WHEEZING IN BLACK PRETERM INFANTS: IMPACT OF VIT. D SUPPLEMENTATION STRATEGY

SPECIFIC AIMS: The over-arching goal of this project is to assess the comparative effectiveness of two vitamin D supplementation strategies in promoting pulmonary health in black infants born preterm. In 2006, 13% of all infants were born preterm and 18% of black infants were preterm.[1] Although it is well known that the smallest preterm patients experience long-term pulmonary morbidity, larger preterm infants also experience a significant burden of wheezing illnesses in infancy and childhood.[2-6] While the majority of the neonatal literature focuses on very low birth weight (VLBW <1500g) and very preterm (<32 wks gestational age (GA)) infants, they account for less than 2% of all births. We focus on a relatively understudied population of preterm infants born at $28^{0/7}$-$36^{6/7}$ wks GA, who represent the majority of preterm births, and whose long-term morbidities therefore have significant public health implications. Wheezing in infancy (with or without viral infections) increases healthcare utilization, and in many infants is a harbinger of asthma.

First recognized as a vitamin important for bone health and the prevention of rickets, vit. D is also a hormone with the potential to mediate a wide range of disease states, response to infection, regulation of inflammation, and propensity to allergy. The D pathway regulates lung inflammation and impacts morphogenesis, structure, cell growth, and survival in bronchial smooth muscle.[7-10] **Due to their developmental immaturity, preterm infants may be particularly vulnerable to any positive or negative effects of vit. D supplementation on the lung, airway, and immune system.** The vit. D requirements for larger preterm infants have not been well established, nor have the specific needs for black preterm infants.

We focus on black infants because of (1) higher rates of prematurity, (2) higher rates of wheezing illnesses, and (3) evidence of racial differences in the response to vit. D. Our preliminary data, supported by the literature,[2] suggest that overly aggressive vitamin supplementation of black infants may result in an increase in wheezing episodes in infancy. However, black infants are also more likely to have rickets in infancy and low serum vit. D levels throughout their life, so it is imperative to identify a supplementation strategy that both meets the nutritional needs of preterm black infants and promotes pulmonary and immune health.

Recommendations for preterm infants are primarily intended for VLBW infants and vary widely from approximately 200-1600 IU/day of vit. D,[11-14] but there is a paucity of outcome data or specific recommendations for relatively healthy preterm infants ≥28 wks GA. For the general infant population, the AAP recommended dose of vit. D increased from 200 to 400 IU/day in 2008, based on evidence of higher serum levels with higher doses, rather than on documented impact on clinical outcomes.[15] Reports of benefits of high doses in other populations and concerns about lower serum levels in black patients have created a trend towards using even higher doses in preterm and black infants. Historically, preterm infants have often been harmed by therapeutic exuberance or seemingly rational extrapolation of therapies from other populations.[16-21] Despite the push to maximize enteral vit. D, overly-aggressive supplementation may inadvertently cause harm and predispose infants to life-long diseases such as asthma and allergy. For most vitamins and hormones that have benefit at optimal levels, both excess and deficiency are associated with disease states; trials of aggressive vitamin supplementation often find unanticipated adverse effects.[22-25] We will test two strategies: (I) sustained supplementation until 6 months of age adjusted for prematurity, and (II) cessation of supplementation when a minimum dietary intake of 200 IU/day is reached. We hypothesize that strategy II will be more effective in promoting pulmonary health and will be sufficient to prevent clinical vit. D deficiency.

Aim 1: Determine the impact of a sustained versus diet-limited dosing strategy for vit. D supplementation on recurrent wheezing in black infants born preterm. *Hypothesis: Based on provocative preliminary data supported by the literature, we predict that black infants receiving diet-limited supplementation (strategy II) will experience less wheezing in infancy, as measured by validated questionnaires, corroborated by exams by a pediatric pulmonologist.*

Aim 2: Identify the impact of each dosing regimen on allergy and atopy. *Hypothesis: Black infants receiving sustained supplementation (strategy I) will experience more allergic sensitization and atopic dermatitis in infancy, as measured by sensitive and specific biomarkers, validated questionnaires, and skin exams.*

Aim 3: Characterize the association between 25(OH)D3 levels and recurrent wheezing in black infants born preterm. *Hypotheses: There will be a U-shaped association between 25(OH)D3 levels and wheezing. Optimal levels in black preterm infants may be lower than published goals.*

A. SIGNIFICANCE

A1. Wheezing is a common and long-lasting complication of prematurity.[4 26-32] In a cohort of infants born at less than 29 weeks GA, 42% of parents reported their child wheezed at 12 months adjusted age.[27] Hack et al. have reported that at eight years of age, 21% of extremely low birth weight survivors and 9% of controls had asthma.[28] Similarly, Palta et al. found that 26% of very low birth weight (VLBW) 8 year olds had wheezed in the previous 12 months, compared to 14% of controls.[31] Although most studies have focused on the outcomes of VLBW infants, particularly those with bronchopulmonary dysplasia (BPD), **increased wheezing, asthma, respiratory infections, and healthcare utilization are also seen in larger premature infants**.[5 33-35] In a New Zealand cohort study, 23.8% of infants born at <33 weeks experienced recurrent wheezing by 12 months corrected age.[4] At 3 years of age, 10.5% of all parents in the National Maternal and Infant Health Survey reported that their children had a physician diagnosis of asthma by 3 years of age. The odds of asthma were increased in premature infants, with odds ratios of 1.41 (1.04-1.92) for infants born at 32-34 wks, and 2.59 (2.04-3.30) for infants born at 28-31 wks.[2] Even infants of late-preterm and low-normal GA may be at increased risk for wheezing. In the Home Allergens and Asthma Study, 18.9% of infants born at 36-38.5 wks had asthma at 6 years of age compared to 6.1% of infants born at 38.5-40.5 wks.[3] Late preterm infants (34-36 wks GA) also have high rates of viral illness, emergency room visits, and hospitalizations in infancy.[5] In these larger preterm infants without overt neonatal lung injury, early exposures may be perturbing the development of the lung, airway, or immune system and lead to recurrent wheezing later in life.[36]

A2. One such candidate with the potential to impact lung and immune development is vit. D. Long known to regulate calcium and phosphorous homeostasis, vit. D is also a hormone implicated in a wide range of physiological processes and disease states, particularly those with an inflammatory or immune component. Polymorphisms in the vit. D receptor (VDR) have been associated with inflammatory response, immune function, fetal and postnatal growth, bone density, cancer, diabetes, cardiovascular diseases, and susceptibility to tuberculosis.[37-53] While the impact of vit. D on the immune system is controversial, some authors have claimed that the beneficial effects of stimulating the D pathway include decreased inflammation and enhanced defense against pathogens.[54-57] Vit. D has become a candidate therapy to decrease inflammation in lung diseases, such as asthma and cystic fibrosis; however, the VDR also plays a key role in the induction of lung inflammation.[8 52 53 56 58-62] Furthermore, exposure of the immature immune system to D may skew T-cells towards a more allergic TH-2 cytokine expression profile.[9 10 63-66] Vit. D also has the potential to alter lung and airway development; in bronchial smooth muscle cells, VDR plays a role in the up-regulation of genes involved in morphogenesis, cell growth, and survival, as well as structural proteins.[7] In mice, *in utero* vit. D deficiency is associated with subsequent decreased lung volume.[67] **Preterm infants, with developmentally immature pulmonary and immune systems that have already been perturbed by preterm birth, may be particularly vulnerable to any positive and negative effects of vit. D on immune or pulmonary development.**

The primary form of vit. D available over-the-counter for infants is cholecalciferol (D3). It is hydroxylated in the liver to calcifediol (25(OH)D3), and then in the kidney to the active form calcitriol (1,25(OH)2D3). Typically, 25(OH)D3 levels are measured to determine sufficiency and insufficiency. Optimal levels in term or preterm infants, however, have not been established.[15] Some authorities recommend a level >50 ng/ml, while others consider 15-30 or 20-30 ng/ml insufficient and <15 or 20 deficient.[51 59 68-70] Infants born to vit. D deficient mothers will also be deficient. Although in adults, vit. D supplementation has been proposed to improve a wide range of disease states,[71-73] the safety and efficacy of aggressive supplementation in adults has also been questioned.[74 75] However, even if aggressive supplementation were of proven benefit in adults, **therapeutic effects and doses cannot safely be extrapolated from older populations to preterm infants**.[16-21]

For the general infant population, the American Academy of Pediatrics (AAP) recently increased its recommended enteral vit. D intake from 200 IU/day to 400 IU/day, based on a goal of achieving higher serum levels of 25(OH)D3, but not based on evidence of differences in clinical outcomes in infants.[15] Breast milk generally has very low levels of vit. D (≤25 IU/L),[76] and so many recommendations have focused on exclusively or partially breast-fed infants. Among breastfed term infants in Berlin (a city with low sunlight exposure), supplementation with either 250 or 500 IU/day for 6 wks was sufficient to correct low serum levels.[77] **There is a paucity of data on which to base recommendations for preterm infants ≥28 wks GA,** especially after discharge from the NICU, with most recommendations focusing on term infants or acutely ill VLBW infants. Infants born preterm are more likely to receive formula or human milk fortifiers designed for

convalescing preterms which contain higher levels of vit. D and calcium than term formulas. Thus, as their volume of intake increases with growth and maturation, they will reach higher levels of vit. D in their diet faster than term infants, thereby increasing the potential to reach a total intake much higher than 400 IU/day when also receiving supplemental vitamins. Similarly, black infants are less likely than white infants to be breast-fed,[78] and therefore they are more likely to be receiving vit. D fortified formulas.

This study will test the comparative effectiveness of two different vit. D supplementation strategies in black infants born preterm: (I) sustained supplementation with 400 IU/day of D3 until 6 months of age adjusted for prematurity, or (II) cessation of supplementation when the infant is taking ≥ 200 IU/day from diet. We will focus on a specific population, black preterm infants, with a unique potential for both positive and negative effects from vit. D supplementation, who may require a tailored approach to supplementation that may differ from the general infant population. This study will focus on clinical outcomes as well as surrogate markers of pulmonary health, allergy, and bone health. While arguments can be made in support of either supplementation strategy (summarized below), very few studies have targeted this population. The proposed study will therefore fill a large gap in the literature and have important public health implications.

