Enteral Vitamin A for Reducing Severity of Bronchopulmonary Dysplasia: A Randomized Trial

Abhijeet A. Rakshasbhuvankar, FRACP,[a,b,c,d] Karen Simmer, FRACP, PhD,[a,b,c] Sanjay K. Patole, FRACP, DrPH,[a,b,c] Benjamin Stoecklin, MD,[a,c,d,e] Elizabeth A. Nathan, MBiostate,[f,g] Michael W. Clarke, PhD,[h,i] J. Jane Pillow, FRACP, PhD[c,d]

abstract

BACKGROUND AND OBJECTIVES: Evidence suggests that intramuscular vitamin A reduces the risk of bronchopulmonary dysplasia (BPD) in preterm infants. Our objective was to compare enteral water-soluble vitamin A with placebo supplementation to reduce the severity of BPD in extremely preterm infants.

METHODS: We conducted a double-blind randomized controlled trial in infants <28 weeks' gestation who were to receive either enteral water-soluble vitamin A (5000 IU per day) or a placebo. Supplementation was started within 24 hours of introduction of feeds and continued until 34 weeks' postmenstrual age (PMA). The primary outcome was the severity of BPD, assessed by using the right shift of the pulse oximeter saturation versus the inspired oxygen pressure curve.

RESULTS: A total of 188 infants were randomly assigned. The mean ± SD birth weight (852 ± 201 vs 852 ± 211 g) and gestation (25.8 ± 1.49 vs 26.0 ± 1.39 weeks) were comparable between the vitamin A and placebo groups. There was no difference in the right shift (median [25th–75th percentiles]) of the pulse oximeter saturation versus inspired oxygen pressure curve (in kilopascals) between the vitamin A (11.1 [9.5–13.7]) and placebo groups (10.7 [9.5–13.1]) (P = .73). Enteral vitamin A did not affect diagnosis of BPD or other clinical outcomes. Plasma retinol levels were significantly higher in the vitamin A group versus the placebo group on day 28 and at 34 weeks' PMA.

CONCLUSIONS: Enteral water-soluble vitamin A supplementation improves plasma retinol levels in extremely preterm infants but does not reduce the severity of BPD.

[a]*Neonatal Clinical Care Unit and* [f]*Women and Infants Research Foundation, King Edward Memorial Hospital, Subiaco, Western Australia, Australia;* [b]*Neonatal Clinical Care Unit, Perth Children's Hospital, Perth, Western Australia, Australia;* [c]*Centre for Child Health Research, Medical School, The University of Western Australia and Telethon Kids Institute, Perth, Western Australia, Australia;* [g]*Division of Obstetrics and Gynaecology, Faculty of Health and Medical Sciences,* [h]*Metabolomics Australia, Centre for Microscopy, Characterization, and Analysis,* [i]*School of Biomedical Sciences, Faculty of Health and Medical Sciences, and* [d]*Division of Anatomy and Human Biology, School of Human Sciences, Faculty of Science, The University of Western Australia, Perth, Western Australia, Australia; and* [e]*Department of Neonatology, University Children's Hospital Basel, Basel, Switzerland*

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WHAT'S KNOWN ON THIS SUBJECT: Evidence suggests that intramuscular vitamin A improves plasma vitamin A levels and reduces the risk of bronchopulmonary dysplasia (BPD) in extremely low birth weight infants. Enteral vitamin A supplementation for the prevention of BPD has not been investigated adequately.

WHAT THIS STUDY ADDS: Enteral water-soluble vitamin A supplementation in extremely preterm infants improves plasma vitamin A levels but does not reduce the severity of BPD, as assessed by the right shift of the pulse oximeter saturation versus inspired oxygen pressure curve.

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Bronchopulmonary dysplasia (BPD) is one of the most common complications of extremely preterm birth and is associated with increased short- and long-term morbidities. BPD increases the risk of rehospitalization from respiratory causes, and the need for inhaled pulmonary medications and tracheostomy in the first 2 years of life, impairs lung function in children and young adults and likely predisposes preterm infants to chronic obstructive pulmonary disease in older age.[1–4] Additionally, BPD is associated with adverse long-term neurodevelopmental outcomes.[5] BPD is the most important perinatal-neonatal factor associated with lower IQ seen in very and extremely preterm infants.[6] The effect of BPD on infants' cognitive abilities may persist lifelong, affecting their higher education and employment prospects.[7]

Caffeine and vitamin A are the only safe and effective drugs currently available for the prevention of BPD.[8,9] Mechanism of action of vitamin A for the prevention of BPD is likely to be multifactorial (Fig 1).[10–14] Despite the evidence, the use of vitamin A to prevent BPD is limited because of reluctance to prescribe repeated intramuscular (IM) injections. Enteral vitamin A may be an alternative approach but has not been studied well in clinical trials.

