Mothers' own Milk Optimization for Preterm Infants Project (MoMO PIP): Maternal Diet and Vitamin D Supplementation Effects on Preterm Infants, a Randomized Control Pilot Study.

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PROTOCOL TITLE:

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PRINCIPAL INVESTIGATOR:

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1.0 Objectives / Specific Aims

The overall goal of this study is to assess maternal vitamin D status along with diet, and milk composition to, in turn, evaluate if infant vitamin D status can be improved with increased maternal supplementation and diet education. Our **central hypothesis** is that by maximizing maternal vitamin D status, there will be an increase infant 25(OH)D concentration in breastfed, preterm infants. This hypothesis will be tested by performing the following Specific Aims.

Aim 1: Determine the efficacy, effectiveness, and safety of maternal and premature infant vitamin D supplementation as a function of maternal vitamin D status, lactation status, and ethnicity.

H_1: Maternal supplementation of high dose (6,600 IU) vitamin D_3/day will be safe for lactating mothers.

H_2: It is anticipated that only high dose (6,600 IU) vitamin D_3/day supplementation of lactating mothers will provide sufficient antirachitic activity in breast milk to prevent vitamin D deficiency in nursing premature infants, regardless of ethnicity/race.

H_3: High-dose maternal supplementation will be safe and more effective than infant vitamin D supplementation alone, without any adverse side effects or health consequences in the mother or infant.

Consented lactating women who delivered preterm infants <35 weeks' gestation admitted to the MUSC NICU will be randomized 3-7 days following delivery to either 1 of 2 study groups: Group A (standard therapy of 600 IU vitamin D_3/day), Group B (high dose supplementation of 6,600 IU Vitamin D_3/day). As such, infants whose lactating mothers are randomized to the 600-IU/day group will receive 400-IU/day oral vitamin D_3 (recommended practice). Those infants of lactating mothers assigned to Group B will receive placebo. Calcium homeostasis will be monitored in all groups. Safety will be assessed throughout the study on a case-rather than a treatment arm basis using cautionary levels as the stop rule with intervention prior to exceeding normal urinary calcium/creatinine ratios in mothers and serum calcium concentrations in both mothers and infants. Through these proposed studies, the prevalence of vitamin D deficiency among mothers and their infants, and the utility of maternal therapeutic intervention with vitamin D_3 will be assessed.

Aim 2: Aim 2: Evaluate and maximize maternal milk composition.

For this aim, we will obtain maternal food dietary intake using the Diet History Questionnaire III (DHQ III). This is a validated tool that is based on a 24-hour dietary recall database from the National Health and Nutrition Examination Surveys (NHANES) in people 19 and older. This survey will be administered between post-partum days 3-7 at the time a milk sample is obtained and at 1-, 2-, and 3-month visits. We then will utilize a milk composition analyzer (name, city, state) in MUSC's Milk Bank to evaluate maternal milk sample composition at the initial visit and again at 1, 2 and 3 months of infant age. Prior to discharge from the NICU, the infant will undergo a body mass analysis using the PEA POD and Bone ultrasound. Analysis will be performed on maternal diet, milk composition, infant body

composition, and vitamin D status for any correlations. Samples will also be archived for metabolomics and cytokine analysis.

2.0 Background

Human milk remains the favorable form of nutrition for the neonate and infant (1). Neonatal vitamin D stores are inversely related to gestational age (2). Along with this, two-thirds of fetal calcium is accumulated in the third trimester. Postnatal risk factors for vitamin D deficiency (VDD) of preterm neonates include decreased sunlight exposure, difficulties with adequate enteral nutrition, and higher requirements for vitamin D. All these factors along with use of diuretics and steroids, lead to an increased risk. Many studies have shown the importance of vitamin D in neonatal and infant health. Munshi et al. found that 80% of ELBW and VLBW infants at Albany Medical Center were deficient or insufficient in vitamin D at 1 month of age (3). Along with deficiency, the incidence of metabolic bone disease is still elevated at 32% of VLBW and 54% of ELBW (4, 5). There is also a significant association between respiratory distress syndrome (RDS) and vitamin D deficiency, especially in infants ≤ 30 weeks(6).

Maternal breast milk is the recommended source of nutrition for both full and preterm infants; however, if mother is deficient in vitamin D, her milk provides limited vitamin D, with roughly 3-4 IU/kg/day provided (7, 8). It has also been found that mothers and their preterm infants were more likely to be vitamin D deficient than mothers and their infants who carried to term. To further worsen this, those preterm infants who are not fed mother's own milk (MoM) are given donor breast milk. Unfortunately, through pasteurization there is 10-20% less vitamin D (9).

Maternal vitamin D status as measured by total circulating 25(OH)D concentration is influenced by many factors including ethnicity, season, high BMI, gestational diabetes, socioeconomic status (poor diet), and prenatal intake (10). From an ethnicity standpoint, African American, Middle Eastern, and Asian women are at highest risk for vitamin D deficiency. To no surprise, these are also the populations at increased risk of preterm delivery. In South Carolina in 2010, our group showed that vitamin D deficiency [defined as 25(OH)D <20 ng/mL (50 nmol/L)] was around 48% overall, with 68.3% of African-American women being deficient (11). In our NICHD vitamin D supplementation trial, there were similar findings with significant deficiency in African American and Hispanic women at baseline (12).