A3. Argument for continuing vit. D supplementation to promote higher total intake (strategy I):

The rationale for more aggressive vit. D supplementation springs from a growing body of evidence that low levels of 25(OH)D3 are associated with disease states in older children and adults. Vit. D is thought to have anti-inflammatory properties, and is hydroxylated to its active form, $1,25(OH)_2$ D3, in T-cells, B-cells, and macrophages.[54 63] In addition, vit. D deficiency and polymorphisms of genes in the D pathway have been associated with increased susceptibility to viral respiratory illnesses, tuberculosis, and HIV.[37 38 40 41 48 50 79 80]

Vit. D deficiency is associated with wheezing illnesses in children. Among 6-14 year old Costa Rican children with asthma, lower vit. D levels are associated with markers of increased severity of allergy and asthma.[59] Lower 25(OH)D levels are associated with poorer asthma control.[60 81] Among American inner city youth, the prevalence of D deficiency is higher in children with asthma than in those without asthma.[52] Several studies have also found an association between low D levels or rickets and acute lower respiratory infections in children.[55 82-85] A polymorphism associated with decreased rates of transcription of vit. D receptor (VDR) RNA has been linked to increased rates of acute lower respiratory infections in infants.[79] *In vivo*, D decreases the NF-κB-mediated inflammatory response to respiratory syncytial virus (RSV) without impairing viral clearance.[57] Cord blood vit. D deficiency is associated with wheezing and RSV bronchiolitis in infancy.[86 87]

Although lower vit. D levels or polymorphisms decreasing sensitivity to D are associated with pulmonary infections and asthma, neither the presence nor direction of causality has been established. Results of supplementation trials have been mixed, although many are still ongoing.[72 74 75] For instance, in a recent trial, supplementation did not speed treatment of tuberculosis.[88] No studies, however, have targeted the association between vit. D status and wheezing with or without respiratory infection in black preterm infants.

A4. Argument for stopping vit. D supplementation when a minimum dietary intake is reached (strategy II):

While *in vivo* data show a role for vit. D in immune defenses, and epidemiologic studies have shown an association between low serum D levels and certain disease states, some authors have cautioned that claims about a role for D supplementation in combating infection are not well justified.[74] In a cohort of infants at 6-36 mo. of age, 25(OH)D level was not associated with illness in infancy, although vit. D binding protein genotype was, suggesting that simply targeting higher levels of vitamin D may not in fact promote health.[89] In response to the "deficiency/disease model" which interprets associations between low 25(OH)D3 levels and several infectious and inflammatory disease processes in adults as proof that vit. D deficiency promotes disease, an "alternative hypothesis" also has been proposed.[90] This hypothesis notes that vit. D metabolism is intricately regulated, and lower levels may actually be secondary to the disease process. This theory interprets short-term benefits of D supplementation in certain disease states, particularly auto-immune diseases, as resulting from short-term anti-inflammatory effects; a corollary of this theory mandates measurement of long-term outcomes in studies of vit. D supplementation. Finally, apparent associations between low vit. D levels and disease states may not result from a causal link, but rather from confounding by other related disease states, nutritional deficiencies, or socioeconomic disparities.

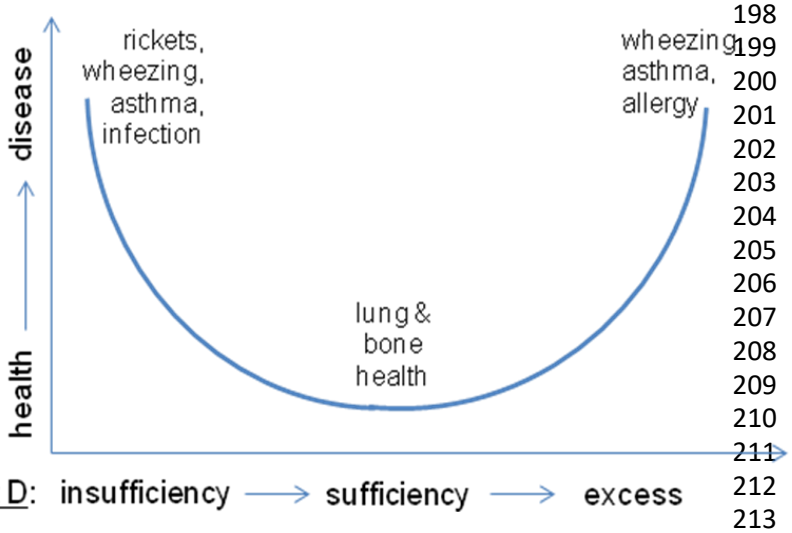
In the preterm population, there is also reason for concern about the safety of aggressive supplementation. Exposing the immature immune system to vit. D may predispose infants to asthma and allergy later in life. *In vitro* studies and mouse models suggest that vitamins may skew T cells towards either less allergic T-helper 1

(vitamins B_6, E, and C) or a more allergic T-helper 2 (vitamins D and A) phenotypes.[9 10 64 65 91-95] There may be a critical developmental window for such an effect.[36] If this is the case, premature infants could be more or less vulnerable than term infants. Milner et al. (2004) found that black infants exposed to multivitamins in the first 6 mo. of life had a higher risk of asthma in early childhood, and that formula-fed vitamin-exposed infants of all races experience more food allergies.[2] Vit. D supplementation at 3 years of age was not associated with asthma, **suggesting that a critical window for exposure exists in early infancy, a time of rapid pulmonary and immunologic maturational changes**.[36] The study by Milner et al. assessed a cohort not yet impacted by the 2003 recommendations for a minimum of 200 IU of D from formula or vitamin supplements. The authors hypothesized that vit. D and A exposure in infancy were potentially skewing T-cells towards a more allergic T-helper-2 (Th2) cytokine profile.[2 10 65 66] Vit. D exposure in infancy was also associated with allergy and asthma among Finnish young adults.[96] These data suggest that infants' developing immune systems may be vulnerable to immunomodulation,[97] and we hypothesize that the preterm immune system may be even more vulnerable to the immunomodulatory effects of vit. D. In addition, preterm infants are more likely than term infants to receive vit. supplementation during this potentially critical window.[2] The results of studies assessing the association between maternal vit. D status during pregnancy and respiratory disease in the offspring have been mixed, but fetal exposure and enteral exposure *ex-utero* should not be equated.[61 98-100]

Furthermore, there is evidence for racial differences in the relationship between vit. D status and health. More may not always be better for black patients. In black diabetic adults, 25(OH)D levels are positively correlated with calcified atherosclerotic plaque.[75] Among postmenopausal women, black women have less bone turnover than white women despite lower 25(OH)D levels and higher PTH levels.[101] Consistent with our preliminary data, Milner et al. found that black, but not white, infants supplemented with multivitamins in the first 6 months of life had an increased risk of asthma at age 3 years.[2] In a study of black children with asthma, patients with a certain polymorphism in CYP24A1 (an enzyme that inactivates $1,25(OH)_2D$) had lower vit. D levels but improved pulmonary function (as measured by FEV1/FVC), suggesting that in black children, higher 25(OH)D levels may not always be better for pulmonary health.[102] Finally, among white mothers, both low and very high serum levels of vit. D are associated with having a small for GA infant (a U-shaped relationship), whereas for black mothers there is no association between maternal serum vitamin D levels and having a small infant.[103]

Although low 25-OH D levels have been associated with pulmonary diseases in adult and pediatric populations, it is not known whether (1) this relationship exists in black infants born preterm, especially with regards to recurrent wheezing in infancy, (2) whether this is a causal relationship amenable to amelioration with supplementation, or (3) whether supplementation beyond that necessary to prevent rickets benefits or harms pulmonary health in this population.

Figure 1: Proposed U-shaped relationship between vit. D status and health.



Modulation or stimulation of the vit. D pathway has the potential to positively or negatively impact lung and airway development, response to respiratory infections, pulmonary inflammation, airway reactivity, and immune development, particularly in the developmentally immature preterm. Preterm infants could be particularly vulnerable to either the positive or negative effects of supplementation, because of their developmental immaturity and their elevated risk of chronic pulmonary morbidity. Furthermore, there are potentially competing outcomes, with one dosing strategy being preferable to minimize the risk of some disease states, but not others. For instance, the strategy II dosing approach could be superior for pulmonary health, but not bone health. It is important to study the actual effects of supplementation strategies, with a focus on clinically meaningful outcomes.

Terminology: In our review of the relevant literature, data are presented on both **wheezing** in infancy and **asthma**, but this should not be interpreted as a claim of equivalency. The focus of this study will be persistent

wheezing in the first year of life, although we will calculate an asthma predictive index as a secondary outcome. Furthermore, from both economic and psychosocial perspectives, recurrent wheezing during infancy itself is a clinically meaningful outcome.

A5. The optimal dose of Vit. D for bone health in black preterm infants is not known:

The consequences of any vit. D supplementation regimen on bone health must also be considered. While insufficient vit. D supplementation may lead to rickets or impaired resolution of osteopenia of prematurity, hypervitaminosis D may lead to hypercalcemia, hypercalciuria, polyuria, dehydration, hypertension, urinary tract stones, nephrocalcinosis, or metastatic calcifications.[104 105] The goal of supplementation should be to provide adequate vit. D to maintain bone health without incurring the sequelae of hypervitaminosis. **The AAP recently increased its recommended enteral vit. D intake from 200 IU/day to 400 IU/day, based on a goal of achieving higher serum levels of 25(OH)D3, but not based on evidence of more clinical rickets at the 200 IU/day dose**.[15] This statement acknowledges that this change was made primarily due to extrapolation from studies in older patients, and is intended to target 25(OH)D3 levels ≥50 nmol/L (~20 ng/ml), although this target level is also extrapolated from older populations; the ideal level for even term infants not known. Furthermore, while the AAP statement addresses the fact that black patients have lower levels of 25(OH)D3 than white patients, it does not address what portion of this may be due to racial differences in the "set-point" or secondary to comorbidities, and what portion may be due to true deficiency amenable to supplementation.

Because preterm infants largely fail to match third trimester calcium and phosphorous accretion rates *ex-utero* due to limitations in parenteral and enteral nutrition and exposure to calcium wasting drugs, preterm infants often experience osteopenia. Therefore, some authorities recommend higher vit. D dosing in this subpopulation, although the primary treatment is provision of adequate enteral calcium and phosphorous, and osteopenia of prematurity is most common in infants <30 wks GA, not the population proposed in this study. Data on the optimal dose of vit. D for preterm infants are limited, and mostly focus on the nutritional management of very low birth weight infants in the NICU. Recommendations range widely from 200 IU to 1600 IU.[11-14 106] In a randomized trial of 39 infants born at <33 weeks GA, Backström et al found that subjects randomized to a supplement of 200 IU/kg up to 400 IU/day did not have significant differences in serum inorganic phosphate, plasma ionized calcium, alkaline phosphatase concentration, serum intact parathyroid concentration, or bone mineral content and density when compared to the group randomized to 960 IU/day.[107] Serum 25(OH)D3 levels were non-significantly higher in the 960 IU group at 6 weeks of age, and the authors concluded that 200-400 IU/day was sufficient to maintain bone health in preterm infants. In another study of 70 preterm infants, no difference was found in bone mineral status at 3 months or 9-11 years of age among infants who had been randomized to 500 IU/day versus 1000 IU/day supplementation in early infancy.[108] Interestingly, exposure to breast-milk was positively correlated with lumbar bone mineral density at school age despite the lower levels of vit. D in breast milk compared to formula.