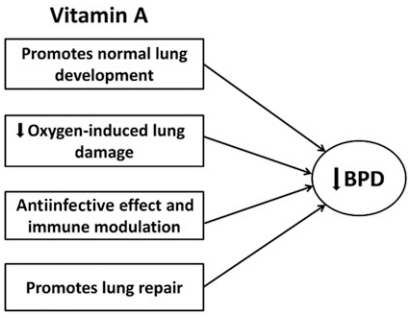

FIGURE 1
Mechanism of action of vitamin A for the prevention of BPD.

The 2 randomized controlled trials (RCTs) investigating enteral vitamin A supplementation for the prevention of BPD revealed no significant benefit.[15,16] Poor absorption by the enteral route could explain these findings and may relate to decreased hydrolysis of retinyl esters, reduced availability of bile salts required for the formation of micelles, or inadequate availability of carrier proteins needed for the absorption of vitamin A in enterocytes.[17] We postulated that the smaller particle size of vitamin A in the water-soluble form might be advantageous for improved absorbance by passive diffusion compared with the larger particle size of the fat-soluble preparations that require carrier-mediated transport.[18] Very low birth weight infants supplemented with the water-soluble form of vitamin A have increased serum retinol levels.[19] The water- versus fat-solubility of vitamin A preparations may be critical for interpreting the results of RCTs of enteral vitamin A: Wardle et al[16] and Calisici et al[15] did not report the form of vitamin A used in their RCTs. Given these data, we aimed to investigate the efficacy of enteral water-soluble vitamin A for reducing the severity of BPD in extremely preterm infants.

The severity of lung disease is assessed by using the shift test, which detects a change in the pulse oximetry saturation (Spo_2) versus inspired oxygen pressure (PIo_2) curve.[20–22] The change may be in the form of the right shift of the entire curve (shift) or displacement of the top part of the curve downward (shunt) or a combination of the two.[23] Shift is measured in kilopascals, whereas shunt is measured in percentages. Shift indicates a decreased ventilation/perfusion ratio, whereas shunt indicates a right-to-left intracardiac or intrapulmonary shunt. A decreased ventilation/perfusion ratio is the predominant mechanism of impaired gas exchange in BPD and can be measured on a continuous scale by using the shift test.[24,25] Hence, the shift test offers better sensitivity and discriminatory capacity compared to BPD defined as a categorical outcome. We reported the discriminatory capacity of shift[25] for the widely used 2001 National Institutes of Health *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) BPD definition.[26] Shift offers the additional benefit of being unaffected by variations in clinician-prescribed saturation targets affecting the use of supplemental oxygen.

We hypothesized that compared with placebo, enteral water-soluble vitamin A supplementation would decrease the severity of BPD, as measured by the right shift in the Spo_2 versus PIo_2 curve.

METHODS

We conducted a double-blind placebo-controlled RCT in our tertiary NICU. We recruited infants who were born at <28 weeks' gestational age and <72 hours of age after obtaining informed parental consent. Infants with major congenital gastrointestinal or respiratory tract abnormalities were excluded. The study was approved by the hospital ethics committee (2016028EW). The study was registered prospectively with the Australian New Zealand Clinical Trial Registry (ACTRN12616000408482) before recruitment commenced. The study protocol was published in a peer-reviewed journal for transparency.[18] We followed Consolidated Standards of Reporting Trials guidelines for reporting the results.[27]

Primary Outcome

The primary outcome was the severity of BPD, as measured by the right shift of Spo_2 versus PIo_2 curve at

36 weeks' postmenstrual age (PMA). Simultaneous measurements of Spo_2 and P_{IO_2} were obtained while the P_{IO_2} level was decreased stepwise in 2 kPa (15 mm Hg or 2% inspired oxygen at sea level) steps at 5-minute intervals. At least 5 Spo_2 measurements in the range of 86% to 97% were obtained; the lowest permissible P_{IO_2} level was 14 kPa (105 mm Hg or 14% inspired oxygen at sea level). Paired measurements of Spo_2 versus P_{IO_2} were plotted, and the right shift was determined by using an algorithm described by Lockwood et al.[24,28]