There are several studies evaluating various supplementation regimens in preterm infants; however, considerable attention has not been given to the role of maternal vitamin D supplementation and its effects on preterm neonate's vitamin D status. Many groups have recommendations on vitamin D supplementation that have failed to form a consensus or have differing recommendations for preterm neonates. The AAP Committee on nutrition recommends 200-400 IU of vitamin D for very low birthweight infants (VLBW). On the other hand, the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) recommends 800-1600 IU daily (13). Due to the lack of substantial data, these various groups fail to make recommendations on arguably those at highest risk--preterm, very low birthweight (VLBW) infants, and extremely low birthweight (ELBW) infants. Along with variations in supplementation recommendations, there are also variations among scientific groups as to an ideal target 25(OH)D level. The AAP recommends 25(OH)D > 20 ng/mL (50 nmol/L) (14) and in the same year another group published a paper with recommended 25(OH)D > 30 ng/mL (75 nmol/L) (15). The Endocrine Society targets >30 mg/mL (75 nmol/L) (16) while ESPGAN >32 ng/mL (80 nmol/L) (13). Previous groups have looked at the effects of various vitamin D supplementation regimens in preterm infants, many of which have not shown significant changes in infant 25(OH)D concentrations, but have shown that higher supplementation leads to improved length, head circumference, and possibly immune function (17). Previous studies have shown safety of increased vitamin D supplementation with 800 IU and 1000 IU without significant toxicity (18-20).

In a previous randomized control trial performed at this institution by Dr. Carol Wagner and colleagues, maternal supplementation with 6400 IU/day of vitamin D_3 was more effective in achieving

vitamin D sufficiency in mothers without increased risk to infants or mothers (21, 22). This present pilot study plans to replicate this in mothers and their preterm infants (22). See IRB application attachments for this paper that is *in press*.

To our knowledge, there are no studies specifically investigating maternal vitamin D supplementation on preterm infant vitamin D status. There has been investigation into maternal vitamin D supplementation on term infant vitamin D stores and data does suggest increased infant serum 25(OH)D levels with increased maternal supplementation (21, 23, 24). The objective of this study is to not only improve preterm infant vitamin D status but also maternal vitamin D status, as this continues to be an area of need in the Charleston, SC area.

The proposed pilot study will aim to improve 25(OH)D concentrations in mothers and their preterm infants, and therefore, allow us to further investigate and understand effects of maternal supplementation in preterm infants.

3.0 Intervention to be studied

Cholecalciferol or vitamin D_3 is an FDA approved medication for treatment and prevention of hypovitaminosis D.

Per Lexicomp**, "Dosing: Adult**

Note: 1 mcg = 40 units

Hypoparathyroidism (off-label use): Note: Active vitamin D preparations (ie, alfacalcidol**,** calcitriol) in conjunction with calcium supplementation are recommended therapy. Addition of cholecalciferol (or ergocalciferol) may be considered for supplemental therapy (25).

Osteoporosis, prevention (off-label use): Adults ≥50 years of age: Oral: 800 to 1,000 units/day is recommended, through dietary sources and/or supplementation if needed (26).

Vitamin D insufficiency/deficiency treatment (off-label use): Note: Repletion strategies may vary depending on desired target serum 25(OH)D levels as well as the clinical status of the patient. The optimal serum 25(OH)D level is controversial; the Institute of Medicine recommends a 25(OH)D level >20 ng/mL (50 nmol/L) as sufficient in nearly all persons (27), whereas others have suggested targeting a level of ~30 ng/mL (75 nmol/L) to minimize the risk of fractures, particularly in patients with osteoporosis (28); (26). During pregnancy, the conversion of 25(OH)D to $1,25(OH)_2D$, the active hormone of vitamin D is optimized at 40 ng/mL (100 nmol/L) and greater. However, some data suggest levels >40 ng/mL (>100 nmol/L) (median level in one trial: ~48 ng/mL) are associated with increased risk of falls in postmenopausal women (29, 30).

Therefore, some experts recommend a range of 20 to 40 ng/mL (50-100 nmol/L) as a reasonable target in most patients (31). In patients with normal absorption, for every 100 units/day of cholecalciferol, the serum 25(OH)D concentration is expected to increase by ~0.7 to 1 ng/mL (1.75 to 2.5 nmol/L) after a few weeks (32); (31). The dose-response declines as the 25(OH)D concentration increases above 40 ng/mL (100 nmol/L) (31). The following recommendations are based primarily on expert opinion and clinical experience:

Initial dosing (according to baseline serum 25(OH)D level):

Serum 25(OH)D 20 to 30 ng/mL (50 to 75 nmol/L): Initial: Supplementation dosing: Oral: 600 to 800 units once daily; a repeat serum 25(OH)D concentration is not required (31) **or** 1,000 to 2,000 units once daily; may consider a repeat serum 25(OH)D concentration in ~3 months to determine if the target level has been achieved (33).

Serum 25(OH)D 10 to <20 ng/mL (25 to 50 nmol/L): Initial:

Supplementation dosing: Oral: 800 to 1,000 units once daily (31) **or** 2,000 units once daily (33); a repeat serum 25(OH)D level should be drawn after ~3 months. If target serum 25(OH)D level has not been achieved, may increase to 2,000 units once daily or administer therapeutic dosing of 50,000 units once weekly for 6 to 8 weeks (31).

OR

Therapeutic dosing (ie, high-dose cholecalciferol): Oral: 50,000 units once **weekly** (or 5,000 to 7,000 units once **daily**) for ~8 weeks, followed by decreased maintenance dosing as needed to maintain target serum 25(OH)D level (34); (26).

***Serum 25(OH)D <10 ng/mL (< 25 nmol/L) or in patients with deficiency symptoms*:** Initial: Therapeutic dosing (ie, high-dose cholecalciferol): Oral: 50,000 units once **weekly** (or 5,000 to 7,000 units once **daily**) for 6 to 8 weeks to achieve target serum 25(OH)D level; a repeat serum 25(OH)D level should be drawn after ~3 months to assure target serum 25(OH)D level has been met (34); (31);(26).

Maintenance dosing: Maintenance dosing is highly patient specific and dependent on target 25(OH)D level, and may range from: 600 to 800 units/day (31) to 1,000 to 2,000 units/day (34);(26).

Special populations (obese patients, patients on medications known to affect vitamin D metabolism, patients with malabsorption syndromes or gastrectomy): Higher doses or longer durations may be necessary for adequate repletion (34); (31).