There is very little evidence that either the higher or lower range of current recommendations is superior for bone health in preterm infants. These studies have largely not addressed (1) impact on pulmonary health, (2) the potentially unique needs of black infants, or (3) the needs of healthy infants born moderately preterm and late preterm. This study aims to identify a supplementation strategy that will minimize recurrent wheezing in infancy while safely maintaining bone health. We hypothesize that strategy II (diet-limited) will be sufficient to prevent rickets and the impaired response to infection associated with D deficiency, and will also result in less recurrent wheezing in infancy than strategy I (sustained).

A6. PRELIMINARY STUDIES

Our preliminary data are based on an ongoing cohort study prospectively following infants without BPD born at $28^{0/7}$-$34^{6/7}$ wks GA at Rainbow Babies and Children's Hospital (RB&C) (K23 HD056299). This study will enroll 300 infants, and as of 6/13/11, 297 subjects had been enrolled from this single center. A questionnaire is administered at 3, 6, 9, and 12 mo. adjusted age to assess respiratory symptoms. Black infants make up 50% of this cohort. Our enrollment rate is on-target and our follow-up rate is 92%, demonstrating the ability to recruit and retain subjects, including a high percentage of minorities and socioeconomically disadvantaged participants. Until the time of this preliminary analysis, the policy of the Rainbow NICU was to supplement infants with 400 IU/day of vit. D if they were receiving less than 200 IU from formula or fortifier; this strategy is intermediate between the 2003 and 2008 AAP recommendations (table 4). Among the infants in this study, 55% were discharged from the NICU on a vitamin supplement. After discharge, management was at the discretion of the family and their primary care provider.

Overall, black infants experienced more wheezing than white infants. At each assessment time point, black infants who had been discharged on vitamins reported more wheezing than black infants not on vitamins; white infants discharged on vitamins had less reported wheezing than white infants not on vitamins (table 1). Among those discharged on vitamins, 76% were still taking them at 3 months, and 49% at 6 months. Among infants not discharged on vitamins, 17% were taking them at 3 months, and 16% at 6 months.

Table 1. Preliminary Data: Relationship Among Race, Vitamin Supplementation, and Wheezing.

	Percent reporting wheezing since previous assessment at each follow-up timepoint.			
	3 month	6 month	9 months	12 months
Black infants discharged on vitamins	34% (16/47)	42% (15/36)	45% (13/29)	44% (12/27)
Black infants not discharged on vitamins	33% (15/46)	26% (10/39)	29% (10/34)	33% (10/30)
Relative risk	1.0	1.6	1.6	1.3
White infants discharged on vitamins	16% (9/55)	26% (14/54)	20% (10/51)	20% (9/45)
White infants not discharged on vitamins	39% (16/41)	43% (16/37)	29%(10/34)	30% (7/27)
Relative risk	0.4	0.6	0.7	0.7

The above table includes all children at the time of the interim analysis in Aug. 2010, including those in the process of the year-long follow-up. Next, we focus on the subset of 143 infants who had completed the study at the time of the analysis, so that we can restrict our analysis to a group of infants that has had an equal opportunity to experience recurrent wheezing. In a logistic regression adjusted for GA and exposure to breast milk, the odds ratio of recurrent wheezing in white infants (n=73) discharged on vitamins compared to those not discharged on vitamins was 0.63 (95% CI: (0.22, 1.78)), and the adjusted odds ratio of recurrent wheezing in black infants (n=70) discharged on vitamins compared to those not discharged on vitamins was 1.33 (95%CI: (0.44, 4.02)). **The effect seems to be strongest in the more mature preterm infants, with the adjusted odds ratio for recurrent wheezing in black infants ≥30 wks GA of 1.55 (95% CI: (0.46, 5.26)), and for black infants >32 weeks of 3.32 (0.553, 19.98).** This apparent maturity effect could potentially be due to a critical developmental window, or because the less mature infants have more competing causes of wheezing. Although these numbers are not statistically significant, we would not expect them to be since this is a preliminary analysis of an ongoing study that was not powered for a stratified analysis by race, and infants are still being enrolled and followed. The direction of the estimates for recurrent wheezing in the subset of infants who have completed the study is consistent with the cross-sectional data for all of the enrolled infants (table 1). Furthermore, this consistent pattern is seen despite some cross-over in vitamin exposure after discharge, which we would expect to bias our results towards the null.

Our preliminary data also give us insight into the outpatient feeding practices for healthy black preterm infants in Cleveland. At enrollment (within ~2 wks of anticipated hospital discharge), 36% were receiving only maternal milk. At 3 mo. adjusted age, 4.5% were receiving only maternal milk, 82% were receiving only formula, and 13.5% were receiving a combination of formula and maternal milk. By 6 mo. adjusted age, 0% were exclusively breast fed and 95.9% were exclusively formula fed. Therefore, the majority of infants in this population, despite efforts to promote breast feeding, are receiving the bulk of their nutrition from formula enriched with vit. D. Therefore, the question of whether to stop vit. D supplementation when infants reach a threshold dietary intake or leave them on supplementation in addition to their dietary sources is particularly relevant.

In a separate pilot study, we enrolled 80 infants born preterm and term, and administered respiratory questionnaires and genotyped the patients for VDR polymorphisms. The best-enrolling site in this pilot was the University Hospitals clinic, which primarily treats socioeconomically disadvantaged black children from Cleveland; we received several calls from parents seeking out our recruiter if they had missed her during their child's visit and expressing willingness to return to the hospital just to participate in the study. This again demonstrates our ability to recruit a population demographically similar to the one in the proposal.

B. INNOVATION

The proposed study is innovative because it (1) focuses on an understudied population of infants 28-36 wks GA, and (2) addresses the unique needs of black preterm infants, whereas most nutritional studies in preterm infants have not accounted for potential differences by race, and (3) focuses on the impact of vit. D supplementation on clinically meaningful measures of pulmonary health and overall health, whereas prior

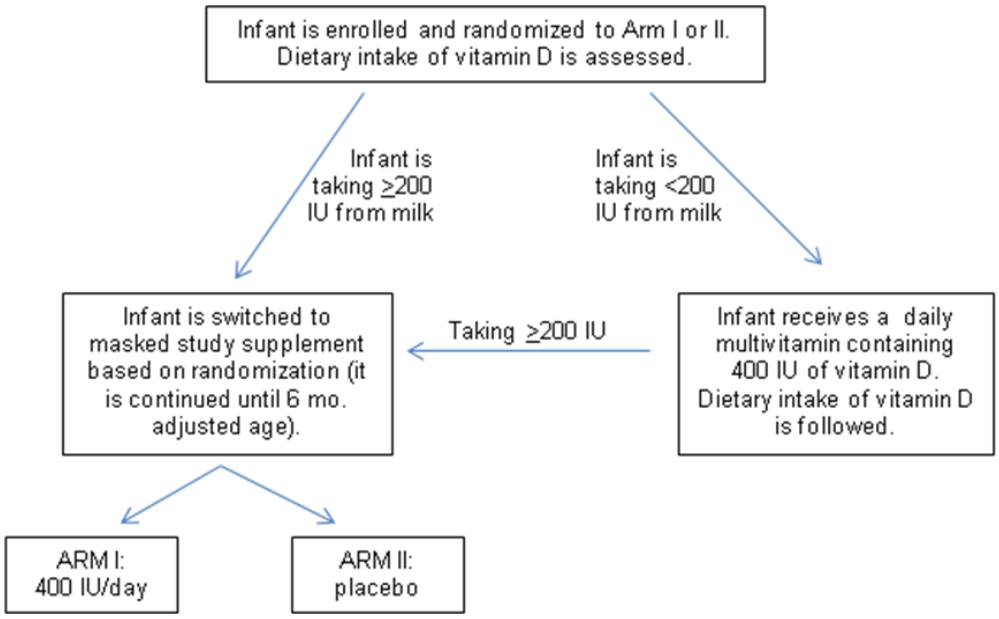
studies of vit. D in preterm health have mostly (a) focused exclusively on bone health and (b) measured short-term surrogate endpoints as opposed to clinical outcomes.

In 2006, the IOM highlighted the cost of moderately preterm infants, or those delivered between 30 and 34 weeks gestation, as a significant contributor to the $26 billion dollars spent annually on prematurity in the United States.[109 110] Despite the lack of current data on the vit. D needs of black infants born at 28-36 wks GA, and the paucity of information on how vit. D positively or negatively influences pulmonary and immune health, ~100,000 black children per year are born at 30-36 wks GA in the U.S.,[1] and choices about vit. D supplementation must be made for all of them. Therefore, this study has the potential to significantly **impact** public health recommendations. The results of this study could lead to a supplementation strategy that minimizes long-term morbidities in this population, including chronic wheezing and allergic diseases.

C. APPROACH

C1. We are proposing a masked placebo-controlled randomized clinical trial of the comparative effectiveness and safety of two different vit. D supplementation strategies in 300 black infants born preterm at $28^{0/7}$-$36^{6/7}$ weeks GA. Infants will be enrolled in the NICU or newborn nursery when they are tolerating full volume feeds and are off oxygen. All infants will receive a daily standard infant multivitamin containing 400 IU of D3 until they are taking at least 200 IU/day of vit. D daily from formula or fortifier. Most infants taking formula will reach this amount by 0-1 mo. adjusted age. Infants will then be randomized to receive either 400 IU of D3/day (Strategy I) or placebo (Strategy II). Study drug will be administered until 6 mo. adjusted age, at which time supplementation will be stopped. Although rates of exclusive breastfeeding beyond 6 mo. are extremely low in this population (0% in our preliminary data), we will continue to provide any exclusively breast-feeding infants with a 400 IU/ml D3 supplement. Study visits will occur at 3, 6, 9, and 12 mo. adjusted age.