Secondary Outcomes

The following secondary clinical outcomes were measured at the time of discharge or death: moderate to severe BPD (therapy with supplemental oxygen for ≥28 days plus requirement of supplemental oxygen and/or continuous positive airway pressure [CPAP] and/or mechanical ventilation at 36 weeks' PMA)[29]; death; use of postnatal steroids for BPD; durations (hours) of supplemental oxygen, mechanical ventilation, and positive pressure support (mechanical ventilation plus CPAP plus humidified high flow); the proportion of infants discharged from the hospital with home oxygen; weight gain (grams per day) during the period of study medication supplementation; retinopathy of prematurity requiring treatment in the form of laser ablation or bevacizumab injection; diagnoses of culture-positive (blood or cerebrospinal fluid) or suspected sepsis (C-reactive protein level >25 mg/L and treatment with antibiotics for at least 5 days); grade 3 or 4 intraventricular hemorrhage (IVH) or periventricular leukomalacia[30]; stage 2a or greater necrotizing enterocolitis (NEC)[31]; and vitamin A adverse effects (bulging fontanel in the absence of IVH, hepatomegaly, and skin changes). MRI of the brain was performed at 38 weeks' PMA and reported as abnormal if it showed sequelae of IVH or intracerebellar hemorrhage or white matter loss.

Vitamin A Levels

We measured plasma retinol and relative dose response (RDR) in 35 infants at day 28 of life. Plasma retinol and RDR were also measured in 36 consecutive infants at 34 weeks' PMA. Measurements were obtained 24 hours after the last dose of the trial medication in infants who had not received steroids in the preceding 2 weeks because steroids can influence blood retinol levels.[32] A blood sample (0.5 mL) was collected in a lithium heparin tube (BD Microtainer; Becton, Dickinson and Company, Franklin Lakes, NJ) by either heel prick or venipuncture. The method of collection does not significantly influence plasma retinol values.[33] Plasma for retinol measurement was obtained immediately by centrifugation of the collected blood at 3000 rpm for 10 minutes. Plasma was stored at −80°C and protected from light until further analysis. We measured plasma retinol levels using high-performance liquid chromatography–tandem mass spectrometry. A plasma retinol level of <10 μg/dL suggests depleted body stores, whereas levels between 10 and 20 μg/dL are considered to be low. An RDR value of >20% indicates deficient liver vitamin A stores.[34]

Randomization and Allocation Concealment

Infants were randomly assigned by a hospital pharmacist not involved in clinical care. Randomization followed a computer-generated randomization table using blocks of 6. Randomization was performed within the Research Electronic Data Capture randomization module, ensuring allocation concealment throughout the study period.[35] Randomization was stratified for sex and gestational age (23^0–25^6 and 26^0–27^6 weeks' PMA). Siblings of multiple births were randomly assigned individually.

Blinding

The pharmacy dispensed vitamin A and the placebo in identical amber-colored containers. Both preparations were indistinguishable by their appearance, smell, and other physical properties.

Intervention

The treatment group received enteral water-soluble vitamin A (Bio-Logical Vitamin A Solution; Biological Therapies, Braeside, Victoria, Australia) containing 5000 IU (0.5 mL) of retinyl palmitate, which was administered once daily through the gastric tube, followed by a feed. The preparation included pegylated castor oil as an emulsifier. The osmolarity of the solution was 1025 mOsm/kg. To further reduce the osmolarity, the medication was administered with at least 1 mL of breast milk. The control group received an identical volume (0.5 mL) of the placebo solution (normal saline mixed with a safe coloring agent: 2.5 mg/dL quinoline yellow). The study medications were started within 24 hours of commencement of enteral feeds and continued until 34 weeks' PMA.

The attending neonatologist determined the day-to-day management of the infants. Routine unit policies for preterm infants <28 weeks' gestation include the commencement of caffeine within 24 hours after birth and prescription of probiotics alongside starting minimal enteral feeds.