Vitamin D deficiency/insufficiency in patients with chronic kidney disease (off-label use): Oral:

Note: In patients without severe and progressive hyperparathyroidism, including chronic kidney disease stages G3 to G5 and dialysis or transplant patients, KDIGO guidelines recommend correcting vitamin D deficiency and insufficiency with treatment strategies recommended for the general population using cholecalciferol (or ergocalciferol) while avoiding hypercalcemia and ensuring phosphate levels are in the normal range. An individualized monitoring approach to direct treatment is also recommended (35);(36). In patients in whom serum parathyroid hormone levels are progressively rising and remain persistently elevated despite correction of modifiable factors (eg, hyperphosphatemia, vitamin D deficiency), calcitriol or vitamin D analogs are suggested instead of cholecalciferol (or ergocalciferol) (37).

Dosing: Geriatric

Refer to adult dosing.

Dosing: Renal Impairment: Adult

There are no dosage adjustments provided in the manufacturer's labeling

Dosing: Hepatic Impairment: Adult

There are no dosage adjustments provided in the manufacturer's labeling.

Dosing: Pediatric

Vitamin D deficiency, prevention (eg, Rickets prevention): AAP (14); (38):

Oral:

Breastfed infants (fully or partially): Oral: 400 units/day beginning in the first few days of life. Continue supplementation until infant is weaned to ≥1,000 mL/day or 1 qt/day of vitamin D-fortified formula or whole milk (after 12 months of age)

Formula-fed infants ingesting <1,000 mL of vitamin D-fortified formula: Oral: 400 units/day

Children and Adolescents without adequate intake: Oral: 400 to 600 units/day. **Note:** Children with increased risk of vitamin D deficiency (chronic fat malabsorption, maintained on chronic antiseizure medications) may require higher doses; use laboratory testing [25(OH)D, PTH, bone mineral status] to evaluate

Vitamin D deficiency, treatment: Oral: **Note:** Treatment should also include calcium and phosphorus supplementation; some patients with chronic fat malabsorption, obesity, or who are maintained on chronic antiseizure medications, glucocorticoids, HIV medications, or antifungals may require higher doses of cholecalciferol (39); monitor vitamin D status closely.

Infants: Oral: 2,000 units daily for 6 weeks to achieve a serum 25(OH)D level >20 ng/mL (>50 nmol/L); followed by a maintenance dose of 400 to 1,000 units daily. **Note:** For patients at high risk of fractures a serum 25(OH)D level >30 ng/mL (75 nmol/L) has been suggested (39).

Children and Adolescents: Oral: 2,000 units daily for 6 to 8 weeks to achieve serum 25(OH)D level >20 ng/mL (> 50 nmol/L); followed by a maintenance dose of 600 to 1,000 units daily. **Note:** For patients at high risk of fractures a serum 25(OH)D level >30 ng/mL (>75nmol/L) has been suggested (39).

Vitamin D deficiency in cystic fibrosis, prevention and treatment: Oral:

CF guidelines (40):

Recommended initial daily intake to maintain serum 25(OH)D level ≥30 ng/mL (>75 nmol/L):

Infants: Oral: 400 to 500 units/day

Children ≤10 years: Oral: 800 to 1,000 units/day

Children >10 years and Adolescents: Oral: 800 to 2,000 units/day

Dosing adjustment for serum 25(OH)D level between 20 to 30 ng/mL (50 to 75 nmol/L) and patient adherence established (Step 1 increase):

Infants: Oral: 800 to 1,000 units/day

Children ≤10 years: Oral: 1,600 to 3,000 units/day

Children >10 years and Adolescents: Oral: 1,600 to 6,000 units/day

Dosing adjustment for serum 25(OH)D level <20 ng/mL (<50 nmol/L) or persistently between 20 to 30 ng/mL (50 to 75 nmol/L) and patient adherence established (Step 2 increase):

Infants: Increase up to a maximum 2,000 units/day

Children ≤10 years: Increase to a maximum of 4,000 units/day

Children >10 years and Adolescents: Increase to a maximum of 10,000 units/day

Alternate dosing (41):

Initial dose: Serum 25(OH)D level ≤30 ng/mL (≤ 75 nmol/L)

Infants: Oral: 8,000 units/**week**

Children and Adolescents: Oral: 800 units/day

Medium-dose regimen: Serum 25(OH)D level remains ≤30 ng/mL (≤ 75 nmol/L) and patient compliance established

Infants and Children <5 years: Oral: 12,000 units/week for 12 weeks

Children ≥5 years and Adolescents: Oral: 50,000 units/week for 12 weeks

High-dose regimen: Repeat 25(OH)D level remains ≤30 ng/mL (≤ 75 nmol/L) and patient compliance established

Infants and Children <5 years: Oral: 12,000 units twice weekly for 12 weeks

Children ≥5 years and Adolescents: Oral: 50,000 units twice weekly for 12 weeks

Vitamin D insufficiency or deficiency associated with CKD (stages 2 to 5, 5D), treatment; serum 25 hydroxyvitamin D [25(OH)D] level ≤30 ng/mL (≤ 75 nmol/L) (42): Oral:

Serum 25(OH)D level 16 to 30 ng/mL (40 to 75 nmol/L): Infants, Children, and Adolescents: 2,000 units/day for 3 months or 50,000 units every month for 3 months

Serum 25(OH)D level 5 to 15 ng/mL (12.5 to 37.5 nmol/L): Infants, Children, and Adolescents: 4,000 units/day for 12 weeks or 50,000 units every other week for 12 weeks

Serum 25(OH)D level <5 ng/mL (< 12.5 nmol/L): Infants, Children, and Adolescents: 8,000 units/day for 4 weeks then 4,000 units/day for 2 months for total therapy of 3 months or 50,000 units/week for 4 weeks followed by 50,000 units 2 times/month for a total therapy of 3 months

