stages I–III, plus disease, or disease requiring treatment), rate of weight gain, proven necrotizing enterocolitis (Bell's Stage II and III), and intraventricular hemorrhage (grade I–IV) did not differ between the groups.

Discussion

The majority of ELGAN had biochemical evidence of vitamin D deficiency at birth. The AAP recommended intake of 400 IU/day (200 IU/day supplement + 200 IU in parenteral and enteral nutrition) did not significantly increase the serum 25(OH) vitamin D concentration at 14 days of age although most infants had adequate concentrations by 28 days of age. Use of the ESPGHAN recommended intake of 800–1000 IU/day led to adequate concentrations at 14 days of age but higher than desired concentrations of serum 25(OH) vitamin D at 28 days of age without observed toxicity. No significant differences were noted in clinical outcomes among the groups, although the trial was not powered to determine an effect on clinical outcomes.

The strengths of this study, a double-blinded, randomized, multi-dose trial evaluating the effect of vitamin D supplementation in ELGAN, include masking of the intervention and assessment of outcomes, the multi-dose regimen used, and the early initiation of enteral vitamin D supplementation within the first week after birth. Data were analyzed by intent to treat, which included infants who were discontinued from study intervention because of feeding intolerance. The sample size, which was larger than previous studies of vitamin D supplementation in preterm infants,² was sufficient to determine pharmacologic outcome. Infants in this trial were also more preterm and at lower birth weight and at higher risk of mortality and morbidity as compared with previous trials. Limitations were that adequate blood samples were not collected on all infants as sampling was limited to clinical indications, and these missing values limit the validity of the results to some extent. The sample size was adequate for the biochemical outcome but was inadequate for assessment of clinical outcomes as well as differences in baseline patient characteristics between infant groups. Additionally, bone mineral content or calcium and phosphate intake were not measured in this study. It is important to note that multiple methods are used for quantitative analysis of 25 (OH) vitamin D concentrations (ELISA, Radio-Immuno Assay, High-Performance Liquid Chromatography etc), and differences in methods of analysis may lead to variation in results.

Preterm infants are at higher risk of lower 25 (OH) vitamin D levels, compared with more mature infants.² Preterm infants with lower 25(OH) vitamin D are also at higher risk of acute respiratory morbidity³ and BPD. ELGAN are at high risk for low serum vitamin D concentrations not only because transfer of vitamin D occurs later in gestation,⁴ but perhaps also because of maternal insufficiency. In a study of 200 pregnant African American women, 29.2% had serum 25(OH) vitamin D concentrations of < 15 ng/mL immediately prior to

delivery, and 47% of their infants had 25(OH) vitamin D concentrations at birth of < 15 ng/mL. This vitamin D deficiency may have important consequences in ELGAN. Vitamin D is necessary for bone health and it also is important for the saccular and alveolar stages of lung development, the dysregulation of which contributes to bronchopulmonary dysplasia in ELGAN. Pups of vitamin D deficient mice have reduced lung volumes and fewer alveoli compared with pups of vitamin D sufficient dams, a phenotype similar to the “new BPD.”