Other Sources of Vitamin A in the Study Infants

Separate to the prescribed medication or placebo solution, all study infants received 966 IU/kg per day of vitamin

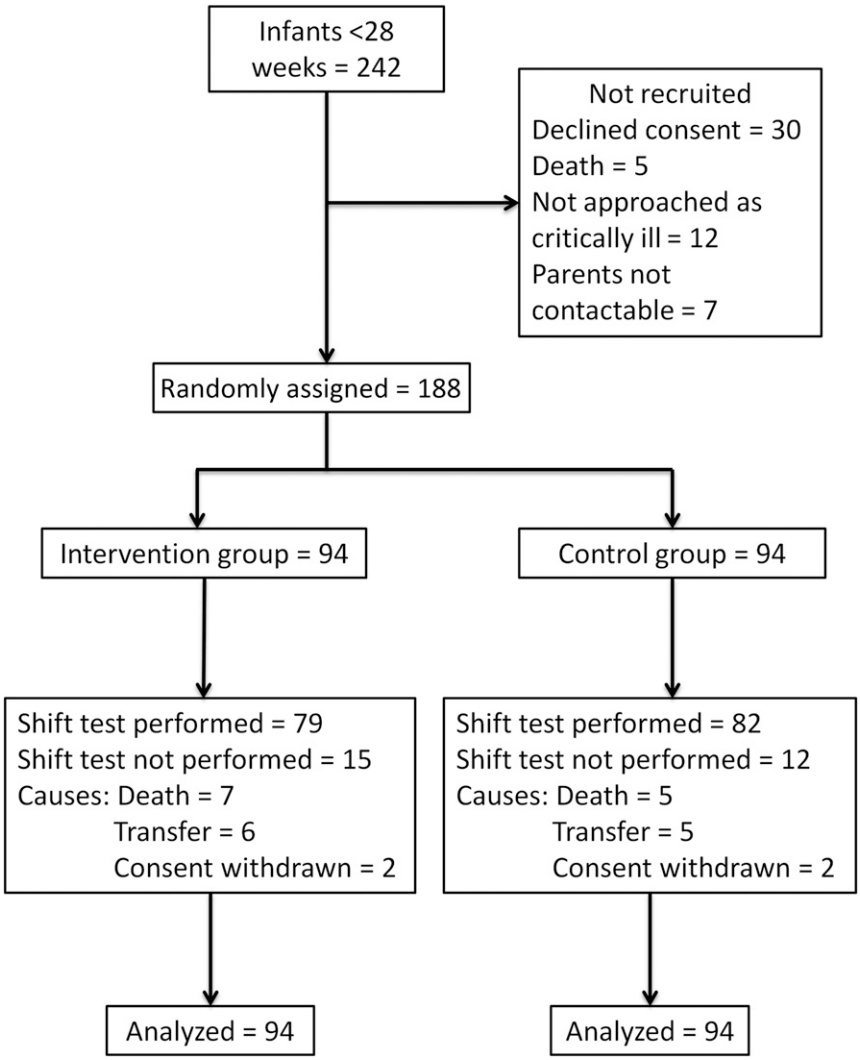

FIGURE 2
Study flow diagram.

A (in lipid emulsion) while on parenteral nutrition and 1820 IU/kg per day of vitamin A when entirely fed with fortified maternal or donor human milk.

Data Collection and Management

Deidentified data were stored in a secure, Web-based, and password-protected Research Electronic Data Capture system.[35] An independent data monitoring committee reviewed the trial periodically for adverse effects. One formal interim analysis was performed after the recruitment of 100 infants; no correction was made to the reported *P* value for this interim test. Criteria for early stopping of the trial ($P < .001$) were based on the Haybittle-Peto approach.[36]

Sample Size

The normal Spo_2 versus Pio_2 curve in adults is shifted to the right of the oxygen-hemoglobin dissociation curve by 6 kPa.[20] Preterm infants with (moderate to severe) BPD had a mean (SD) shift of 16.5 (4.7) kPa at a mean (SD) PMA of 37.2 (0.62) weeks.[24] A 20% (3.3 kPa) change in the shift would require 64 infants with moderate to severe BPD (calculated by using the sample size calculator available at https://clincalc.com/stats/samplesize.aspx). With a reported incidence of ~40% to 45% for moderate to severe BPD in extremely preterm infants[37,38] and accounting for a 20% loss to follow-up, we required 188 extremely preterm infants to achieve a study power of 80% with a two-tailed test and 5% significance. The 20% loss to follow-up estimate was based on our hospital data: estimated mortality of 10% to 15% and transfer of 5% to 10% of the recruited infants before 36 weeks' PMA.

