Supplementary Online Content

Hibbs AM, Ross K, Kerns LA, et al. Effect of vitamin D supplementation on recurrent wheezing in black infants who were born preterm: the D-Wheeze randomized clinical trial. *JAMA*. doi:10.1001/jama.2018.5729

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods.

Changes to eligibility lab ranges and follow-up definition of normal ranges in the first year of enrollment

Changes to the acceptable ranges for phosphorus were made. Initial normal ranges were based on clinical laboratory normal ranges (3.1-7.2 mg/dl). Due to phosphorous levels <4 ml/dl being considered high risk for rickets in the 2013 AAP clinical report on calcium and vitamin D requirements for enterally fed preterm infants,[1] and the high end cut-off causing a large number of clinically well subjects to be ineligible, the normal ranges for phosphorus in the study were changed to those indicated in the table below.

The eligibility threshold for 25(OH)D was lowered to 10 ng/ml to improve generalizability of the sample and improve accrual, as it was noted that the prior level of 15 ng/ml was excluding a high number of otherwise healthy asymptomatic neonates with normal calcium, phosphorous, and alkaline phosphatase levels. After an infant was enrolled in the study, an out of range lab value was treated as an adverse event and referred to the medical monitor. The medical monitor was authorized to repeat the lab, refer the infant for further clinical care, or remove the infant from the study, as needed to ensure infant safety.

Summary of final laboratory values treated as normal for eligibility and follow-up:

Laboratory Test	Range treated as normal for eligibility or follow-up
25(OH)D (ng/mL)	10-80 initially 15-80 at 3 month follow-up and later
Calcium (mg/dl)	6.9-11 at ≤40 weeks adjusted GA 8.5-10.7 at >40 weeks adjusted GA
Phosphorous (mg/dl)	4-9.5 at ≤40 weeks adjusted GA 4-9 at >40 weeks adjusted GA
Alkaline Phosphatase (U/L)	<700 at <40 weeks adjusted GA <500 at >40 weeks adjusted GA

Missing data sensitivity analysis using a pattern mixture modeling approach

Some participants missed the 12 month follow up visit with a recurrent wheezing outcome not yet determined (more than one wheezing incident had not been reported as of their last visit). There were 8 such subjects, all from different families. However, one of the subjects, in the diet-limited group, did have a twin with an observed positive recurrent wheezing outcome. We first note that since we are considering only the randomization group as a covariate, the resultant p-value in a Poisson GEE model after imputing the 8 missing outcomes essentially depends only on the number of positive recurrent wheezing outcomes in each of the groups. We generate corresponding p-values for each imputation pattern. We then computed the estimates of the probability for recurrent wheezing for each of these subjects using logistic GEE regression, with the same covariates as in the adjusted model (except days in study, which is not a baseline variable). For the one twin member among those with missing outcomes, we did not adjust for fact that the other twin is observed as having recurrent wheezing, which is conservative. The averaged p-value, with p-values weighted by the corresponding imputation pattern probability, was 0.023.

We then vary the probabilities for a positive recurrent wheezing outcome for the missing data cases, increasing them by factors of 2 and 3 (or up to 0.99 if the multiplied value exceeds 0.99), and finally then setting all of the values to 0.99. Corresponding average p-values were 0.029, 0.033, and 0.043. These results indicate that the p-value being less than the final significance threshold of 0.047 is supported even if the missing values are not missing at random.

eReference

1. Abrams SA, Committee on Nutrition. Calcium and vitamin D requirements of enterally fed preterm infants. *Pediatrics.* 2013;131(5):e1676-1683.