The primary outcome will be parent-reported **recurrent wheezing** (≥2 episodes of wheezing with or without an infection) between initial hospital discharge and 12 mo. corrected age. Recurrent wheezing will be defined as one report of >1 wheezing episode since the last interview, or reports of single wheezing episodes at multiple follow-up visits.



Secondary outcomes will include: history of upper and lower respiratory tract infections, pulmonary medication use, the Phadiatop Infant assay of allergic sensitization, hospitalization and ER visits, parental report of allergies and eczema, skin exams for eczema, a calculation of the asthma predictive index, 25(OH)D3 levels, serum markers of bone health (alkaline phosphatase, calcium, phosphorous,), and a speed-of-sound bone density assessment. Economic data will be gathered for use in future studies and for availability for meta-analysis, but will not be analyzed as part of this study.

Table 1. Summary of Study Design]

Intake of vit. D. from formula	Study vitamin
<200 IU/day	Open label multivitamin containing 400 IU/day of vit. D
≥200 IU/day	Randomized to 400 IU/day of vit. D (strategy I, n=150) or placebo (strategy II, n=150)

Table 2 Outcome Measures

	Outcome Measure	Measurement Tools	Comments
Aims 1 and 3	Recurrent Wheezing	International Study of Asthma and Allergies (ISAAC) questionnaire wheezing module.	The ISAAC questionnaire has been validated for use in children.[111 112] A slightly modified version to reflect the shorter assessment intervals and younger children in this study will be used. **The phrasing of the core question about wheezing, "wheezing or whistling in the chest," is standardized wording that has also been used in the American Thoracic Society child questionnaire ATS-DLD-78-C,[113] and in the follow-up assessments of multi-center trials studying preterm infants** (e.g., NO CLD[114 115] and SUPPORT[116 117], **and in the study on which or preliminary data are based** (K23-HD056299)). Parent-identified wheezing has been shown to have good agreement with concurrent physician auscultation.[118] Additional questions based on the ATS-DLD-78-C will further identify whether wheezing occurred with or without a respiratory infection, for secondary analyses of wheezing episodes by apparent etiology. **Such questions are used in clinical practice and are meaningful to providers and patients.** Pulmonary medications, hospitalization, ER visits, and outpatient sick visits will also be captured.
Aim 2	Aeroallergen and Food Sensitivities	Phadiatop Infant assay ISAAC questionnaire allergy module	The Phadiatop® Infant assay, an *in vitro* test for IgE antibodies to food and inhalant allergens, has been recommended by the NHLBI and NIAID as the biomarker to measure sensitization in infants, It is 96% sensitive and specific.[123 124] The assay yields a positive or negative result for sensitization but does not report the specific antigen. Clinical allergy symptoms will be assessed by questionnaire. The ISAAC questionnaire has been validated for use in children; a modified version to reflect the shorter assessment intervals and younger children will be used. Additional questions about types of allergies and food allergies based on the ATS-DLD-78-C will also be used. Food sensitization in infancy is strongly associated with the risk of childhood asthma.[125]
	Eczema	SCORAD and ISAAC eczema module.	The validated European Taskforce on Atopic Dermatitis SCORAD eczema assessment tool will be completed during the follow-up visits.[126-128] A question about prior diagnoses of eczema excerpted from the ISAAC eczema module will also be used. Eczema in infancy is associated with allergy in childhood.[125]
	Laboratory Values	Eosinophil count.	Eosinophilia at 9-11 mo. has been associated with increased risk of childhood asthma,[129] and may also be elevated with food allergies. It will be assessed at the 12 mo visit, and used to calculate the Asthma Predictive Index,]
	Modified Asthma predictive index (API)	Composite score comprised of other outcomes[129]	The modified API is determined by recurrent wheezing, history of eczema, parental history of asthma, eosinophilia, sensitization to aeroallergens and foods, and wheezing apart from colds. [129-131] The API will be used to describe differences in risk in the study arms. We expect a similar distribution of family history in the arms, but will examine that assumption.
Safety	Adverse Events	e.g., fracture, kidney, stones, rickets, etc.	Clinical adverse events potentially related to vit. D supplementation will result in unblinding and referral to specialty care. Adverse events will be reported to the DSMB, IRB, and NIH in compliance with local and federal guidelines.
	Laboratory values	Alkaline phosphatase, , calcium, phosphorous, 25(OH)-D	Differences in serum markers of bone health will be compared. Hypocalcemia, hypercalcemia, hypophosphatemia, or hyperphosphatemia will result in unblinding, removal from the protocol, and referral to specialty care. Because the optimal serum levels of vit. D are not known, and because of potentially competing positive and negative clinical outcomes, serum levels of vitamin D will not be used to unblind a patient or remove them from the protocol except in cases of extreme deficiency (level <15 ng/ml) or excess (>80 ng/ml).
	Bone Density	Tibial speed of sound	Norms have been published for infant and pediatric speed of sound (ultrasound) measurements of bone density.[132-137]

Randomization: Infants will be randomized 1:1 to a dosing strategy using permuted blocks. Block size will be randomly varied and investigators and study personnel will be blinded to block size. Randomization will be stratified by site and maternal milk exposure (receiving any maternal breast milk at enrollment). Families, treating physicians, and study staff will be blinded to assignment. Randomization will be performed by the CTSC-supported Case Western Reserve University Center for Clinical Investigation Statistical Sciences Core.

Inclusion criteria: (1) $28^{0/7}$-$36^{6/7}$ wks GA at birth; (2) family identifies the child as black or African American; (3) ≤28 days of supplemental oxygen (subsequent oxygen therapy for <72 hrs for a brief subsequent illness or surgery will be allowed); (4) admitted to the NICU, special care nursery, transitional care nursery, or well-baby nursery as a neonate; and (5) ≤40 wks corrected GA at enrollment.

Exclusion criteria: (1) BPD (>28 days of supplemental oxygen); (2) pre-existing diagnosis of moderate to severe osteopenia of prematurity and/or alkaline phosphatase ≥700; (3) history of fracture; (4) gastrointestinal surgery, including for NEC; (5) known gastrointestinal malabsorption; (6) major congenital anomaly; (7) congenital pulmonary or airway disorder (e.g., cystic fibrosis, tracheomalacia, swallowing disorder, bronchopulmonary sequestration); (8) documented wheezing or stridor prior to enrollment; (9) previous vit. D supplementation with >400 IU/day; (10) family plans to move more than the pre-determined catchment area at each site (60 miles from CWRU, to be determined before enrollment at each additional site); (11) baseline hypo- or hypercalcemia, hypo- or hyperphosphatemia; and (12) baseline 25(OH)D level <10 ng/ml or >80 ng/ml.

We will exclude infants born at <28 wks GA or those with BPD (oxygen requirement >28 days), as these infants do not represent the majority of NICU patients, and they are likely to have more early lung injury and architectural developmental abnormalities pre-dating randomization; they are also more likely to have osteopenia of prematurity requiring treatment with higher than usual doses of vit. D, calcium, or phosphorous. The apparently “healthy” infants included represent the majority of preterm infants born in the U.S..

Strategy I: Infants will receive 400 IU/day of D3 in the form of a standard infant multivitamin until they are taking 200 IU/day of vit. D from formula or human milk fortifier. This will also ensure sufficient intake of other vitamins, such as vit. A, until the volume of intake is sufficient. Once they are taking ≥ 200 IU of vit. D from their diet, **infants will be supplemented with 400 IU/day of D3** (D-Vi-Sol™) until 6 mo. corrected age.

Strategy II: Infants will receive 400 IU/day of D3 in the form of a standard infant multivitamin until they are taking ≥200 IU/day of vit. D from formula or human milk fortifier. Subsequently, they **will receive placebo** (color-matched commercially available pediatric compounding solution).

Study procedures: Infants will be assessed by home-visits at 3, 6, and 9 mo. adjusted age (If participants are uncomfortable having the study team come to their home or prefer to come to the clinic for any reason, they can be seen in clinic). If additional sites outside of Cleveland are added, the sites may choose to conduct the 3, 6, and 9 month visits in the clinic if home visits are not feasible due to that site’s infrastructure. A scheduled clinic visit will occur at 12 mo. for physical exam and lab work. A dietary history and reinforcement of study supplement instructions will be done over the phone monthly between study visits until 6 mo adjusted age. The appropriate study medication (multivitamin, D3 supplement, or placebo) will be delivered to the home. A 1 ml syringe will be provided for administration to avoid any unintentional overdoses.[105]

Summary of 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

Table 3. Study procedures.

	Enrollment	Hospital discharge	3 mo. adjusted age	6 mo. adjusted age	9 mo. adjusted age	12 mo. adjusted age
Assessment of dietary vit. D intake.*	X	X	X	X	x	x
Home visit***			X	X	X	
Respiratory questionnaire			X	X	X	X
calcium, phosphorous, alkaline phosphatase**	X		X	X		
25(OH)D3,	X		X	X		X
Lung exam	X					X
Skin Exam for eczema			X	X	X	X
Phadiatop® Infant assay						X
Eosinophil Count						X
Bone Density (tibial speed of sound ultrasound)						X

*Also be assessed monthly by phone., **When possible, lab tests prior to hospital discharge will be run on scavenged blood or coordinated with scheduled blood draws to avoid an additional needle stick for the infants. *** If additional sites outside of Cleveland are added, the sites may choose to conduct the 3, 6, and 9 month visits in the clinic if home visits are not feasible due to that site's infrastructure.

C2. Dosing: This effectiveness study compares supplementation strategies that can be feasibly followed by a family and primary care provider in a busy practice. D3 will be used, as this is the most common form of vit. D in over-the-counter infant supplements. Our preliminary data show that very few black preterm infants in Cleveland are exclusively breast-fed and most mothers stop breast-feeding by 3 mo. adjusted age, so most infants will be receiving a significant amount of vit. D from formula. Intake in both arms is within the current wide range of recommendations for preterm infants.[11 14]

Due to a great heterogeneity in practice springing from the paucity of evidence for the needs of this population, both strategies represent vit. D exposures currently experienced by black preterm infants. Once an infant is taking a minimum of 200 IU/day (typically achieved by 0-1 mo. adjusted age in formula-fed premature infants), the daily intake of the two arms differs by 400 IU/day. Arm I results in a minimum daily intake of 400 IU, and an overall intake that exceeds the 2008 AAP recommendations. Arm II results in a minimum daily intake of 200 IU, and total intake over 6 months that is intermediate between the 2003 and 2008 AAP recommendations. Infants in arm I may slightly exceed the 2010 IOM recommendations for a total intake of <1000 IU/day in infants less than 6 months, and infants in arm II will likely remain within this range (table 4).[138]

Table 4. Comparison of proposed trial arms and other supplementation strategies.