Statistical Analysis

An intention-to-treat analysis was performed. The Spo_2 versus Pio_2 curve shift measurements were not normally distributed and were compared between treatment groups by using the Mann–Whitney *U* test with Hodges-Lehman 95% confidence intervals (CI). Outcomes were compared by using independent *t* tests, χ^2 tests, or Fisher's exact tests as appropriate. The duration of respiratory support outcomes was analyzed by using survival techniques and compared by using the log-rank test. Deaths and transfers that occurred while patients were on respiratory support were censored in the analysis. Median Kaplan-Meier survival estimates and 25th to 75th percentiles were reported. The relationship between plasma retinol levels and RDR and the shift test was assessed by using the Spearman correlation coefficient. Post hoc sensitivity analyses were performed to assess the consistency of treatment effects when the correlation between siblings was accounted for and when adjusting for baseline imbalance. Stata Release 16 (Stata Corp, College Station, TX) software was used for sensitivity analyses by using a generalized estimating equations approach on log-transformed shift measurements. SPSS version 25.0 (IBM SPSS Statistics, IBM Corporation, Armonk,

TABLE 1 Baseline Maternal and Neonatal Demographic and Clinical Characteristics

	Vitamin A (n = 94)	Placebo (n = 94)
Maternal characteristics		
Ethnicity, n (%)[a]		
White	52 (55)	57 (60)
Aboriginal or TSI	15 (16)	20 (21)
Other	27 (28)	17 (18)
Antenatal steroids, any dose, n (%)	79* (84)	93* (98)
Antenatal steroids, 2 doses, n (%)	64 (68)	66 (70)
Histologic chorioamnionitis, n (%)[b]	62 (72)	58 (66)
Diabetes, n (%)	8 (8)	8 (8)
Hypertension, n (%)	7 (7)	11 (11)
Cesarean delivery, n (%)	48* (51)	65* (69)
Neonatal characteristics		
Gestational age, wk, mean (SD)	25.8 (1.4)	26.0 (1.3)
Birth weight, g, mean (SD)	853 (201)	852 (211)
Birth weight z score, mean (SD)	0.35 (0.87)	0.25 (0.94)
Male sex, n (%)	49 (52)	49 (52)
IUGR, n (%)	4 (4)	7 (7)
Apgar score <7 at 5 min, n (%)	29 (31)	27 (28)
CRIB[39] score, median (25th–75th percentiles)	4 (1–7)	4 (1–7)
SNAPPE-2[40] score, median (25th–75th percentiles)	32 (23–48)	32 (22–46)

CRIB, Clinical Risk Index for Babies; IUGR, intrauterine growth restriction; SNAPPE-2, Score for Neonatal Acute Physiology–Perinatal Extension 2; TSI, Torres Strait Islander.

[a] Because no numbers beyond decimal points are reported, percentages may not add up to 100.

[b] Placenta not available for 6 women in the placebo group and 9 women in the vitamin A group.

* $P < .05$.

NY) statistical software was used in all other data analyses. All hypothesis tests were two-sided, and P values <.05 were considered statistically significant.

RESULTS

A total of 188 infants were recruited into the study between December 2016 and May 2019 (Fig 2). Twenty-seven infants did not have a shift test at 36 weeks' PMA (15 in the vitamin A group and 12 in the placebo group) for various reasons, as shown in Fig 2. The remaining 79 infants in the vitamin A group and 82 infants in the placebo group had shift measurements and were included in the analysis of the primary outcome. More than 80% of the expected doses of the study medications were administered in 82% of the infants in the vitamin A group and 85% of the infants in the placebo group. The reasons for not receiving doses were death, withdrawal of consent, transfer to the step-down unit before 34 weeks' PMA, and nil per os order.

Baseline maternal and neonatal characteristics were comparable between treatment groups (Table 1). The median (25th–75th percentiles) postnatal age when vitamin A was started was 2 (1–3) days, and the median (25th–75th percentiles) duration of vitamin A supplementation was 48 (42–57) days. No infant developed feed intolerance with the study medication, and the study medication was not stopped in any infant because of adverse effects. Exposures to antenatal steroids and cesarean delivery were less frequent in the vitamin A group than in the placebo group.