eTable 1. Overview of Maternal Milk and Formula Feeding, By Randomization Group

	Sustained n/N (%)	**Diet-Limited** n/N (%)
Ever Received Maternal Milk (at or Prior to Study Entry)	134/152 (88.2%)	129/147 (87.8%)
Maternal Milk in 24 hours prior to Randomization[a]	107/153 (69.9%)	104/147 (70.7%)
Baseline Diet[b]		
Maternal milk only	63/153 (41.2%)	52/146 (35.6%)
Maternal milk with formula or donor milk	42/153 (27.5%)	47/146 (32.2%)
Formula or donor milk only	48/153 (31.4%)	47/146 (32.2%)
One Month after Randomization		
Maternal milk only[c]	17/148 (11.5%)	19/139 (13.7%)
Maternal milk and formula	40/148 (27.0%)	35/139 (25.2%)
Formula only	91/148 (61.5%)	85/139 (61.2%)
3 Months Adjusted Age		
Maternal milk only[c]	6/139 (4.3%)	7/137 (5.1%)
Maternal milk and formula	20/139 (14.4%)	12/137 (8.8%)
Formula only	113/139 (81.3%)	118/137 (86.1%)
6 months Adjusted Age		
Maternal milk only[c]	6/137 (4.4%)	3/134 (2.2%)
Maternal milk and formula	7/137 (5.1%)	5/134 (3.7%)
Formula only	122/137 (89.1%)	124/134 (92.5%)
Formula and cow's milk	0/137 (0%)	1/134 (0.7%)
Cow's milk only	2/137 (1.5%)	1/1374(0.7%)
9 months Adjusted Age		
Maternal milk only[c]	4/136 (2.9%)	4/131 (3.1%)
Maternal milk and formula	4/136 (2.9%)	1/131 (0.8%)
Formula only	119/136 (87.5%)	115/131 (87.8%)
Formula and cow's milk	5/136 (3.7%)	2/131 (1.5%)
Cow's milk only	4/136 (2.9%)	9/131 (6.9%)
12 months Adjusted Age		
Maternal milk only[c]	1/132 (0.8%)	0/132 (0%)
Maternal milk and formula	6/132 (4.5%)	3/132 (2.3%)
Formula only	23/132 (17.4%)	14/132 (10.6%)
Formula and other milk (cow, almond, or soy)	8/132 (6.1%)	8/132 (6.1%)
Cow, almond, or soy milk only	94/132 (71.2%)	107/132 (81.1%)

[a] In order to balance infants likely to receive long-term maternal milk feeding between randomization groups, randomization was stratified based on whether the infant had received maternal milk in the 24 hours prior to enrollment. Because subsequently enrolled siblings in twin and triplet groups received the same randomization number as the first sibling, they were assigned to the same strata as the first enrolled siblings. Only their own mother's milk (not donor milk) was not counted for this purpose, as donor milk feeds were generally transitioned to formula feeds prior to discharge.

[b] The baseline dietary assessment was conducted within a week of randomization.

[c] Exclusive maternal milk feeding included infants who were receiving maternal milk with additives, such as formula powder or human milk fortifiers, to add calories or nutrients to maternal milk. It does not include infants receiving any feeds containing only formula or formula with other nutritional additives. Infants ingesting only maternal milk received open-label multivitamins containing 400 IU/day of vitamin D in both study arms.

eTable 2. Full Estimates for Preplanned and *Post Hoc* Adjusted Models

	Pre-Planned Relative Risk (95% CI)	**Post Hoc** Relative Risk (95% CI)
Recurrent Wheezing	0.62 (0.44, 0.87)	0.63 (0.45, 0.89)
Site 1 versus 4	0.37 (0.13, 1.06)	0.38 (0.13, 1.11)
Site 2 versus 4	0.66 (0.36 1.19)	0.55 (0.25, 1.21)
Site 3 versus 4	1.05 (0.25, 4.39)	0.99 (0.22, 4.42)
Receiving Maternal Breast Milk at Randomization	1.29 (0.88, 1.90)	1.12 (0.73, 1.71)
Gestational Age 28-33 weeks	0.97 (0.64, 1.47)	0.94 (0.66, 1.44)
Sex Female	0.68 (0.49, 0.94)	0.67 (0.451, 0.997)
Days in Study	0.996 (0.993, 0.999)	0.995 (0.92, 0.999)
History of Ventilator Support	1.07 (0.75, 1.54)	0.91 (0.58, 1.43)
History of Any Oxygen Treatment in NICU	1.22 (0.86, 1.74)	1.49 (0.79, 2.80)
Family history of hay fever	1.30 (0.88, 1.93)	1.41 (0.93, 2.15)
Family history of food allergy	1.31 (0.93, 1.86)	1.08 (0.69, 1.69)
Family history of eczema	1.07 (0.72, 1.58)	1.04 (0.67, 1.62)
Home not owned or rented	1.27 (0.92, 1.76)	1.28 (0.90, 1.82)
Referred for Outpatient Palivizumab	1.11 (0.69, 1.81)	0.97 (0.56, 1.67)
Family History of Asthma	-----	1.02 (0.68, 1.52)
Multiple Birth	-----	1.02 (0.65, 1.61)