Maintenance dose [once repletion accomplished; serum 25(OH)D level >30 ng/mL (>75 nmol/L)]: Infants, Children, and Adolescents: 200 to 1,000 units/day

Nutritional rickets, treatment: Limited data available (38): Administer in combination with calcium supplementation:

Daily therapy (preferred):

Infants: Oral: 2,000 units daily for ≥3 months, followed by maintenance dose of 400 units daily

Children: Oral: 3,000 to 6,000 units daily for ≥3 months, followed by maintenance dose of 600 units daily

Adolescents: Oral: 6,000 units daily for ≥3 months, followed by maintenance dose of 600 units daily

Single-dose therapy:

Infants ≥3 months: Oral: 50,000 units once, or in divided doses over several days; after 3 months, initiate maintenance dose of 400 units daily

Children: Oral: 150,000 units once, or in divided doses over several days; after 3 months, initiate maintenance dose of 600 units daily

Adolescents: Oral: 300,000 units once, or in divided doses over several days; after 3 months, initiate maintenance dose of 600 units daily

Dosing: Renal Impairment: Pediatric

There are no dosage adjustments provided in the manufacturer's labeling; however, cholecalciferol is not renally eliminated to a significant extent and dosage adjustment is not necessary.

Dosing: Hepatic Impairment: Pediatric

There are no dosage adjustments provided in the manufacturer's labeling.

Use: Labeled Indications

Dietary supplement: As a vitamin D dietary supplement

Doses for this study are under IND 66,346 and it's safety has been proven in this referenced study that is currently *in press* in Breastfeeding medicine (22).

Maternal and infant placebos will be used in this study. Maternal placebo will be sugar gummies from the same manufacturer (Church & Dwight). Infant placebo vitamin D drops will be from the same company (D Drops) and contain the eluent without the vitamin D.

4.0 Study Endpoints

- Primary outcomes will be:
  - Infant total circulating 25(OH)D concentration at 3 months
  - Mother total circulating 25(OH)D concentration at 3 months
- Primary or secondary safety endpoints
  - Maternal serum calcium concentration initially and at months 1-, 2-, and 3
  - Maternal urine calcium/creatinine ratios initially and at months 1-, 2-, and 3
  - Infant serum calcium concentration at time points initially and at months 1-, 2-, and 3

5.0 Inclusion and Exclusion Criteria/ Study Population

Only women 18-45 years who are lactating and their preterm infants (<35 weeks) within the first week postpartum will be eligible for the study. Men will be excluded on the basis of the study criterion of lactation. Infants of both genders will be followed along with their mothers. Women who deliver at MUSC will be eligible for participation.

a. **Inclusion Criteria for Lactating Women**: Women with documentation of fully breastfeeding who present within one month of delivery, who are 18-45 years old and in good general health will be eligible for participation.

b. **Exclusion Criteria for Lactating Women**: Women with preexisting type I or type II diabetes, hypertension requiring medication or parathyroid disease will not be eligible for participation in the study. Those women with active thyroid disease will not be eligible for inclusion in the study; however, women who have normal thyroid parameters on thyroid replacement hormone will be eligible for participation in the study. In addition, because of

the potentially confounding effect of multiple gestations/births (e.g. twins, triplets), women will multiple gestations/births will not be eligible for participation in the study.

No racial or ethnic groups will be excluded from this study.

6.0 Number of Subjects

For this initial Pilot study, we will enroll 50 mothers/infant dyads in total, 25 in the control and 25 in the placebo group.

7.0 Setting

Mothers and their infants will be initially seen in the MUSC Shawn Jenkins Children's Hospital NICU. If still inpatient for the remainder of their 2-3 visits, they will continue to be seen there. Following discharge, study visits will either be conducted in infant High-Risk Clinic or the Research Nexus clinic (MUSC Clinical Sciences Building, suite 214). The infant High-Risk Clinic has exam rooms, registration personnel and nursing staff. The NEXUS outpatient clinic has exam rooms, nursing staff, registration staff and lab personnel. We will meet the subjects at any of these sites and conduct the visits. There is only potential for 3 outpatient visits.

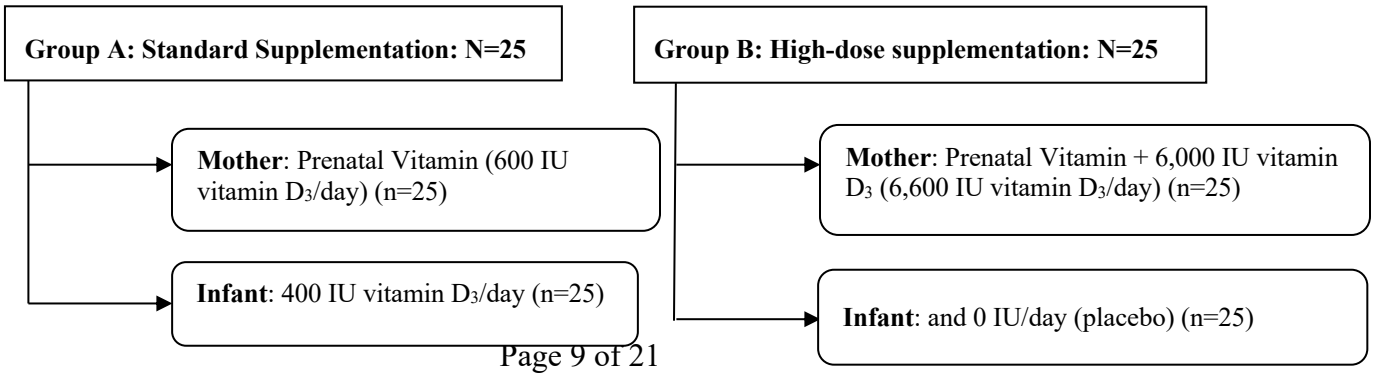
8.0 Recruitment Methods

Mothers of infants and their infants who both meet entry criteria will be approached by someone directly involved in the infant's care which could potentially also be a study personnel. The mother will then be consented by the study personnel within the first week of their infants' NICU stay. This could be by telephone if mom has been discharged or in person. Chart review will be used to determine eligibility.