The shift did not differ between study groups (Table 2). The Hodges-Lehman median difference estimate was 0.10 (95% CI: −0.60 to 0.90; P = .730). Secondary clinical outcomes did not differ between groups (Table 2). No adverse effects of enteral vitamin A were seen in any participant. Eight infants in the vitamin A group and 5 infants in the placebo group died before discharge from the hospital. The causes of death are shown in Table 3.

Plasma retinol levels were significantly higher in the vitamin A group compared with the placebo group on day 28 and at 34 weeks' PMA (Table 4). Plasma vitamin A levels and RDR at 28 days were not correlated with the right shift of the Spo_2 versus Pio_2 curve. The Spearman correlation between plasma vitamin A levels at 28 days and the right shift was 0.259 (P = .139). The correlation between RDR at 28 days and the right shift of Spo_2 versus Pio_2 curve was 0.010 (P = .955).

We performed additional analyses to account for clustering due to twins and baseline imbalance in antenatal steroids in the placebo and vitamin A groups. A sensitivity analysis of the primary outcome to allow for sibling cluster intracorrelation revealed no substantive change to results (mean effect 1.06; 95% CI: 0.97 to 1.15; P = .204): a mean effect of 1.06 represents a 6% increase (95% CI: 3% decrease to 15% increase) in shift in the treated group compared with the placebo group. An adjustment for antenatal steroid use (yes or no) to assess the influence of this baseline imbalance between the placebo and treatment groups did not change the results (results not shown).

DISCUSSION

Our RCT revealed that supplementation of water-soluble vitamin A did not reduce the severity of BPD, as assessed by the shift test, despite the increased plasma vitamin A levels. To our knowledge, this is the first RCT of enteral water-soluble vitamin A in extremely preterm infants.

Our trial is based on the encouraging finding in an adequately powered RCT revealing decreased risk of BPD

TABLE 2 Primary and Secondary Outcomes

	Vitamin A (n = 94)	Placebo (n = 94)	P
Primary outcome			
Right shift of Spo_2 versus PIo_2 curve, kPa, median (25th–75th percentiles)[a]	11.1 (9.5–13.7)	10.7 (9.5–13.1)	.730
Secondary outcomes			
Death before discharge, n (%)	8 (8)	5 (5)	.388
Duration of oxygen, h, median (25th–75th percentiles)[b]	1195 (567–2461)	1156 (370–1911)	.582
Duration of PP support, h, median (25th–75th percentiles)[b]	1597 (1205–2084)	1620 (1339–2024)	.302
Duration of MV, h, median (25th–75th percentiles)[b]	66 (19–644)	80 (20–436)	.422
Duration of CPAP and/or HHF, h, median (25th–75th percentiles)[b]	1432 (1126–1645)	1421 (1222–1598)	.417
Moderate-severe BPD[29] at 36 weeks' gestation, n (%)	36 (38)	35 (37)	.988
Postnatal steroids for BPD,[29] n (%)	14 (15)	12 (13)	.651
ROP requiring treatment, n (%)	6 (6)	6 (6)	.898
Sepsis suspected or proven, n (%)	31 (33)	30 (32)	.876
IVH[30] grade 3 or 4 or PVL, n (%)	8 (8)	7 (7)	.788
MRI, n (%)			
Not abnormal	57 (61)	54 (58)	.918
Abnormal	20 (21)	22 (24)	.918
Scan not done	17 (18)	18 (19)	.851
NEC[31] stage 2a or more, n (%)	4 (4)	3 (3)	.989
Shunt, median (25th–75th percentiles)	6 (0–12)	5 (0–12)	.636
Oxygen support on discharge from the hospital, n (%)			
No oxygen	49 (52)	53 (56)	.825
Oxygen	8 (8)	8 (8)	.825
Not discharged from the hospital	37 (39)	33 (35)	.546
Wt gain, g/d, mean (95% CI)	22 (21 to 23)	23 (22 to 24)	.702
Discharge wt, g, mean (95% CI)	3099 (2940 to 3260)	3081 (2930 to 324)0	.877
Discharge wt z score, mean (95% CI)	−0.70 (−0.90 to −0.49)	−0.77 (−0.95 to −0.58)	.653
Duration of hospitalization, d, median (25th–75th percentiles)[b]	108 (89–129)	102 (87–126)	.664

HHF, humidified high flow; MV, mechanical ventilation; PP, positive pressure (includes MV, CPAP, and HHF); PVL, periventricular leukomalacia; ROP, retinopathy of prematurity.