Relative risk (RR) values for recurrent wheezing are sustained versus diet-limited arms. Days in study is a considered as a continuous variable. For other variables, relative risk is associated with condition being "yes" versus "no".

Pre-planned adjustment includes gestational age, variables that were considered in randomization stratification (site and maternal breast milk at randomization), time in study, plus those found to be associated with the recurrent wheezing outcome (p-value < 0.10): gender, ventilator support, oxygen, family history of hay fever, family history of food allergy, family history of eczema, home not rented or owned, and Palivizumab referral. Highly correlated variables were not included in the model together.

Full factorial Poisson GEE models of recurrent wheezing were fit with randomization group and maternal milk, and with randomization group and gender. Interactions between randomization group and maternal milk at randomization (p=0.20), and randomization group and sex (p=0.32), were not statistically significant. Interaction terms were not included in the final adjusted models.

Post hoc adjustment included the same covariates as the pre-planned model, plus those found to differ significantly between randomization groups (p-value < 0.05): family history of asthma and multiple birth (family history of hay fever was already included). Models were fit with Poisson GEE regression and within family exchangeable correlation (n= 267 for both models). For numerical stability with model fit, we randomly removed a one of the triplets in each of the two sets, so that the sample size is two less than for the unadjusted model fit. The variance inflation factor was no higher than 1.41 for all cases in the *post hoc* model. Given the pre-planned model was contained in the *post hoc* model, there was thus no multicollinearity in either model. Also, the quasi-likelihood under the independence model criterion (QIC) and the QICu GEE model fit criteria for the pre-planned and *post hoc* models were respectively 582.03 and 582.97, and 566.38 and 561.05. For the unadjusted model, the QIC and QICu values were 622.34 and 621.76. Smaller values indicate better fit.

The p-value for randomization group was 0.005 in the pre-planned adjusted model and 0.009 in the *post hoc* model.

eTable 3. Outcomes by Reporting Time-Point. Medically attended illnesses are characterized as being for any cause (including respiratory), and also for a respiratory cause. Results expressed as n/N (%) or median (interquartile range). Follow-up visits occurred at 3, 6, 9, and 12 months of age, adjusted for prematurity.