9.0 Consent Process

Before performing any procedure, informed consent will be obtained from the mother enrolled in this study for both her and her infant child. This consent will be obtained by the PI, co-investigators, or the Study Coordinator. Consent will be given following an informative discussion period and then either by reading the Informed Consent Agreement to the subject or by allowing the subject to read the consent, then reviewing it with the subject. This will take place at MUSC, either in the NICU or on Labor and Delivery. There could potentially be no waiting period between informing the prospective subject and obtaining the consent.

10.0 Study Design / Methods



Mother

1. Visits: Each mother accompanied by her breastfeeding infant will be instructed to either present to the infant's room in the NICU, Infant High-Risk Clinic, or NEXUS clinic for evaluation.

2. Sociodemographic and Medical History: Following a woman's informed consent, each woman will have generated a study record that will include identifying information such as name, address, sociodemographic, ethnic and racial background, and medical information that will include past medical history and history about the most recent pregnancy, delivery, and postpartum state. This information will be collected from the mother via interview and from her clinical medical record and will be collected for research purposes only.

3. Questionnaires: Each woman will be asked to complete a series of questionnaires throughout the study that pertain to her health status, activity level, clothing use, dietary intake, and labor/delivery and breastfeeding history.

Initial Visit	1 Month Follow Up	2 Month Follow Up	3 Month Follow Up
Sociodemographic (Q1)			
Pregnancy & Delivery (Q2)			
Newborn History (Q3)			
Physical Activity (Q4)	Physical Activity (Q4)	Physical Activity (Q4)	Physical Activity (Q4)
Maternal Clothing (Q5)	Maternal Clothing (Q5)	Maternal Clothing (Q5)	Maternal Clothing (Q5)
Breast Feeding Log (Q6)	Breast Feeding Log (Q6)	Breast Feeding Log (Q6)	Breast Feeding Log (Q6)
	Breastfeeding Review (Q7)	Breastfeeding Review (Q7)	Breastfeeding Review (Q7)
	Infant Health/Diet (Q8)	Infant Health/Diet (Q8)	Infant Health/Diet (Q8)
Maternal/Infant Health Assessment (Q9)	Maternal/Infant Health Assessment (Q9)	Maternal/Infant Health Assessment (Q9)	Maternal/Infant Health Assessment (Q9)
Edinburgh PPD Screen (Q10)	Edinburgh PPD Screen (Q10)	Edinburgh PPD Screen (Q10)	Edinburgh PPD Screen (Q10)
Online DHQ-III	Online DHQ-III	Online DHQ-III	Online DHQ-III

4. Body Mass Index (BMI): Based on a woman's weight and her height, a woman's body mass index (BMI) will be calculated at her initial visit and all subsequent visits, and will be recorded in Redcap

5. Daily Vitamin D and Prenatal Vitamins: Using a web-based computer program, each mother will be randomized to receive one of two vitamin D_3 regimens (600 or 6,600 IU/day). The 600 IU of vitamin D group will receive this from their prenatal vitamin. The experimental group will continue their prenatal vitamin (600IU vitamin D), and 6,000 IU vitamin D which will be from taking six, 1,000IU Vitamin D gummies.

6. Laboratory Data from Blood and Urine Samples: At initiation of the study and at each monthly visit (1, 2, and 3), each woman will have blood sample drawn (20 mL) for detailed analysis of vitamin D and calcium homeostasis. The total blood to be drawn from woman during the 3-month study period will be 60mL (<1/2 cup). In addition, she will be asked to provide a urine sample to measure her urinary calcium/creatinine. A total of up to 4 blood and urine samples will be obtained from each mother, depending on continued participation in the study.

7. Milk Samples: At time of initiation of the study and at each monthly visit (1, 2, and 3), each mother will provide a milk sample from her left breast. The skin will be washed with warm water prior to pumping. The amount obtained will be less than 20 mL at each visit. This milk can be pumped that day and kept refrigerated or pumped at time of visit. This will then be run on the Calais Milk Analyzer to be tested for Fat, Total Protein, True Protein, Lactose, and Calorie/ounce.

8. Degree of Skin Pigmentation will be measured at each visit using the Smart Probe 400, a commercially available spectrometer, which is placed lightly on the skin. Sites to be measured include the inner and outer lower arm and the buttocks.

9. Depression/Anxiety Screening: Each mother enrolled in the study will complete the Edinburgh Postnatal Depression Scale (EPDS) at each monthly study visit. Women who score above the threshold consistent with possible depression will be referred to the appropriate trained professional. Any woman who responds "yes" to the suicide related statements in the EPDS will be immediately referred to the Emergency Room for further evaluation and treatment.

Infant

1. Outpatient Visits: Each mother will be asked to bring her infant as a scheduled outpatient at her monthly visits. If still admitted to the NICU, these monthly visits will be held in the MUSC NICU. If discharged, visits will be held at Infant High-Risk Clinic or NEXUS clinic. These visits will be the initial at approximately 3-7 days of life, then at months 1, 2, and 3.

2. Questionnaires: We will ask each mother to complete a questionnaire about her baby's dietary type (breastfeeding, formula-supplementation), and feeding frequency for the week prior to each outpatient visit. Each questionnaire will take approximately 5 minutes to complete. See maternal section table.

3. Daily Vitamin D Supplementation: Infants of mothers who receive 600 IU of vitamin D_3 will receive 400 IU of vitamin D_3 in the form of drops. Infants of mothers who receive 6600 IU of vitamin D_3 will receive placebo drops.

3. General Health Assessment of Infant: At 1 week after delivery and then monthly until 3-months of age, each enrolled infant will undergo a growth (measuring head circumference, weight, length, head circumference, Ponderal index, body mass index [BMI]) and general health assessment.