[a] Seventy-nine infants in the vitamin A group and 82 infants in the placebo group had a shift test.

[b] Data summaries represent Kaplan-Meier survival estimates.

at 36 weeks' PMA (relative risk: 0.85; 95% CI: 0.73 to 0.98) in extremely low birth weight infants treated with IM vitamin A (5000 IU, 3 times per week for 4 weeks).[41] However, this parenteral treatment approach is routinely not used because it involves repeated IM injections in preterm infants. Enteral vitamin A offers an alternative approach to preventing BPD. The RCTs by Wardle et al[16] and Calisici et al[15] revealed no benefit of enteral vitamin A for improving plasma retinol levels or prevention of BPD at 36 weeks' PMA.

Our findings are consistent with these 2 studies, except we found improved plasma retinol levels with vitamin A supplementation, confirming that the water-soluble preparation is absorbed in extremely preterm infants. Our findings are consistent with those reported by Basu et al.[19] Furthermore, they confirm that an increase in plasma retinol levels is achievable in extremely preterm infants as opposed to the more mature infants reported by Basu et al[19] (median gestation: 30.5 weeks; only 26 of 196 infants <28 weeks).

TABLE 3 Causes of Death in the Study Groups

Cause of Death	Vitamin A Group, n	Placebo Group, n
Severe BPD	2	0
Pulmonary hemorrhage	1	1
NEC	3	2
IVH	1	0
Sepsis	1	2

The lack of benefit of enteral vitamin A for reducing the severity of BPD in our study is unlikely to be due to inadequate plasma retinol levels for the following reasons: (1) the plasma retinol levels in our study were comparable to those after IM supplementation in the study by Tyson et al,[41] which revealed benefit; (2) plasma retinol levels and RDR at day 28 were not correlated with the severity of BPD; and (3) our duration of vitamin A supplementation was longer compared to that in previous studies (6–11 weeks versus 4 weeks).[16,41]

The ineffectiveness of vitamin A in our study compared to previous studies is more likely related to the overall less severe lung disease in our cohort, as indicated by values for the right shift of the Spo_2 versus PIo_2

TABLE 4 Subgroups of Infants Who had Vitamin A Levels Tested at Day 28 (n = 35) and 34 Weeks' PMA (n = 36)

	Vitamin A		Placebo		*P*
	Median	25th–75th Percentiles	Median	25th–75th Percentiles	
Day 28[a]					
Plasma retinol, μg/dL	26.4	18.1–33.3	14.9	9.8–27.3	.023
RDR, %	18.2	6.8–28.1	28.7	6.3–39.0	.271
34 wk PMA[b]					
Plasma retinol, μg/dL	21.5	18.4–23.5	18.1	11.8–19.7	.014
RDR, %	9.0	6.9–17.9	12.9	5.6–27.7	.375

[a] n = 20 in the vitamin A group; n = 15 in the placebo group.
[b] n = 18 in the vitamin A group; n = 18 in the placebo group.

curve. Indeed only 27 (34%) infants in the vitamin A group and 26 (32%) infants in the placebo group had shift values consistent with moderate to severe BPD.[25,26] Furthermore, the major vitamin A supplementation trials (by Tyson et al[41] and Wardle et al[16]) for BPD were conducted 2 decades ago. Four contemporary lung-protective management strategies adopted in our unit may explain the lower severity of BPD in our study versus these earlier studies:

1. Routine use of a lung-protective ventilation strategy (early preferential use of CPAP, prophylactic or low threshold (inspired oxygen ≤30%) for surfactant administration, volume-targeted ventilation, and early extubation)[42,43]: The median duration of ventilation in the control group of our study was 80 hours (3.3 days). Although Tyson et al[41] did not report the duration of ventilation or ventilation strategy, 48% of infants in their control group were ventilated on day 28 of life. The increasing use of noninvasive ventilation and other lung-protective strategies for preterm infants is reported in many parts of the world.[42,43]
2. Optimal vitamin A intake: the baseline vitamin A intake was higher in our study (1820 IU/kg per day) compared with the study by Tyson et al[41] (1000 IU/kg per day), indicating optimal vitamin A intake as per the current guidelines.[44–47]
3. The routine use of prophylactic caffeine[48,49]: Details of caffeine supplementation were not reported by Tyson et al.[41] Because prophylactic caffeine has emerged only over the last 15 years, the benefits of this intervention may not have been exploited in the trial of Tyson et al.[41,48,49]
4. Probiotic supplementation: Considering the role of inflammation in the pathogenesis of BPD, probiotics may reduce the risk of BPD by altering the gut microbiota.[50] Current evidence from systematic reviews of RCTs is inadequate for extremely low birth weight infants to support this hypothesis.[51]