	Sustained	**Diet-Limited**	**Risk Difference** % (95% CI)
Any Wheezing Reported[a]			
3 month	17/140 (12.1%)	41/137 (29.9%)	-17.8 (-28.8, -8.5)
6 month	26/137 (19.0%)	36/135 (26.7%)	-7.7 (-18.9, 2.4)
9 month	30/136 (22.1%)	42/131 (32.1%)	-10.0 (-24.5, -1.3)
12 month	30/132 (22.7%)	32/132 (24.2%)	-1.5 (-14.4, 7.2)
Respiratory Infection			
3 month	53/140 (37.9%)	67/137 (48.9%)	-11.0 (-24.2, 0.6)
6 month	67/137 (48.9%)	72/134 (53.7%)	-4.8 (-20.2, 5.3)
9 month	68/135 (50.4%)	74/131 (56.5%)	-6.1 (-21.8, 3.8)
12 month	72/132 (54.5%)	79/132 (59.8%)	-5.3 (-17.7, 7.9)
Respiratory Medications Reported			
3 months	3/140 (2.1%)	10/137 (7.3%)	-5.2 (-10.0, -0.1)
6 months	14/137 (10.2%)	22/135 (16.3%)	-6.1 (-17.4, -0.3)
9 months	25/136 (18.4%)	23/131 (17.6%)	0.8 (-13.4, 6.6)
12 months	17/132 (12.9%)	26/132 (19.7%)	-6.8 (-18.5, 0.3)
Any Pediatrician Sick Visit			
3 months	28/139 (20.1%)	32/137 (23.4%)	-3.2 (-14.2, 6.8)
6 months	15/137 (10.9%)	27/134 (20.1%)	-9.2 (-18.4, -0.5)
9 months	35/135 (25.9%)	28/131 (21.4%)	4.6 (-6.7, 15.7)
12 months	32/132 (24.2%)	28/132 (21.2%)	3.0 (-9.4, 11.5)
Respiratory Pediatrician Sick Visit			
3 month	16/139 (11.5%)	19/137 (13.9%)	-2.4 (-11.5, 5.0)
6 month	11/137 (8.0%)	15/134 (11.2%)	-3.2 (-11.1, 3.8)
9 month	27/135 (20.0%)	15/131 (11.5%)	8.5 (-1.3, 17.6)
12 month	15/132 (11.4%)	15/132 (11.4%)	0.0 (-11.0, 4.6)
Any ED Visits			
3 months	33/140 (23.6%)	47/137 (34.3%)	-10.7 (-22.0, 1.0)
6 month	40/137 (29.2%)	37/135 (27.4%)	1.8 (-11.3, 12.2)
9 month	49/136 (36.0%)	51/131 (38.9%)	-2.9 (-16.7, 8.4)
12 month	48/132 (36.4%)	50/131 (38.2%)	-1.8 (-12.3, 12.6)

	Sustained	Diet-Limited	Risk Difference % (95% CI)
Respiratory ED Visits			
3 months	21/140 (15.0%)	29/137 (21.2%)	-6.2 (-17.2, 2.6)
6 months	30/137 (21.9%)	24/135 (17.8%)	4.1 (-8.2, 12.4)
9 months	33/136 (24.3%)	32/131 (24.4%)	-0.2 (-12.1, 10.6)
12 months	27/132 (20.5%)	26/131 (19.8%)	0.6 (-9.8, 10.9)
Any Hospital Admission			
3 months	18/140 (12.9%)	24/137 (17.5%)	-4.7 (-14.0, 4.0)
6 months	6/137 (4.4%)	10/135 (7.4%)	-3.0 (-8.0, 3.3)
9 months	2/136 (1.5%)	13/131 (9.9%)	-8.5 (-14.0, -2.9)
12 months	10/132 (7.6%)	4/132 (3.0%)	4.5 (-1.1, 10.2)
Respiratory Hospital Admission			
3 months	7/140 (5.0%)	12/137 (8.8%)	-3.8 (-9.7, 2.2)
6 months	2/137 (1.5%)	7/135 (5.2%)	-3.7 (-7.9, 0.8)
9 months	1/136 (0.7%)	9/131 (6.9%)	-6.1 (-10.8, -1.5)
12 months	5/132 (3.8%)	3/132 (2.3%)	1.5 (-3.0, 5.5)
SCORAD Eczema Exam Score ≥ 25[b]			
3 months	8/140 (5.7%)	0/136 (0.0%)	5.7 (1.9, 9.6)[c]
6 months	2/137 (1.5%)	5/135 (3.7%)	-2.2 (-6.0, 1.6)
9 months	3/132 (2.3%)	2/128 (1.6%)	0.7 (-2.6, 4.0)
12 months	3/128 (2.3%)	5/124 (4.0%)	-1.7 (-6.0, 2.6)
Calcium (mg/dL)			
3 months	10.1 (9.9-10.5) (n=135)	10.2 (9.8-10.5) (n=134)	___
6 months	10.1 (9.8-10.3) (n=134)	10.1 (9.7-10.5) (n=132)	
Phosphorous (mg/dL)			
3 months	6.7 (6.2-7.1) (n=135)	6.6 (6.2-6.9) (n=134)	___
6 months	6.1 (5.8-6.4) (n=134)	6.1 (5.8-6.4) (n=131)	
Alkaline Phosphatase (U/L)			
3 months	302 (260-364) (n=135)	312 (248-375) (n=134)	___
6 months	259 (222-309) (n=134)	261 (224-312) (n=131)	