4. PEAPOD Assessment: Prior to discharge from the NICU, the infant will also be placed in the PEAPOD® for body mass composition analysis. The material and data will be specifically obtained for research purposes. There is minimal risk. This is a safe device in which the infant lays in for 2 minutes.

5. Blood Sample from Infant: At initial visit, 1-, 2- and 3-months of age, each infant will have a blood sample 4mL (<1tsp) taken from either a vein (infant's hand, arm or foot) or the heel. Total amount obtained will be less than 12mL (<1 1/2 tablespoon). The serum sample will be analyzed for vitamin D and calcium concentration. Parents will be counseled that if their infant is vitamin D deficient, the infant's primary care physician and they will be contacted regarding the finding(s) with recommended follow-up.

11.0 Specimen Collection and Banking

Blood, urine and milk specimens will be collected. The specimen will be marked with patient name, subject ID, date and time of collection as well as MRN. This is necessary to ensure integrity of the data is maintained. These specimens will be stored in a double

locked laboratory maintained by Dr. Carol Wagner and Dr. John Baatz in the Darby Children's Research Institute (DCRI), Room 304. The specimens will be stored for up to 5 years. The PI and the co-investigators as listed on the IRB application will have access to the specimens and associated data and will be responsible for receipt or transmission of them. Specimens will not be sent to an outside facility.

Specimen/Banking for Future Use

- Describe the linkages to subjects and who will have access to subject identities.
    - Specimens will be stored in -80ºC freezer with study ID and participant name, MRN on samples. The samples will be located in the double locked lab of Drs. Carol Wagner and John Baatz. These samples will be destroyed after 5 years.
    - This information will be maintained in a database and all other data, including results of the study will be in a separate database that is password protected with the results by subject ID.
- List the data to be stored or associated with each specimen.
    - In one password-protected database: subject ID, participant name, MRN
    - In a separate password-protected database: subject ID, results
- State whether samples could be used for genetic analysis.
    - Yes, blood samples will be used for genetic polymorphisms of vitamin D binding proteins as this can affect vitamin D metabolism and processing and well as overall vitamin D status.
- Can specimens be withdrawn? If so, what is the process.
    - Yes, if a mother wants her samples withdrawn, she will inform the PI in writing of her request and sample(s) will be destroyed. This will be documented in the database and in the participant research record.

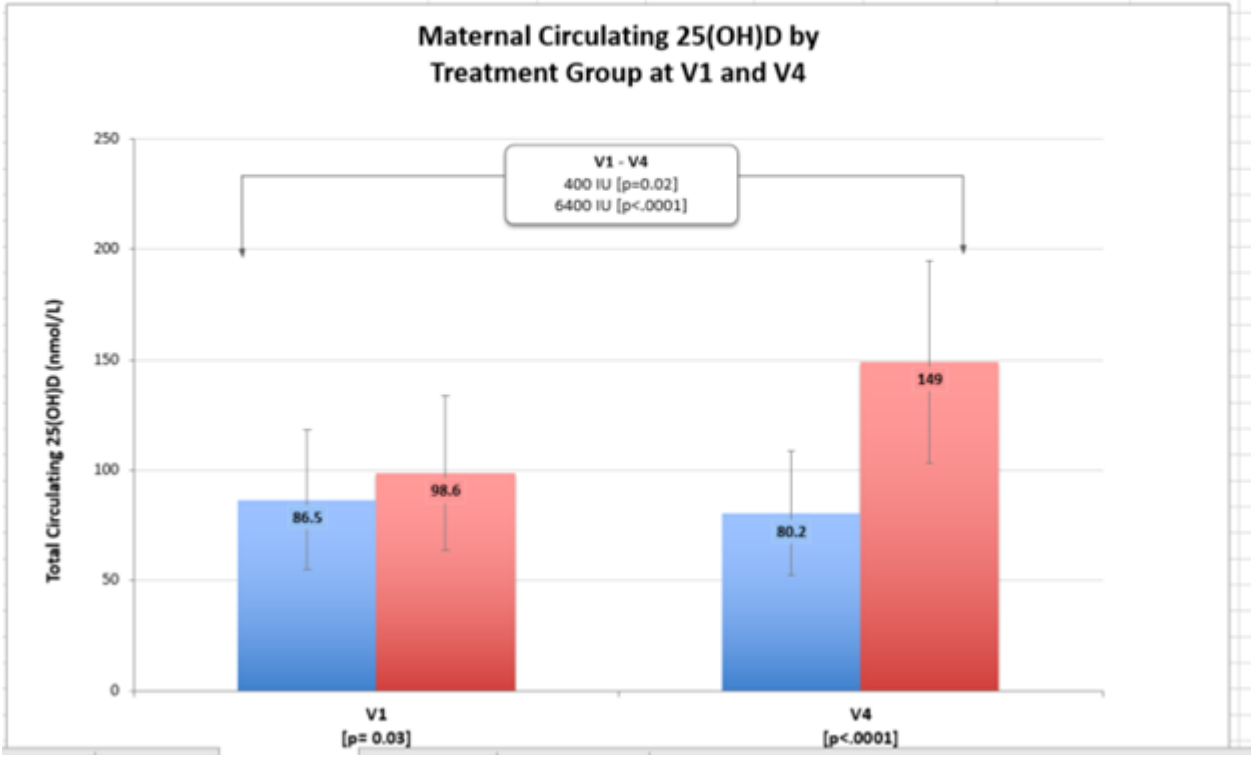
12.0 Data Management

All maternal and infant blood and urine samples obtained will be labeled with the patient's name, subject ID number, and medical record number to ensure proper tracking. Each study subject and her infant then will be assigned a specific study number without any reference to the woman or infant's name, which will be used to enter all data into RedCap. We will follow all HIPAA guidelines to maintain protection of patient/subject information. With these precautions in place, there is a remote risk that the samples and data could be linked to the woman and her infant; however, the study number and the name will be kept in a separate file in the locked office of Dr. Irons. The data will be entered into a secured database with only the study number entered, thus ensuring that the number and data will be kept separately from the woman and infant's names.