	Trial Arms		Other Strategies for Comparison		
	Strategy I. Sustained supplementation	**Strategy II. Supplementation titrated based on dietary intake**	AAP 2003 report	AAP 2008 report	RB&C practice at time of Preliminary Data
Minimum total daily intake (IU/day)	400	200	200	400	200
Dose of D supplement (IU/day)	400	400	200	400	400
Initiation of D	When on full feeds	When on full feeds	≤2 mo. old	≤1 wk old	On full feeds
Dietary intake of D to start supplement (IU/day)	All receive D	< 200	<200	< 400	<200
Dietary intake of D to stop supplement (IU/day)	Continue until 6 mo adjusted age	≥200	≥200	≥ 400	Per family/ provider
Supplementation if taking >1L/day term formula (IU/day)	400	Placebo	None	None	None

Supplementation if taking 800 ml/day of term formula (IU/day)	400	Placebo	None	400	None
Supplementation if taking 800 ml/day Enfacare (IU/day)	400	Placebo	None	None	None
Supplementation if taking 400 ml/day Enfacare (IU/day)	400	Placebo	None	400	None
Supplementation if taking 300 ml/day Enfacare (IU/day)	400	400	200	400	400
Supplementation if exclusively breast-fed (unfortified) (IU/day)	400	400	200	400	400
Total daily enteral D intake (IU/day of diet + supplement) for infant drinking:*					
250 ml term formula	~500	~500	~300	~500	~100-500**
250 ml transititional formula	~548	~548	~348	~548	~148-548**
500 ml term formula	~600	~200	~200	~600	~200-600**
500 ml transititional formula	~895	~295	~295	~895	~295-895**
1000 ml term formula	~800	~400	~400	~400	~400-800**
1000 ml transititional formula	~990	~590	~590	~590	~590-990**
1500 ml term formula	~1000	~600	~600	~600	~600-1000**
1500 ml transititional formula	~1285	~885	~885	~885	~885-1285**

* Term formula generally contains 400-410 IU/L, transitional or post-discharge preterm formulas contain 520-590 IU/L of vit. D. **A range is presented to represent infants on and off supplements after discharge. Total milk intake increases over time, with the volumes listed representing a typical progression in the first several months of age.

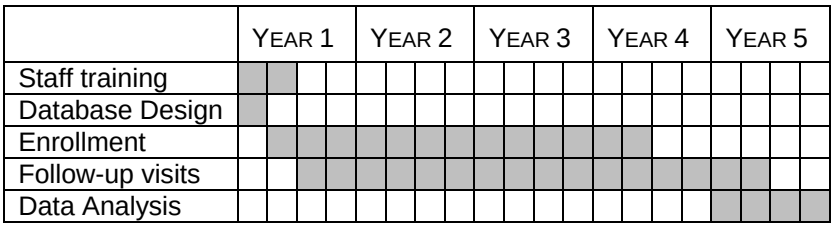
We have selected the current supplementation dosing because we do not feel that a dosing strategy with a total daily intake of <200 IU is safe or ethical due to the risk of rickets. Conversely, although studies in a northern Finland cohort showed an association between supplement doses ≥2000 IU/day and allergy and atopy later in life, our preliminary data in black infants and the Milner (2004) cohort study showing increased asthma and allergy among supplemented black infants suggest an association at much lower doses in this population; both of these studies were based on over-the-counter vitamin use, which corresponded to recommended doses of 200-400 IU/day.[2 94-96] With concerns about adverse effects at supplementation doses of 400 IU/day, and the current IOM recommendations to limit total (diet + supplement) intake to ≤1000 IU/day, we also do not feel that it would be ethical to expose this population to higher doses before the safety of the 400 IU supplement is established with regards to wheezing and allergy. The current dosing strategy nevertheless allows for separation of total dietary intake of vit. D of 400 IU/day between the treatment groups (table 4).

The vitamin supplements that will be used (multivitamins and D-only preparations) with a concentration of 400 IU/ml of D3 are commercially available over-the-counter and are generally considered to be safe. Inclusion and exclusion criteria listed in the protocol are intended to exclude infants for whom the dosing strategies in the protocol may not be appropriate (for instance, those with severe osteopenia). Infants on study supplement with levels <15 ng/ml or >85 ng/ml at follow-up will stop their masked supplement and their levels will be reported to their primary care provider for management; they will continue with their follow-up visits.

In Cleveland, study supplements will be dispensed by the MetroHealth investigational pharmacy for the MetroHealth inpatients, and by the University Hospitals investigational pharmacy for the University Hospitals inpatients and all outpatients, in order to standardize the packaging and delivery of masked supplement. At other sites, the local research pharmacists will dispense study drug in accordance with study, site, and federal procedures. Masked supplement will be prepared under standardized sterile conditions and in compliance with pediatric pharmacy best practices and regulations. The placebo will be Ora-Sweet SF commercially available flavored pediatric compounding solution that is sugar-free and alcohol-free. Since infants in both arms will be switched from a multivitamin to either the masked vit. D or placebo, no family will receive both the D-only product and the placebo for direct comparison. Study meds will be delivered to the home monthly by the research nurse or FedEx. The first time an infant is taking >200 IU/day from diet and is switched to masked D/placebo, the study supplement will be given to the family by the research nurse in person to reinforce medication dosing and teaching. Any remaining supplements from the previous months will be weighed by the research nurse at each visit to assess compliance.

Performance Sites: Infants will be recruited from two hospitals that are affiliated with CWRU that have level III NICUs and a track-record for collaborative neonatal research: University Hospitals Case Medical Center (containing the Rainbow Babies and Children's Hospital and the MacDonald Women's Hospital) and MetroHealth Medical Center. Combined, these centers admit over 900 preterm infants/year born at $30^{0/7}$-$36^{6/7}$ wks GA, >50% of whom are black. CWRU is located Cleveland, OH, which is at 41^{o} N latitude and averages 66 sunny days, 97 partly cloudy days, and 202 cloudy days per year.[139] Additional sites may be added if approved by the DSMB and NIH.

Table 5. Study Timeline. 300 infants will be recruited over 39 mo. (~7-8 /mo.) at the two CWRU hospitals.



C3. Statistical Analysis:

Overall modeling strategy: Analyses will be based on an **intent-to-treat** approach. To assess the success of randomization, standard summary statistics will be used to compare baseline characteristics of interest (*e.g.*, potential confounders) between the two arms. For instance, frequencies and proportions will be used to summarize GA (28-33 vs. 34-36 wks), birth weight, family history of asthma and allergy, socioeconomic status, daycare, number of children in the family, season of birth, and Synagis exposure. Pearson chi-squared tests will also be used to compare proportions between arms. Alternatively, means, standard deviations, medians, and inter-quartile ranges will be used to summarize continuous characteristics and outcomes. A two-sample Welch's t-test will then be used to compare the mean of the variable between arms.

Because the incidence of binary composite outcomes, such as recurrent wheezing, is not likely to be small, relative risks (RR) and odds ratios (OR) will not be similar, and so binary outcomes will be modeled using a Poisson regression model to estimate RR and a logistic regression model to estimate OR. Secondary analyses will include an interaction between GA and arm to investigate if the treatment effect on each outcome differs by GA. Continuous or binary outcomes that are measured at repeated times (such as allergy or eczema) will be modeled using generalized linear mixed models that model the correlated data among an infant over time using random intercepts and slopes. These models will include treatment arm and time since randomization to allow us to estimate the average treatment effects on responses over time. Additional models will include an interaction between treatment arm and GA, as well as treatment arm and time, to estimate the average treatment effect on the response at 3, 6, 9, and 12 mo. adjusted age. Unless otherwise specified, a 0.05 two-sided significance level will be used for all analyses. All analyses will be performed using SAS 9.2.

Covariate adjustment: Although imbalance between the arms will only occur by chance due to randomization, secondary analyses will include models that adjust for time since randomization, enrollment site, breast milk exposure at enrollment, Synagis, and GA. While covariate adjustment in a clinical trial setting may ensure achievement of the correct significance level as well as increase statistical power, if a covariate is not strongly related to an outcome but is imbalanced between arms, adjustment for an imbalanced covariate is not required and in fact, will decrease the precision and dilute the impact of the treatment when the outcome is binary (such as recurrent wheezing).[140] Consequently, baseline variables that have a statistically significant unadjusted association with the outcome of interest (based on a significance level of 0.10) will also be included as covariates in secondary analyses, regardless of whether or not the variable is "balanced" among treatment arms.

Adherence: Adherence will be measured at 3, 6, 9, and 12 mo. using the amount of remaining supplement during the previous 3 months. Because this study also blinds families, treating physicians, and study staff to the assigned strategy, we expect adherence to be similar between the two arms. Using a generalized mixed model, we will test this assumption by estimating the average difference in adherence between the two arms over time. We do not plan to use adherence as a covariate in the analyses or to perform subgroup analyses in adherent patients only as it is well known that such analyses will produce a biased treatment effect.[141]

Model fit: The fit of each model will also be tested using residual analysis and appropriate action will be taken if there is evidence of lack of fit (e.g. transformations or categorization of covariates or outcomes, sensitivity analyses to gauge effect of outliers on outcomes of interest, etc.).

Accounting for correlated data within twin pairs: We expect ~10% of all mothers will have twins where both twins will be randomized to the same arm. Standard methods for accounting for correlated data among twins

(GEE, mixed models, etc.) may not converge due to the small number of twin pairs. Consequently, multiple outputation will be used to ensure that correlated data among twins is appropriately modeled.[115 142]

Interim analysis: One interim analysis will be performed after one-half of the sample completes the 6-month follow-up. Recurrent wheezing will be the primary focus of planned interim analyses of efficacy and safety, although secondary outcomes will also be presented. Details of the interim analysis are in the Data Safety Monitoring Plan (DSMP).

Missing data: We estimate a 15% rate of drop-out throughout the study. For composite outcomes (such as recurrent wheezing), the models discussed assume that data are completely missing at random while for outcomes measured at multiple time points, the models discussed assume that data are missing at random. However, it is likely that data will be missing not at random and so sensitivity analyses will be conducted to ascertain the effect of missing data on the estimated treatment effects. In particular, a model based approach that jointly models the response to treatment and the drop-out process will be used.[143]

Aim 1: We will model the RR (or OR) of recurrent wheezing between the two arms using a Poisson (or logistic) regression model. Assuming that the rate of recurrent wheezing with Strategy II is 30%, and a two-sided significance level of 0.05, a sample size of 115 infants per group will detect a RR of 1.6 with 80% power. Allowing for ~15% loss to follow-up and ~10% of all mothers having twins where both twins are randomized to the same arm, it is expected that a **sample size of 300** infants (230/0.85*1.1) will be obtained. Consequently, we expect that this estimate of power is conservative.