In summary, it is likely that improved standards of care over the last 2 decades have reduced the severity of BPD, making it difficult to replicate the beneficial effects of IM vitamin A on BPD reported by Tyson et al.[41] Adoption of lung-protective strategies and an aggressive approach to the nutrition of extremely preterm infants may explain the findings of a recent study in which a national shortage of vitamin A was not associated with an increase in the incidence of BPD.[52]

The strengths of our study include a robust methodology, the use of the shift test as the objective scale to measure the severity of BPD, and the use of a water-soluble vitamin A in extremely preterm infants at highest risk for BPD.

The limitations of our study need to be acknowledged. These include the availability of plasma vitamin A levels in only a fraction of participants. We did not monitor vitamin A levels in all infants because of the safety of supplementation reported in previous studies, the uncertainty of normal levels in extremely preterm infants, the lack of clinical utility, the and increased risk of iatrogenic anemia.[41] Another limitation was the small sample size for the outcome of moderate to severe BPD as per NICHD criteria, with the NICHD criteria being more widely used and more readily interpreted than the shift test.[26] However, the NICHD criteria for BPD have limitations, as discussed earlier.[18,25] The shift test is suitable for use as an outcome indicator of BPD severity in clinical trials given its scientific and proven ability to discriminate from the semiquantitative NICHD disease severity classification at a population level.[23,24] It is being implemented across Australian and New Zealand Neonatal Network units for benchmarking.[53]. The process will provide data on the correlation between the shift test with long-term developmental and respiratory outcomes.

Our study does not provide a direct comparison between IM vitamin A and enteral vitamin A supplementation for the prevention of BPD. IM vitamin A was not included as a third intervention for the following reasons: (1) IM vitamin A for prevention of BPD is not widely practiced because of discomfort and risk of trauma on a background of only modest benefit,[54,55] (2) it would have been ethically challenging to include this painful procedure as a control arm because IM vitamin A is not a routine practice in Australia, (3)

blinding of the intervention would have been difficult, and (4) we anticipated difficulty in obtaining parental consent and clinician acceptance of the trial.

CONCLUSIONS

Our study reveals that enteral water-soluble vitamin A improves plasma retinol levels in extremely preterm infants but does not reduce the severity of BPD. The lack of benefit of enteral vitamin A is likely related to the overall low severity of BPD in our study population. Further studies of enteral water-soluble vitamin A are essential in preterm infants with suboptimal vitamin A intake and high severity of BPD.

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ABBREVIATIONS

BPD: bronchopulmonary dysplasia
CI: confidence interval
CPAP: continuous positive airway pressure
IM: intramuscular
IVH: intraventricular hemorrhage
NEC: necrotizing enterocolitis
NICHD: *Eunice Kennedy Shriver* National Institute of Child Health and Human Development
P_{IO_2}: inspired oxygen pressure
PMA: postmenstrual age
RCT: randomized controlled trial
RDR: relative dose response
Spo_2: pulse oximeter saturation

Deidentified individual participant data will be made available in addition to study protocols, the statistical analysis plan, and the informed consent form. The data will be made available after publication to researchers who provide a methodologically sound proposal for use in achieving the goals of the approved proposal. Proposals should be submitted to the corresponding author.

This trial has been registered with the Australian New Zealand Clinical Trials Registry (https://www.anzctr.org.au/) (identifier ACTRN12616000408482).

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Address correspondence to Abhijeet A. Rakshasbhuvankar, FRACP, Neonatal Clinical Care Unit, King Edward Memorial Hospital, 374 Bagot Rd, Subiaco, WA 6008, Australia. E-mail: abhijeet.rakshasbhuvankar@health.wa.gov.au

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