- *Describe the data analysis plan, including any statistical procedures.*

Power Analysis

Treatment Group	V1 [p= 0.03]	V4 [p<.0001]	V1 SD	V4 SD	n	Cohen's D	f
400 IU Vit D/day Mother & Infant Placebo Group	86.5	80.2	31.42	28.19	74	1.668	0.834
6400 IU Vit D/day Mother & Infant Group	98.6	149	35.31	45.88	74		



F tests - ANOVA: Repeated measures, within-between interaction based on effect size calculated from figure above.

Analysis: *A priori*: Compute required sample size
Input: Effect size f = 0.834
α err prob = 0.05
Power (1-β err prob) = 0.80
Number of groups = 2
Number of measurements = 3
Corr among rep measures = 0.5
Nonsphericity correction ε = 1
Output: Noncentrality parameter λ = 25.0400160
Critical F = 4.4589701
Numerator df = 2.0000000
Denominator df = 8.0000000
Total sample size = 6 (3 per group)
Actual power = 0.9620629

13.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

A. Creation of a Data and Safety Monitoring Board (DSMB): We will have a Data and Safety Monitoring Committee (DMSC) in place. Two of the members of the DSMB are physician scientists external to MUSC well known in the field of neonatology and nutrition. Dr. Sarah Taylor, MD a neonatologist at Yale University, and Dr. Rita Ryan, MD, a neonatologist at Rainbow Babies and Children's Hospital. One member will be a biostatistician at MUSC. He will maintain the database of the study, follow

HIPAA guidelines, and conduct ongoing interim analyses to ensure that the risk: benefit analyses remain in favor of benefit to the subjects.

B. Conduct ongoing monitoring of interventional trial by those who have appropriate expertise to accomplish the trial's mission: The PI will be responsible for checking laboratories of the women within 24 hours of their reporting and then entering the laboratory data into the computer. She will be provided with a set of normative laboratory values as a reference. The PI will review values weeklyor will be notified if a value falls outside of the referent value range. All data will be verified independently by ***Renee Washington, MA. The DSMB will be notified via email with source documents and adverse report sheets if a subject's value falls outside the referent value range. In addition, all adve***rse events will be reviewed quarterly by the DSMC, whose report will be forwarded to the IRB on a quarterly basis. The Investigators will report all Serious Adverse Events by email to the IRB and the DSMC; in addition, the IRB will receive a email report within 10 days of the Clinical Investigators' knowledge of the Event. In addition, the investigators will generate a quarterly report to the DSMC regarding subject enrollment, subject completion, adverse events and serious adverse events. The DSMC will review the report and a summary letter with their findings sent to the IRB.

C. Interim Data Analyses & Monitoring: Interim analyses for the evaluation of safety and efficacy will be conducted based on the recommendations of the Data Safety & Monitoring Board for two reasons: more than 10% of subjects in a given arm had achieved urinary calcium/creatinine ratios outside the acceptable range or there is evidence to the DSMC that there is significant clinical benefit to a given treatment arm over the other arm or arms of the study. The DSMC will serve to monitor for safety and efficacy. DSMB reports will include summary statistics: on mother-child recruitment (expected vs. actual); mother-child follow-up visits (expected vs. actual); data form quality (completion and timeliness of forms); tracking of data editing; demographics of the randomized mothers-children; aggregate safety; aggregate efficacy; and related information. The DSMB also will monitor the trial from the standpoint of futility using the techniques of Lan and Wittes (43).

14.0 Withdrawal of Subjects

- Describe anticipated circumstances under which subjects will be withdrawn from the research without their consent, including stopping participation for safety reasons.
    - Mother and infant move away from the study site
    - Infant is transferred to another facility
    - If mother had baseline hypercalcemia or hypercalciuria or baby had hypercalcemia, they would exit the study
- Describe any procedures for orderly termination of subjects by investigator.
    - The PI would notify the subjects in both writing and verbally if their participation is terminated.
- Describe procedures that will be followed when subjects voluntarily withdraw from the research, including partial withdrawal from procedures with continued data collection.
    - For voluntary withdrawal, the participant would notify the PI in writing. If mother requests to withdrawal either her or the infant, they will both be removed from the study without further data collection.

15 Risks to Subjects

1. **The Risks of Phlebotomy:**
    a. **Maternal:** The total amount of blood to be drawn at each maternal visit is 15 mL. Using aseptic technique, blood will be drawn monthly for a maximum of 4 blood draws (initial visit and monthly beginning at month 1 through month 3) during the study period and the total amount of blood over the study period will not exceed 60mL (<½ cup). Momentary discomfort may occur when skilled technicians or nurses perform phlebotomy with slight pain when the needle is inserted into a vein. Pressure will be applied to the arm following the procedure to minimize the risk of bruising; however, despite these efforts, a bruise may form at the site. Lastly, there is a slight chance of inflammation of the vein and/or blood clot formation, but this is extremely rare.
    b. **Infant:** The total amount of blood to be drawn during the study period (initial visit and months 1, 2, and 3) will be 2 mL at each visit (less than a teaspoon or less than 0.6% of the infant's total blood volume) or 10 mL (<2 tablespoons) over the course of 3 months. Using aseptic technique, blood will be drawn from the infant's hand, arm or heel either via venipuncture or heel stick. As with any blood draw, there may be a slight pain when the needle is inserted into a vein. Momentary discomfort may occur when skilled technicians or nurses perform phlebotomy. Pressure will be applied to the arm following the procedure to minimize the risk of bruising; however, despite these efforts, a bruise may form at the site. Lastly, there is a slight chance of inflammation of the vein and/or blood clot formation, but this is extremely rare.