Observational studies have suggested that current nutritional practices are effective in reducing biochemical evidence of vitamin D deficiency in larger preterm infants, but there has been a lack of data in ELGAN. Four trials compared the vitamin D status in premature infants following random assignment to vitamin D supplementation.²⁷ Pittard et al evaluated 27 low-birth-weight and 25 full-term well infants from birth to 16 weeks after delivery and found that 400 IU of vitamin D was a sufficient daily intake for both premature and full-term well infants. In the study by Koo et al, who evaluated supplementation with three dose regimens (200, 400, and 800 IU/day) of vitamin D in very low birth weight infants, infants in the higher dose group had a 25(OH) vitamin D achieved concentration of only around 31 ng/mL. Koo et al found growth and metabolic outcomes, duration of mechanical ventilation (2–3 days), and oxygen supplementation (8–9 days) did not differ between groups. It is not clear why ELGAN in our trial receiving 800 IU/day of vitamin D had 25(OH) vitamin D concentrations substantially higher than the VLBW infants receiving a similar dose in the study by Koo et al. It is possible that a standard weight-independent dose for all premature infants may not be appropriate, and a weight-based dosing (IU/kg) may improve vitamin D levels. However, we did not notice an inverse correlation between the serum vitamin D concentration and birth weight (data not shown) suggesting that smaller infants are not more likely to have higher concentrations with a given magnitude of supplementation. It is possible that differences in study participants (infants in our trial were substantially smaller and more immature), in form of supplementation (infants in the study by Koo et al had formula supplemented with vitamin D), or timing (were enrolled when clinically stable and recovering from respiratory illness and on feeds of at least 75 kcal/kg/day) may account for these differences. Two small clinical trials evaluating dose-response effect of oral vitamin D after establishment of full enteral feeding had no significant differences in vitamin D status after supplementation in preterm infants. Hanson et al randomized formula-fed late preterm infants born 32 weeks gestational age to receive 400 IU/day or placebo, and both the placebo and supplemented groups had levels considered insufficient at discharge. Backstrom et al studied 39 infants < 33 weeks of gestational age who were randomly allocated to receive vitamin D 200 IU/kg of body weight/day up to a maximum of 400 IU/day or 960 IU/day until 3 months old. Preterm infants in the low vitamin D group received assisted ventilation longer and had a trend for longer duration of oxygen supplementation. These previous studies evaluated VLBW infants, and not ELGAN who are at much higher risk of respiratory morbidity and mortality, and may have much lower vitamin D stores due to birth at an earlier gestational age and a greater impairment in nutrition.

In our study, vitamin D supplementation at 800 IU/day led to many infants with 25(OH) vitamin D concentrations beyond those generally considered optimal (>60ng/mL). Vitamin D toxicity, usually presenting as emesis, paresthesias, and hypercalcemia, is generally not seen until levels of 250–300 ng/mL are reached. Higher 25(OH) vitamin D concentrations

are generally well tolerated as its active metabolite (1,25 (OH) vitamin D) is tightly regulated. We did not notice any evidence of biochemical (vitamin D concentrations > 200 ng/mL) or overt clinical toxicity (higher serum calcium or lower phosphate) in the higher dose group, and in fact observed a trend toward fewer infants with late onset sepsis in this group, as well as a trend for fewer infants on oxygen at 28 days or receiving steroids for BPD. Similar to vitamin A supplementation, where a large randomized controlled trial used higher doses than those recommended in pilot studies and found statistical improvement in chronic lung disease in ELGAN. It is plausible that higher doses of vitamin D may be well tolerated in premature infants, and in fact may improve clinical outcomes. The higher dose given early corrects the biochemical vitamin D deficiency by two weeks of age, but leads to higher than “normal” concentrations by four weeks.

The results of this pilot trial suggest that initial higher doses (800 IU/day) for a shorter duration (1–2 weeks) to restore serum vitamin D concentrations to the normal range followed by a lower dosage (200 IU/day) may be an ideal dosing regimen for ELGAN. A large randomized trial evaluating multiple important clinical outcomes is required to determine if such dosing of vitamin D decreases the risk of lung disease or late onset sepsis in the extremely preterm infant.

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Abbreviations

ELGAN	Extremely low gestational age newborns
AAP	American Academy of Pediatrics
ESPGHAN	European Society for Paediatric Gastroenterology Hepatology and Nutrition
BPD	Bronchopulmonary dysplasia

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Figure 1. Revised template of the CONSORT diagram showing the flow of participants through each stage of a randomized trial

CONSORT diagram illustrates enrollment, allocation, follow-up, and analysis of the randomized controlled trial with two intervention groups (200 IU, 800 IU) and standard supplementation group (placebo) for vitamin D supplementation

IU, international unit; NEC, necrotizing enterocolitis; SIP, spontaneous intestinal perforation; NPO, *nil per os*.

Figure 2.