Aim 2: The main marker for allergic sensitization for this aim will be the Phadiatop Infant® assay. We will estimate the OR of sensitization between Arm I and II using a logistic regression model and the RR using a Poisson regression model. In one study, 21% of two-year-olds had a positive Phadiatop Infant test.[123] Aeroallergen sensitization rates as high as 18-31% have been reported in high risk infant cohorts. Assuming that the rate of sensitization with Arm II is 20% and a two-sided significance level of 0.05, a sample size of 115 infants per group will detect a OR of 2.31 with 80% power. Clinical symptoms of allergy or eczema will be measured by questionnaires. In this case, we will model the average RR of allergy or eczema between the two arms over time using a generalized linear mixed model assuming a Poisson distribution and the average OR of allergy or eczema between the two arms over time using a generalized linear mixed model assuming a binomial distribution. Approximately 15%-18% of infants suffer from eczema.[144-146] Estimates of the prevalence of objectively measured food allergies in infants and toddlers range widely from approximately 2-5%.[125 147] If we conservatively assume an average rate of eczema or allergy in Arm II of 15%, a correlation of 0.5 among repeated measures, and a two-sided significance level of 0.05, a sample size of 115 infants per group will detect an average OR over time of 2.06 with 80% power.

Aim 3: We will use a logistic (or Poisson) regression model to estimate the OR (or RR) of recurrent wheezing per 1 unit increase in 25(OH)D3 level. Because 25(OH)D3 levels are measured at enrollment, 3 months, and 6 months, levels will be entered into the logistic or Poisson regression models in 1 of 2 ways: 1) each measure of 25(OH)D3 will be included in the model (separately or simultaneously); or 2) the level of vit. D received over 6 months will be summarized using the average of the three 25(OH)D3 measures. If all 3 measures of 25(OH)D3 are included in the model simultaneously, spurious results may occur if they are collinear. Although steps will be taken to reduce multi-colinearity (e.g., centering), if the correlation among the three 25(OH)D3 measures is higher than 0.70, each level will only be included in the models separately. To address our hypothesis of a U-shaped association between 25(OH)D3 and recurrent wheezing, the association between 25(OH)D3 and recurrent wheezing will be modeled using a restricted cubic spline with knots defined using the quintiles or quantiles of 25(OH)D3 levels. Using Wald-type contrasts, if our data suggests a simpler association between 25(OH)D3 and recurrent wheezing (*e.g.*, a quadratic or piecewise linear association), this association will be simplified accordingly. Because subjects with lower levels of 25(OH)D3 may not be comparable to subjects with higher levels (despite randomization), adjustment for baseline covariates will be required in the primary analysis for this aim using the criteria for covariate adjustment discussed previously.

C4. Limitations and Rationale for Proposed Approach:

Although some experts have recommended higher levels of supplementation than proposed for strategy II, we feel that safety must be established first, given the preliminary data supported by the literature suggesting harm in this population, given the recent FDA warning about the risk of adverse events in infants unintentionally exceeding 400 IU/day of supplementation, and given the IOM recommendation to limit total intake to <1000 IU/day.[2 105 138] Others might question whether study subjects should be exposed to the enteral

vit. D amounts in strategy I, due to the suggestion of increased wheezing illnesses with vit. D supplementation. However, the population of black preterm infants in Cleveland is at high risk for vit. D deficiency, and we do not believe clinical practice should be changed to restrict vit. D supplementation based on observational data alone. In short, while a rationale exists for both higher and lower dosing, given the suggestion of competing benefits and morbidity with supplementation, and the paucity of studies measuring clinically relevant outcomes in this population, we believe equipoise exists for this study.

This is an effectiveness study comparing two dosing strategies that could practically be used by pediatricians. We have not targeted specific plasma levels of 25(OH)D3, because (1) it would be impractical to routinely finely titrate supplementation dose to a target level on all healthy preterm infants, and (2) the optimal levels in this population are not known. Aim 3 will assess the relationship between levels and recurrent wheezing.

Recruitment and retention are challenges for all clinical studies. The research team has a track record for success with similar populations. Examples of proven strategies include using experienced research nurses with a prior history of serving the local community through clinical care, and frequent contact with families (e.g., sending birthday cards hand-written by the PI). If we are not on-target for recruitment, we will consider additional strategies, including expanding enrollment to other CWRU-affiliated NICUs.

C5. Summary: We propose a comparative effectiveness trial of two clinically relevant vit. D supplementation strategies, focusing on outcomes that are meaningful to patients and providers. The results of this study have the potential to improve care for the >100,000 black infants born at 28-36 wks GA in the U.S. yearly.

REFERENCES

1. National Center for Health Statistics Fnd. www.marchofdimes.com/peristats.

2. Milner JD, Stein DM, McCarter R, Moon RY. Early infant multivitamin supplementation is associated with increased risk for food allergy and asthma. *Pediatrics* 2004;114(1):27-32.

3. Raby BA, Celedon JC, Litonjua AA, Phipatanakul W, Sredl D, Oken E, et al. Low-normal gestational age as a predictor of asthma at 6 years of age. *Pediatrics* 2004;114(3):e327-32.

4. Elder DE, Hagan R, Evans SF, Benninger HR, French NP. Recurrent wheezing in very preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1996;74(3):F165-71.

5. Colin AA, McEvoy C, Castile RG. Respiratory morbidity and lung function in preterm infants of 32 to 36 weeks' gestational age. *Pediatrics* 2010;126(1):115-28.

6. Escobar GJ, McCormick MC, Zupancic JA, Coleman-Phox K, Armstrong MA, Greene JD, et al. Unstudied infants: outcomes of moderately premature infants in the neonatal intensive care unit. *Arch Dis Child Fetal Neonatal Ed* 2006;91(4):F238-44.

7. Bosse Y, Maghni K, Hudson TJ. 1alpha,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. *Physiol Genomics* 2007;29(2):161-8.

8. Wittke A, Chang A, Froicu M, Harandi OF, Weaver V, August A, et al. Vitamin D receptor expression by the lung micro-environment is required for maximal induction of lung inflammation. *Arch Biochem Biophys* 2007;460(2):306-13.

9. Long KZ, Santos JI. Vitamins and the regulation of the immune response. *Pediatr Infect Dis J* 1999;18(3):283-90.

10. Matheu V, Back O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. *J Allergy Clin Immunol* 2003;112(3):585-92.

11. Nutrition and feeding of preterm infants. Committee on Nutrition of the Preterm Infant, European Society of Paediatric Gastroenterology and Nutrition. *Acta Paediatr Scand Suppl* 1987;336:1-14.

12. Cooke R, Hollis B, Conner C, Watson D, Werkman S, Chesney R. Vitamin D and mineral metabolism in the very low birth weight infant receiving 400 IU of vitamin D. *J Pediatr* 1990;116(3):423-8.

13. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr*;50(1):85-91.

14. Tsang RC, R. U, B. K, S.H. Z. *Nutrition of the Preterm Infant: Scientific Basis and Practical Guidelines*. 2nd ed: Digital Educational Publishing, Inc., 2005.

15. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* 2008;122(5):1142-52.
16. Jacobson RM, Feinstein AR. Oxygen as a cause of blindness in premature infants: "autopsy" of a decade of errors in clinical epidemiologic research. *J Clin Epidemiol* 1992;45(11):1265-87.
17. Jhung MA, Sunenshine RH, Noble-Wang J, Coffin SE, St John K, Lewis FM, et al. A national outbreak of Ralstonia mannitolilytica associated with use of a contaminated oxygen-delivery device among pediatric patients. *Pediatrics* 2007;119(6):1061-8.
18. Robertson AF. Reflections on errors in neonatology III. The "experienced" years, 1970 to 2000. *J Perinatol* 2003;23(3):240-9.
19. Robertson AF. Reflections on errors in neonatology: II. The "Heroic" years, 1950 to 1970. *J Perinatol* 2003;23(2):154-61.
20. Robertson AF. Reflections on errors in neonatology: I. The "Hands-Off" years, 1920 to 1950. *J Perinatol* 2003;23(1):48-55.
21. Robertson AF, Baker JP. Lessons from the past. *Semin Fetal Neonatal Med* 2005;10(1):23-30.
22. Xu H, Perez-Cuevas R, Xiong X, Reyes H, Roy C, Julien P, et al. An international trial of antioxidants in the prevention of preeclampsia (INTAPP). *Am J Obstet Gynecol*;202(3):239 e1-39 e10.
23. Hemila H, Kaprio J. Modification of the effect of vitamin E supplementation on the mortality of male smokers by age and dietary vitamin C. *Am J Epidemiol* 2009;169(8):946-53.
24. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *Jama* 2007;297(8):842-57.
25. Myung SK, Kim Y, Ju W, Choi HJ, Bae WK. Effects of antioxidant supplements on cancer prevention: meta-analysis of randomized controlled trials. *Ann Oncol*;21(1):166-79.
26. Evans M, Palta M, Sadek M, Weinstein MR, Peters ME. Associations between family history of asthma, bronchopulmonary dysplasia, and childhood asthma in very low birth weight children. *Am J Epidemiol* 1998;148(5):460-6.
27. Greenough A, Limb E, Marston L, Marlow N, Calvert S, Peacock J. Risk factors for respiratory morbidity in infancy after very premature birth. *Arch Dis Child Fetal Neonatal Ed* 2005;90(4):F320-3.
28. Hack M, Taylor HG, Drotar D, Schluchter M, Cartar L, Andreias L, et al. Chronic conditions, functional limitations, and special health care needs of school-aged children born with extremely low-birth-weight in the 1990s. *Jama* 2005;294(3):318-25.
29. Halvorsen T, Skadberg BT, Eide GE, Roksund OD, Carlsen KH, Bakke P. Pulmonary outcome in adolescents of extreme preterm birth: a regional cohort study. *Acta Paediatr* 2004;93(10):1294-300.
30. Korhonen P, Laitinen J, Hyodynmaa E, Tammela O. Respiratory outcome in school-aged, very-low-birth-weight children in the surfactant era. *Acta Paediatr* 2004;93(3):316-21.
31. Palta M, Sadek-Badawi M, Sheehy M, Albanese A, Weinstein M, McGuinness G, et al. Respiratory symptoms at age 8 years in a cohort of very low birth weight children. *Am J Epidemiol* 2001;154(6):521-9.
32. Vogt H, Lindstrom K, Braback L, Hjern A. Preterm birth and inhaled corticosteroid use in 6- to 19-year-olds: a Swedish national cohort study. *Pediatrics*;127(6):1052-9.
33. Bird TM, Bronstein JM, Hall RW, Lowery CL, Nugent R, Mays GP. Late preterm infants: birth outcomes and health care utilization in the first year. *Pediatrics*;126(2):e311-9.
34. Gunville CF, Sontag MK, Stratton KA, Ranade DJ, Abman SH, Mourani PM. Scope and impact of early and late preterm infants admitted to the PICU with respiratory illness. *J Pediatr*;157(2):209-14 e1.
35. Perez L, Corchado Z, Rodriguez M, Garcia D, Medina L, Vicens A, et al. Respiratory illness in late preterm infants during the first six months of life. *Bol Asoc Med P R*;102(1):18-20.
36. Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. *Birth Defects Res B Dev Reprod Toxicol* 2008;83(6):547-60.
37. Larcombe LA, Orr PH, Lodge AM, Brown JS, Dembinski IJ, Milligan LC, et al. Functional gene polymorphisms in canadian aboriginal populations with high rates of tuberculosis. *J Infect Dis* 2008;198(8):1175-9.
38. Babb C, van der Merwe L, Beyers N, Pheiffer C, Walzl G, Duncan K, et al. Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonary tuberculosis patients. *Tuberculosis (Edinb)* 2007;87(4):295-302.
39. Wilbur AK, Kubatko LS, Hurtado AM, Hill KR, Stone AC. Vitamin D receptor gene polymorphisms and susceptibility M. tuberculosis in native Paraguayans. *Tuberculosis (Edinb)* 2007;87(4):329-37.