[a] At each follow-up visit, parents may have reported one or more wheezing episode(s) since the last visit.

[b] Scoring Atopic Dermatitis (SCORAD) scores of ≥25 are consistent with moderate to severe eczema.

[c] Confidence interval estimated through stratified resampling, with multiple birth and single birth strata. Otherwise, confidence intervals were obtained through Poisson GEE regression with identity link or through linear GEE regression.

eTable 4. Adverse Events by Organ System. Each event could be categorized in more than one organ system, and individual patients may have experienced multiple events, such as multiple respiratory infections.

	Sustained		Diet-Limited	
	Patients affected (N, %)	**Number of events**	**Patients affected (N, %)**	**Number of events**
Respiratory	99/153 (64.7%)	219	101/147 (68.7%)	242
Gastrointestinal	35/153 (22.9%)	46	50/147 (34.0%)	61
Genitourinary	3/153 (2.0%)	3	3/147 (2.0%)	3
Cardiac	2//153 (1.3%)	2	1/147 (0.7%)	1
Endocrine	37/153 (24.2%)	38	38/147 (25.9%)	48
Infectious or Immune	105/153 (68.6%)	251	108/147 (73.5%)	269
Hematologic	10/153 (6.5%)	10	8/147 (5.4%)	9
Dermatologic	38/153 (24.8%)	49	43/147 (29.3%)	57
Neurologic	2/153 (1.3%)	2	1/147 (0.7%)	1
Orthopedic	2/153 (1.3%)	2	1/147 (0.7%)	1
Trauma	2/153 (1.3%)	2	6/147 (4.1%)	6
Other	32/153 (20.9%)	36	25/147 (17.0%)	30
Total Adverse Events	128/153 (83.7%)	417	127/147 (86.4%)	473

eTable 5. *Post Hoc* Analysis of Achieved 25(OH)D Levels by Maternal Breast Milk Status at Randomization. Levels were checked at the 3, 6, and 12 month visits. Levels are reported as median and interquartile range.

Time-Point and Maternal Breast Milk at Randomization	**Sustained** (ng/ml) median (interquartile range), (N)	**Diet-Limited** (ng/ml) median (interquartile range), (N)	p-value[a]
3 months adjusted age			
Maternal Breast Milk	37.7 (33.1-45.3), (n=94)	37.3 (30.4-42.2), (n=97)	0.32
No Maternal Breast Milk	40.5 (34.9-52.3), (n=42)	34.0 (31.0-42.0), (n=39)	0.001
Total	38.0 (33.9-46.5), (n=136)	36.8 (30.6-42.0), (n=136)	<0.001
6 months adjusted age			
Maternal Breast Milk	35.9 (31.3-42.0), (n=91)	38.0 (31.0-41.0), (n=96)	0.65
No Maternal Breast Milk	40.5 (35.0-48.5), (n=44)	33.5 (28.8-40.5), (n=38)	0.004
Total	37.0 (33.0-43.6), (n=135)	37.2 (30.0-41.0), (n=134)	0.17
12 months adjusted age			
Maternal Breast Milk	32.0 (27.0-37.0), (n=87)	33.0 (28.0-38.9), (n=87)	0.40
No Maternal Breast Milk	35.5 (30.8-39.1), (n=38)	31.0 (28.0-36.0), (n=35)	0.02
Total	33.0 (28.0-38.0), (n=125)	32.0 (28.0-38.0), (n=122)	0.64

[a] P-values calculated using linear GEE.