Box Plot illustrating serum 25(OH) vitamin D concentration at birth, day 14, and day 28 in three daily supplementation groups: placebo (total daily intake of approximately 200 IU/day found in formula or TPN), 200 IU/day (total daily intake of approximately 400 IU/day due to 200 IU supplement + 200 IU found in formula or TPN) and 800 IU/day (total daily intake of approximately 1000 IU/day due to 800 IU supplement + 200 IU found in formula or TPN).

IU, international units; d, days.

Table 1

Demographics and characteristics of infants

Maternal Characteristics	All (N = 87)	Placebo (N = 33)	200 IU/day (N = 28)	800 IU/day (N = 26)
Age (years, mean $\pm$ SD)	25.7 $\pm$ 5.4	24.0 $\pm$ 4.0	27.3 $\pm$ 5.6	26.8 $\pm$ 6.2
Married	26(30)	8 (24)	9 (32)	9 (35)
High school education	69 (79)	26 (79)	19 (68)	24 (92)
Medicaid/uninsured	64 (74)	25 (76)	21 (75)	18 (69)
Race				
White	38 (44)	16 (48)	8 (29)	14 (54)
Black	48 (55)	17 (51)	19 (68)	12 (46)
Other	1 (1)	0 (0)	1 (4)	0 (0)
Gravida (Mean $\pm$ SD)	2.7 $\pm$ 1.8	2.2 $\pm$ 1.5	3.3 $\pm$ 1.9	2.7 $\pm$ 1.8
Parity (Mean $\pm$ SD)	2.0 $\pm$ 1.4	1.8 $\pm$ 1.2	2.4 $\pm$ 1.4	2.0 $\pm$ 1.7
Multiple births	9 (10)	3 (9)	3 (11)	3 (12)
Diabetes mellitus	6 (7)	0 (0)	4 (14)	2 (8)
Pregnancy Induced Hypertension	28 (32)	10 (30)	12 (43)	6 (23)
Chronic hypertension	17 (20)	5 (15)	8 (29)	4 (15)
Antepartum Hemorrhage	13 (15)	6 (18)	3 (11)	4 (15)
Clinical chorioamnionitis	14 (16)	6 (18)	4 (14)	4 (15)
Histologic chorioamnionitis	54 (62)	21 (64)	17 (61)	16 (62)
Rupture of Membranes 18 hrs	41 (47)	17 (52)	14 (50)	10 (38)
Duration of rupture of membranes (hours; Median [range])	76 [1–867]	67 [1–480]	59 [1–312]	108 [1–867]
Antenatal steroids	85 (98)	32 (97)	28 (100)	25 (96)
Maternal antibiotics	78 (90)	31 (94)	26 (93)	21 (81)
Magnesium sulfate	83 (95)	31 (94)	27 (96)	25 (96)
Vaginal delivery (vertex)	35 (40)	12 (36)	13 (46)	10 (38)
Vaginal delivery (breech)	6 (7)	2 (6)	1 (4)	3 (12)
Cesarean delivery	46 (53)	19 (58)	14 (50)	13 (50)

Infant Characteristics	All (N = 100)	Placebo (N = 36)	200 IU/day (N = 34)	800 IU/day (N = 30)
Birth Weight (g) Mean $\pm$ SD	770 $\pm$ 215	774 $\pm$ 210	744 $\pm$ 210	796 $\pm$ 228
Gestational age, wk Mean $\pm$ SD	25.3 $\pm$ 1.4	25.5 $\pm$ 1.4	25.3 $\pm$ 1.4	25.1 $\pm$ 1.6
Male sex	45 (45)	16 (44)	15 (44)	14 (47)
Apgar score 3				
At 1 minutes	74 (74)	25 (69)	26 (76)	23 (77)
At 5 minutes	23 (23)	4 (11)	12 (35)	7 (23)
Delivery room care				
Oxygen	99 (98)	35 (97)	33 (97)	30 (100)