40. Selvaraj P, Chandra G, Jawahar MS, Rani MV, Rajeshwari DN, Narayanan PR. Regulatory role of vitamin D receptor gene variants of Bsm I, Apa I, Taq I, and Fok I polymorphisms on macrophage phagocytosis and lymphoproliferative response to mycobacterium tuberculosis antigen in pulmonary tuberculosis. *J Clin Immunol* 2004;24(5):523-32.
41. Roth DE, Soto G, Arenas F, Bautista CT, Ortiz J, Rodriguez R, et al. Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. *J Infect Dis* 2004;190(5):920-7.
42. Selvaraj P, Kurian SM, Chandra G, Reetha AM, Charles N, Narayanan PR. Vitamin D receptor gene variants of BsmI, ApaI, TaqI, and FokI polymorphisms in spinal tuberculosis. *Clin Genet* 2004;65(1):73-6.
43. Samuel S, Sitrin MD. Vitamin D's role in cell proliferation and differentiation. *Nutr Rev* 2008;66(10 Suppl 2):S116-24.
44. Keen RW, Egger P, Fall C, Major PJ, Lanchbury JS, Spector TD, et al. Polymorphisms of the vitamin D receptor, infant growth, and adult bone mass. *Calcif Tissue Int* 1997;60(3):233-5.
45. Kostner K, Denzer N, Muller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. *Anticancer Res* 2009;29(9):3511-36.
46. Lorentzon M, Lorentzon R, Nordstrom P. Vitamin D receptor gene polymorphism is associated with birth height, growth to adolescence, and adult stature in healthy caucasian men: a cross-sectional and longitudinal study. *J Clin Endocrinol Metab* 2000;85(4):1666-70.
47. Morley R, Carlin JB, Pasco JA, Wark JD, Ponsonby AL. Maternal 25-hydroxyvitamin D concentration and offspring birth size: effect modification by infant VDR genotype. *Eur J Clin Nutr* 2009;63(6):802-4.
48. Saadi A, Gao G, Li H, Wei C, Gong Y, Liu Q. Association study between vitamin D receptor gene polymorphisms and asthma in the Chinese Han population: a case-control study. *BMC Med Genet* 2009;10:71.
49. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene* 2004;338(2):143-56.
50. Uitterlinden AG, Fang Y, van Meurs JB, van Leeuwen H, Pols HA. Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. *J Steroid Biochem Mol Biol* 2004;89-90(1-5):187-93.
51. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006;84(1):18-28.
52. Freishtat RJ, Iqbal SF, Pillai DK, Klein CJ, Ryan LM, Benton AS, et al. High prevalence of vitamin D deficiency among inner-city African American youth with asthma in Washington, DC. *J Pediatr*;156(6):948-52.
53. Ginde AA, Mansbach JM, Camargo CA, Jr. Vitamin D, respiratory infections, and asthma. *Curr Allergy Asthma Rep* 2009;9(1):81-7.
54. Chen S, Sims GP, Chen XX, Gu YY, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol* 2007;179(3):1634-47.
55. Karatekin G, Kaya A, Salihoglu O, Balci H, Nuhoglu A. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. *Eur J Clin Nutr* 2009;63(4):473-7.
56. Lange NE, Litonjua A, Hawrylowicz CM, Weiss S. Vitamin D, the immune system and asthma. *Expert Rev Clin Immunol* 2009;5(6):693-702.
57. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol*;184(2):965-74.
58. Litonjua AA. Childhood asthma may be a consequence of vitamin D deficiency. *Curr Opin Allergy Clin Immunol* 2009;9(3):202-7.
59. Brehm JM, Celedon JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009;179(9):765-71.
60. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol* 2010.
61. Carroll KN, Gebretsadik T, Larkin E, Minton P, Woodward K, Van Driest S, et al. Inverse association of vitamin D levels with maternal asthma and no association with respiratory illness severity in their infants. *Pediatric Academic Societies Meeting*. Vancouver, Canada, 2010:PAS4401.77.
62. Hansdottir S, Monick MM. Vitamin D effects on lung immunity and respiratory diseases. *Vitam Horm*;86:217-37.
63. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol* 2005;97(1-2):93-101.

64. D'Ambrosio D. Increased IgE but reduced Th2-type inflammation in vitamin D receptor-deficient mice. *J Immunol* 2005;174(8):4451; author reply 51.
65. Moed H, Stoof TJ, Boorsma DM, von Blomberg BM, Gibbs S, Bruynzeel DP, et al. Identification of anti-inflammatory drugs according to their capacity to suppress type-1 and type-2 T cell profiles. *Clin Exp Allergy* 2004;34(12):1868-75.
66. Jirapongsananuruk O, Melamed I, Leung DY. Additive immunosuppressive effects of 1,25-dihydroxyvitamin D3 and corticosteroids on TH1, but not TH2, responses. *J Allergy Clin Immunol* 2000;106(5):981-5.
67. Zosky GR, Berry LJ, Elliot JG, James AL, Gorman S, Hart PH. Vitamin d deficiency causes deficits in lung function and alters lung structure. *Am J Respir Crit Care Med*;183(10):1336-43.
68. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357(3):266-81.
69. Lee JM, Smith JR, Philipp BL, Chen TC, Mathieu J, Holick MF. Vitamin D deficiency in a healthy group of mothers and newborn infants. *Clin Pediatr (Phila)* 2007;46(1):42-4.
70. Vieth R, Bischoff-Ferrari H, Boucher BJ, Dawson-Hughes B, Garland CF, Heaney RP, et al. The urgent need to recommend an intake of vitamin D that is effective. *Am J Clin Nutr* 2007;85(3):649-50.
71. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am J Clin Nutr* 2007;85(6):1586-91.
72. Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med* 2007;167(16):1730-7.
73. Avenell A, Cook JA, Maclennan GS, Macpherson GC. Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). *Age Ageing* 2007;36(5):574-7.
74. Bruce D, Ooi JH, Yu S, Cantorna MT. Vitamin D and host resistance to infection? Putting the cart in front of the horse. *Exp Biol Med (Maywood)*;235(8):921-7.
75. Freedman BI, Wagenknecht LE, Hairston KG, Bowden DW, Carr JJ, Hightower RC, et al. Vitamin d, adiposity, and calcified atherosclerotic plaque in african-americans. *J Clin Endocrinol Metab*;95(3):1076-83.
76. CDC. http://www.cdc.gov/breastfeeding/recommendations/vitamin_d.htm.
77. Siafarikas A, Piazena H, Feister U, Bulsara MK, Meffert H, Hesse V. Randomised controlled trial analysing supplementation with 250 versus 500 units of vitamin D3, sun exposure and surrounding factors in breastfed infants. *Arch Dis Child*.
78. Racial and ethnic differences in breastfeeding initiation and duration, by state - National Immunization Survey, United States, 2004-2008. *MMWR Morb Mortal Wkly Rep* 2010;59(11):327-34.
79. Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D receptor polymorphisms and the risk of acute lower respiratory tract infection in early childhood. *J Infect Dis* 2008;197(5):676-80.
80. Leow L, Simpson T, Cursons R, Karalus N, Hancox RJ. Vitamin D, innate immunity and outcomes in community acquired pneumonia. *Respirology*;16(4):611-6.
81. Chinellato I, Piazza M, Sandri M, Peroni D, Piacentini G, Boner AL. Vitamin D serum levels and markers of asthma control in Italian children. *J Pediatr*;158(3):437-41.
82. Muhe L, Lulseged S, Mason KE, Simoes EA. Case-control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. *Lancet* 1997;349(9068):1801-4.
83. Najada AS, Habashneh MS, Khader M. The frequency of nutritional rickets among hospitalized infants and its relation to respiratory diseases. *J Trop Pediatr* 2004;50(6):364-8.
84. Walker VP, Modlin RL. The vitamin D connection to pediatric infections and immune function. *Pediatr Res* 2009;65(5 Pt 2):106R-13R.
85. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. *Eur J Clin Nutr* 2004;58(4):563-7.
86. Belderbos ME, Houben ML, Wilbrink B, Lentjes E, Bloemen EM, Kimpen JL, et al. Cord blood vitamin d deficiency is associated with respiratory syncytial virus bronchiolitis. *Pediatrics*;127(6):e1513-20.
87. Camargo CA, Jr., Ingham T, Wickens K, Thadhani R, Silvers KM, Epton MJ, et al. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. *Pediatrics*;127(1):e180-7.
88. Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, et al. High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. *Lancet*;377(9761):242-50.