Infant Characteristics	All (N = 100)	Placebo (N = 36)	200 IU/day (N = 34)	800 IU/day (N = 30)
Bag and mask ventilation	90 (90)	32 (89)	32 (94)	26 (87)
CPAP during resuscitation	29 (29)	13 (36)	7 (21)	9 (30)
Intubated during resuscitation	49 (49)	16 (44)	17 (50)	16 (53)
Hypotension				
24 hours of life	22 (22)	9 (25)	5 (15)	8 (27)
Treated with volume therapy	21 (21)	7 (19)	6 (18)	8 (27)
Treated with inotropes	9 (9)	4 (11)	2 (6)	3 (12)
Base deficit, median [mean]	5 (6.0)	5 (4.8)	5 (6.2)	6 (7.1)
Patent ductus arteriosus				
Diagnosis of patent ductus arteriosus	14 (14)	6 (18)	5 (15)	3 (12)
Treated with indomethacin	3 (3)	1 (3)	2 (6)	0 (0)
Treated with ibuprofen	1 (1)	1 (3)	0 (0)	0 (0)
Treated with surgery	0 (0)	0 (0)	0 (0)	0 (0)
Intraventricular hemorrhage prophylaxis with indomethacin	61 (61)	22 (61)	24 (71)	15 (50)

Data are shown as n (%)

Table 2

Clinical outcomes of enrolled infants

Clinical Outcomes	All Infants (N = 100)	Placebo (N = 36)	200 IU/day (N = 34)	800 IU/day (N = 30)	p
Respiratory Disease, n (%)					
Respiratory distress syndrome	90 (90)	31 (86)	32 (94)	27 (90)	0.71
Received Surfactant < 72 hrs	80 (80)	26 (72)	30 (88)	24 (80)	0.25
Received Nitric oxide	19 (19)	11 (31)	4 (12)	4 (13)	0.09
Pneumothorax	2 (2)	1 (3)	1 (3)	0 (0)	0.65
Pulmonary hemorrhage	8 (8)	1 (3)	5 (15)	2 (7)	0.18
Respiratory support, n (%)					
On > 21% FiO ₂ at 28 days	63 (63)	25 (69)	21 (62)	17 (57)	0.55
On > 21% FiO ₂ at 36 wks	36 (36)	15 (42)	12 (35)	9 (30)	0.61
BPD (> 30% FiO ₂) at 28 days	45 (45)	21 (58)	12 (35)	12 (40)	0.12
Severe BPD (IMV or CPAP or 30% FiO ₂ at 36 wks)	29 (35)	12 (36)	10 (43)	8 (31)	0.84
Steroids for BPD	27 (27)	13 (36)	8 (24)	6 (20)	0.29
Days alive and off respiratory support at median (25 th –75 th Centiles) mean ± SD	0.5 (0–22) 6.53 ± 9.5	1 (0–11) 6.8 ± 9.5	0 (0–8) 5.5 ± 9.1	0.5 (0–22) 7.0 ± 10.3	0.63 0.78
Death or BPD at 36w	46 (46%)	17 (47)	19 (56)	11 (37)	0.63
Oxygen requirement, median (25 th –75 th Centiles)					
Highest FiO ₂ on day 28	30.5 (39.5)	35 (21–50)	29 (22–39)	30 (21–50)	0.55
Highest FiO ₂ on day 36	21 (32.1)	21 (21–38)	23 (21–35)	21 (21–30)	0.62
Necrotizing enterocolitis, n (%)					
All NEC	6 (6)	1 (3)	4 (12)	1 (3)	0.22
NEC Stage I–II	2 (2)	0 (0)	2 (6)	0 (0)	0.14
NEC Stage III	4 (4)	1 (3)	2 (6)	1 (3)	0.78
Spontaneous intestinal perforation, n (%)	6 (6)	1 (3)	2 (6)	3 (10)	0.47
Neurological disease, n (%)					
Head imaging < 10 days	58 (58)	21 (58)	18 (53)	19 (63)	0.70
IVH Grade I	29 (29)	13 (36)	8 (24)	8 (27)	0.48