89. Weber DR, Cole D, Zhang JH, Wong B, Carpenter TO. Analysis of 25-hydroxyvitamin D (25-OHD) level and binding protein genotype as predictors of risk for illness in infancy. *Pediatric Academic Societies Meeting*. Vancouver, Canada, 2010:PAS1451.29.
90. Albert PJ, Proal AD, Marshall TG. Vitamin D: the alternative hypothesis. *Autoimmun Rev* 2009;8(8):639-44.
91. Hoag KA, Nashold FE, Goverman J, Hayes CE. Retinoic acid enhances the T helper 2 cell development that is essential for robust antibody responses through its action on antigen-presenting cells. *J Nutr* 2002;132(12):3736-9.
92. Li-Weber M, Giaisi M, Treiber MK, Krammer PH. Vitamin E inhibits IL-4 gene expression in peripheral blood T cells. *Eur J Immunol* 2002;32(9):2401-8.
93. Li-Weber M, Weigand MA, Giaisi M, Suss D, Treiber MK, Baumann S, et al. Vitamin E inhibits CD95 ligand expression and protects T cells from activation-induced cell death. *J Clin Invest* 2002;110(5):681-90.
94. Wjst M. Allergy risk of vitamin D supplements has been described in various settings. *J Allergy Clin Immunol* 2008;121(4):1065-6; author reply 66.
95. Wjst M. Introduction of oral vitamin D supplementation and the rise of the allergy pandemic. *Allergy Asthma Clin Immunol* 2009;5(1):8.
96. Hypponen E, Sovio U, Wjst M, Patel S, Pekkanen J, Hartikainen AL, et al. Infant vitamin d supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. *Ann N Y Acad Sci* 2004;1037:84-95.
97. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. *Allergy* 2000;55(8):688-97.
98. Camargo CA, Jr., Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, et al. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am J Clin Nutr* 2007;85(3):788-95.
99. Devereux G, Litonjua AA, Turner SW, Craig LC, McNeill G, Martindale S, et al. Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 2007;85(3):853-9.
100. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, et al. Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr* 2008;62(1):68-77.
101. Aloia JF, Mikhail M, Pagan CD, Arunachalam A, Yeh JK, Flaster E. Biochemical and hormonal variables in black and white women matched for age and weight. *J Lab Clin Med* 1998;132(5):383-9.
102. Iqbal SF, Pillai DK, Benton AS, Lerner J, Wiles A, Foerster M, et al. Genetic variants in vitamin D metabolism influence asthma phenotypes in African American children. *Pediatric Academic Societies Meeting*. Vancouver, Canada, 2010:PAS2720.4

103. Bodnar LM, Catov JM, Zmuda JM, Cooper ME, Parrott MS, Roberts JM, et al. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. *J Nutr*;140(5):999-1006.
104. Chesney RW. Requirements and upper limits of vitamin D intake in the term neonate, infant, and older child. *J Pediatr* 1990;116(2):159-66.
105. FDA. http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm214343.htm, 2010.
106. Dall'Agnola A, Beghini L. Post-discharge supplementation of vitamins and minerals for preterm neonates. *Early Hum Dev* 2009;85(10 Suppl):S27-9.
107. Backstrom MC, Maki R, Kuusela AL, Sievanen H, Koivisto AM, Ikonen RS, et al. Randomised controlled trial of vitamin D supplementation on bone density and biochemical indices in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1999;80(3):F161-6.
108. Backstrom MC, Maki R, Kuusela AL, Sievanen H, Koivisto AM, Koskinen M, et al. The long-term effect of early mineral, vitamin D, and breast milk intake on bone mineral status in 9- to 11-year-old children born prematurely. *J Pediatr Gastroenterol Nutr* 1999;29(5):575-82.
109. Waitzman NJ, Rogowski J. Societal costs of preterm birth. In: Behrman R, Stith-Butler A, editors. *Preterm Birth: Causes, Consequences and Prevention*. Washington, D.C.: The National Academies Press, 2006:398-429.
110. Zupancic JAF. A systematic review of costs associated with preterm birth. In: Behrman R, Stith-Butler A, editors. *Preterm Birth: Causes, Consequences and Prevention*. Washington, D.C.: The National Academies Press, 2006.
111. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8(3):483-91.

112. Jenkins MA, Clarke JR, Carlin JB, Robertson CF, Hopper JL, Dalton MF, et al. Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma. *Int J Epidemiol* 1996;25(3):609-16.

113. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis* 1978;118(6 Pt 2):1-120.

114. Ballard RA, Truog WE, Cnaan A, Martin RJ, Ballard PL, Merrill JD, et al. Inhaled nitric oxide in preterm infants undergoing mechanical ventilation. *N Engl J Med* 2006;355(4):343-53.

115. Hibbs AM, Black D, Palermo L, Cnaan A, Luan X, Truog WE, et al. Accounting for multiple births in neonatal and perinatal trials: systematic review and case study. *J Pediatr*;156(2):202-8.

116. Carlo WA, Finer NN, Walsh MC, Rich W, Gantz MG, Laptook AR, et al. Target ranges of oxygen saturation in extremely preterm infants. *N Engl J Med*;362(21):1959-69.

117. Finer NN, Carlo WA, Walsh MC, Rich W, Gantz MG, Laptook AR, et al. Early CPAP versus surfactant in extremely preterm infants. *N Engl J Med*;362(21):1970-9.

118. Bianca AC, Wandalsen GF, Miyagi K, Camargo L, Cezarin D, Mallol J, et al. International Study of Wheezing in Infants (EISL): validation of written questionnaire for children aged below 3 years. *J Investig Allergol Clin Immunol* 2009;19(1):35-42.

119. Bentur L, Beck R, Berkowitz D, Hasanin J, Berger I, Elias N, et al. Adenosine bronchial provocation with computerized wheeze detection in young infants with prolonged cough: correlation with long-term follow-up. *Chest* 2004;126(4):1060-5.

120. Bentur L, Beck R, Berkowitz D, Hasanin J, Berger I, Gavriely N. Pulmonary Acoustics and Esophageal pH Monitoring in Infantile Nocturnal Cough. *Pediatric Asthma, Allergy, and Immunology* 2004;17(4):262-70.

121. Prodhan P, Dela Rosa RS, Shubina M, Haver KE, Matthews BD, Buck S, et al. Wheeze detection in the pediatric intensive care unit: comparison among physician, nurses, respiratory therapists, and a computerized respiratory sound monitor. *Respir Care* 2008;53(10):1304-9.

122. Gavriely N, Vizel E, Avrahami AN, Guryachev Y, Kroin I. Validation of an automatic wheeze detector. *American Thoracic Society Meeting*. New Orleans, 2010.

123. Ballardini N, Nilsson C, Nilsson M, Lilja G. ImmunoCAP Phadiatop Infant--a new blood test for detecting IgE sensitisation in children at 2 years of age. *Allergy* 2006;61(3):337-43.

124. Lau S, Nilsson M, Sulser C, Schulz G, Borres MP, Wahn U. Use of Phadiatop Infant in diagnosis of specific sensitization in young children with wheeze or eczema. *Pediatr Allergy Immunol* 2008;19(4):337-41.

125. Kjaer HF, Eller E, Andersen KE, Host A, Bindslev-Jensen C. The association between early sensitization patterns and subsequent allergic disease. The DARC birth cohort study. *Pediatr Allergy Immunol* 2009;20(8):726-34.

126. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993;186(1):23-31.

127. Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997;195(1):10-9.

128. Oranje AP, Stalder JF, Taieb A, Tasset C, de Longueville M. Scoring of atopic dermatitis by SCORAD using a training atlas by investigators from different disciplines. ETAC Study Group. Early Treatment of the Atopic Child. *Pediatr Allergy Immunol* 1997;8(1):28-34.

129. Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 2000;162(4 Pt 1):1403-6.

130. Guilbert TW, Morgan WJ, Zeiger RS, Bacharier LB, Boehmer SJ, Krawiec M, et al. Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. *J Allergy Clin Immunol* 2004;114(6):1282-7.

131. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. *N Engl J Med* 2006;354(19):1985-97.

132. Bagnoli F, Toti MS, Conte ML, Badii S, Mori A, Tomasini B, et al. [Quantitative ultrasound for the assessment of bone in the preterm infant]. *Minerva Pediatr*;62(2):125-32.

133. Gursoy T, Yurdakok M, Hayran M, Korkmaz A, Yigit S, Tekinalp G. Bone speed of sound curves of twin and singleton neonates. *J Pediatr Endocrinol Metab* 2008;21(11):1065-71.

134. Mercy J, Dillon B, Morris J, Emmerson AJ, Mughal MZ. Relationship of tibial speed of sound and lower limb length to nutrient intake in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2007;92(5):F381-5.

135. Nemet D, Dolfin T, Wolach B, Eliakim A. Quantitative ultrasound measurements of bone speed of sound in premature infants. *Eur J Pediatr* 2001;160(12):736-40.
136. Teitelbaum JE, Rodriguez RJ, Ashmeade TL, Yaniv I, Osuntokun BO, Hudome S, et al. Quantitative ultrasound in the evaluation of bone status in premature and full-term infants. *J Clin Densitom* 2006;9(3):358-62.
137. Zadik Z, Price D, Diamond G. Pediatric reference curves for multi-site quantitative ultrasound and its modulators. *Osteoporos Int* 2003;14(10):857-62.
138. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, D.C.: The National Academies Press, 2011.
139. Center NCD. http://lwf.ncdc.noaa.gov/oa/climate/online/ccd/cldy.html.
140. Pocock SJ, Assmann SE, Enos LE, Kasten LE. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. *Stat Med* 2002;21(19):2917-30.
141. Piantadosi S. *Clinical Trials: A Methodologic Perspective, 2nd edition*. Hoboken, NJ: John Wiley & Sons, Inc., 2005.
142. Follmann D, Proschan M, Leifer E. Multiple outputation: inference for complex clustered data by averaging analyses from independent data. *Biometrics* 2003;59(2):420-9.
143. Carpenter J, Pocock S, Lamm CJ. Coping with missing data in clinical trials: a model-based approach applied to asthma trials. *Stat Med* 2002;21(8):1043-66.
144. Goldstein IF, Perzanowski MS, Lendor C, Garfinkel RS, Hoepner LA, Chew GL, et al. Prevalence of allergy symptoms and total IgE in a New York City cohort and their association with birth order. *Int Arch Allergy Immunol* 2005;137(3):249-57.
145. Kase JS, Pici M, Visintainer P. Risks for common medical conditions experienced by former preterm infants during toddler years. *J Perinat Med* 2009;37(2):103-8.
146. Moore MM, Rifas-Shiman SL, Rich-Edwards JW, Kleinman KP, Camargo CA, Jr., Gold DR, et al. Perinatal predictors of atopic dermatitis occurring in the first six months of life. *Pediatrics* 2004;113(3 Pt 1):468-74.
147. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007;120(3):638-46.