Clinical Outcomes	All Infants (N = 100)	Placebo (N = 36)	200 IU/day (N = 34)	800 IU/day (N = 30)	p
IVH Grade II	21 (21)	7 (19)	10 (29)	4 (13)	0.28
IVH Grade III	12 (12)	3 (8)	6 (18)	3 (10)	0.45
IVH Grade IV	7 (7)	1 (3)	4 (12)	2 (7)	0.34
Seizures > 72 hrs	4 (4)	0 (0)	3 (9)	1 (3)	0.17
Head imaging normal > 28 days	62 (62)	24 (67)	18 (53)	20 (67)	0.41
Retinopathy of prematurity (L or R), n (%)					
ROP diagnosed	43 (43)	18 (50)	10 (29)	15 (50)	0.14
Immature vessels zone I and II	26 (26)	9 (25)	10 (29)	7 (23)	0.85
Immature vessels zone III	11 (11)	4 (11)	6 (18)	1 (3)	0.19
ROP in zone I and II	20 (20)	10 (28)	6 (18)	4 (13)	0.32
Stage I or II; zone III	5 (5)	3 (8)	0 (0)	2 (7)	0.25
ROP stage III or higher	7 (7)	3 (8)	2 (6)	2 (7)	0.92
Plus disease	3 (3)	2 (6)	0 (0)	1 (3)	0.39
Treatment with Avastin	6 (6)	2 (6)	2 (6)	2 (7)	0.98
Other treatments	1 (1)	1 (3)	0 (0)	0 (0)	0.41
Infection, n (%)					
Early onset sepsis (< 72 hrs)	6 (6)	1 (3)	4 (12)	1 (3)	0.22
Antibiotics > 5 days starting < 72hrs	29 (29)	11 (31)	4 (12)	7 (23)	0.16
Late onset sepsis (> 72 hrs)	29 (29)	13 (36)	11 (32)	5 (17)	0.19
Coagulase negative staphylococci	12 (12)	7 (19)	4 (12)	1 (3)	0.13
Escherichia coli	3 (3)	1 (3)	2 (6)	0 (0)	0.39
Methicillin-resistant Staphylococcus aureus	4 (4)	1 (3)	2 (6)	1 (3)	0.78
Other organisms	10 (10)	5 (14)	2 (6)	3 (10)	0.54
Lumbar puncture	62 (62)	24 (67)	20 (59)	18 (60)	0.77
Meningitis, culture positive	7 (7)	3 (8)	1 (3)	3 (10)	0.50
Nutrition					
First feed with human milk, n (%)	91 (91)	34 (94)	30 (88)	27 (90)	0.65
At day 14 on human milk, n (%)	50 (50)	21 (58)	12 (35)	17 (57)	0.11
At day 14 on human milk or formula, n (%)	14 (14)	7 (19)	5 (15)	2 (7)	0.33

Clinical Outcomes	All Infants (N = 100)	Placebo (N = 36)	200 IU/day (N = 34)	800 IU/day (N = 30)	p
At day 28 on human milk, n (%)	37 (37)	17 (47)	8 (24)	12 (40)	0.11
At day 28 on human milk or formula, n (%)	7 (7)	2 (6)	1 (3)	4 (13)	0.24
At day 14, total feeding volumes, mean±SD	113± 50	113 ± 48	102± 58	127± 39	0.22
At day 28, total feeding volumes, mean±SD	131 ± 35	131 ± 34	137± 30	124 ± 41	0.71
Days until BW regained, median (25 th –75 th Centiles)	14 (10–19)	14 (11–18)	16 (12–19)	11 (9–20)	0.39
Days to 120 ml/kg/day, median (25 th –75 th Centiles)	13 (10–18)	14 (11–18)	12 (11–20)	12 (9–15)	0.53
Days on TPN, median (25 th –75 th Centiles)	10 (6–14)	10 (5–13)	11 (7–17)	9 (5–14)	0.46
Hospitalization, n (%)					
In hospital at 36 wks	82 (82)	33 (92)	23 (68)	26 (87)	0.02
Still in hospital at 120 days	32 (32)	13 (36)	10 (29)	9 (30)	0.80
Death 36 wks, n (%)	9 (9)	1 (3)	7 (21)	1 (3)	0.02