2. **The Risks of Randomization in the Study:**
    a. There is a possibility that the treatment that each subject receives may prove to be less effective or to have more side effects than the other study treatment(s) or other available treatments. Because the ideal dose of vitamin D during lactation for mothers of preterm infants is unknown presently, the only way to determine the optimal dose is through a well-controlled, well designed scientific study that we are attempting to initiate.

3. **The Risks of High Dose Maternal Vitamin D Supplementation are hypercalciuria and hypercalcemia.** There is a small risk that the woman's blood calcium level would increase in response to higher dose vitamin D supplementation. The body responds by excreting calcium in the urine to maintain normal calcium levels. If calcium levels in the blood continue to rise, then serum calcium will increase leading to hypercalcemia. This process takes weeks to months to occur. It takes approximately 3 months to reach steady state on an increased vitamin D intake/regimen. In related studies conducted at MUSC comparing maternal vitamin D supplementation with 400, 2400, or 6400 IU of vitamin D_3/day and their infants for 7 months, there were no differences in infant or maternal safety parameters (22). There were no reported episodes of either hypercalciuria or hypercalcemia in any of the treatment groups. In fact, maternal 25 (OH)D levels were higher in the 6400 IU/day treatment group compared to the 400 IU group. In breastfed infants, there was no statistical difference in the average circulating 25 (OH)D in either treatment arm (22).
    a. To ensure that each lactating mother and her breastfeeding infant and each nonlactating mother (and her formula-feeding infant as a control) maintain normal vitamin D status throughout the study period and not develop hypervitaminosis D, both mother and infant will be screened monthly for hypercalciuria, the first indicator of hypervitaminosis D. All study subjects will be monitored closely for hypervitaminosis D. Operationally, we define caution limits as urinary Ca/creatinine ratio > 0.8 mmol/mmol. Whenever any patient

exceeds the caution limit, a specific case study will be initiated by the Data Safety and Monitoring Committee (DSMC) to examine the contribution of sunlight exposure and other confounding factors. Operationally, we define upper limits as a urinary calcium/creatinine ratio > 1.0 mmol/mmol Ca/creatinine ratio. This is a conservative upper limit as circulating levels achieved in individuals living in sun-rich environments achieve this amount without causing toxicity. The DSMC will be convened to review any patient who exceeds any predefined toxic limit for any outcome. If the elevated levels are unexplained, the patient will be withdrawn from supplementation but will continue to be monitored.

4. **The Risk of Maternal Vitamin D Supplementation on the Infant**: The developing infant's vitamin D levels are 50% of maternal serum levels at birth and rise gradually over the next 2-3 months if there is adequate delivery or synthesis (by skin). Because mothers who are breastfeeding and who are deficient in vitamin D produce milk that is deficient in vitamin D, there is a need to supplement either the infant directly or the mother herself. This study is designed to increase maternal circulating 25(OH)D levels of lactating women through the use of high dose supplementation. Approximately ~20% of maternal vitamin D passes into the milk, and therefore, the risk of hypervitaminosis D in infants whose mothers are receiving the 6,600 IU/day vitamin D_3 regimen—based on predicted maternal circulating 25(OH)D is extremely low (22). There is a risk of hypovitaminosis D to the infant whose mother is receiving the 600 IU/day D_3 regimen if that infant is not receiving the infant vitamin D_3 drops that are prescribed as part of that treatment arm (Group A).

   Minimizing Risk of Hypovitaminosis D Identified by an Infant's Participation in this Study: As an additional safety measure, if at visit 4, any infant in any treatment arm, circulating 25(OH)D concentration is found to be below normal [defined *a priori* as <20 ng/mL (<50 nmol/L)], will be supplemented with the 400 IU vitamin D/day. If an infant's circulating 25(OH)D concentration is <10ng/mL (<25 nmol/L) and is associated with a serum calcium level of <7.5 mg/dL (<18.75 nmol/L) at 3 months or older, then compliance of mother with her prescribed vitamin D regimen and that of the infant will be assessed. If mother has been compliant with the prescribed infant vitamin D_3 supplementation and the infant continues to have evidence of vitamin D deficiency with hypocalcemia, the infant will be referred to their pediatrician for further evaluation.

5. **The Risk of Milk Collection:** There is minimal physical risk in donating a milk sample either by using a manual or electric pump.

6. **The Risk of Breach of Confidentiality through Collection of Medical History and Clinical Laboratory Data:** As with any research study, there is a small risk that medical information, laboratory reports and data could become available to individuals outside of the study. All study personnel will adhere to HIPAA guidelines and regulations.

7. **The Risk of Measuring Degree of Skin Pigmentation using a skin tone chart and the Smart Probe 400**, a commercially available spectrometer, is minimal. The probe, from which a beam of visual spectrum light is emitted, and the absorbance measured, lightly touches the skin. Readout of the absorbance corresponds to the degree of pigmentation on a scale from 1 to 12. This technology is similar to what is currently used to measure bilirubin levels in neonates (transcutaneous bilirubin spectrometer) without any known side-effects. The information will be entered as a number into the database that will not be linked to the individual's name or medical record number such that confidentiality is maintained.

8. **The Risk of Postnatal Depression/Anxiety Screening:** The benefits of postnatal depression/anxiety screening far outweigh the risks. There is a risk that if the women are not screened for postpartum depression, they will not receive adequate evaluation and treatment. Asking questions about sadness and depression, anxiety and sleep problems can raise uncomfortable feelings that could precipitate suicidal ideation; however, any woman with clinical depression who goes without medical assessment and treatment is at greater risk. Studies have shown that early intervention has a positive impact on the outcome of the woman and her family.

   Based on the EPDS screening tests, those women who score in the high range for anxiety and depression will be referred to their primary care provider or if having suicidal ideation, to the Emergency Department (as outlined below). Those women who qualify for further evaluation and treatment will receive both written and verbal information regarding postpartum depression and the ill effects it can produce for themselves, their infant and other family members. Women will be referred to therapists at MUSC who specialize in postpartum depression. Any woman responding positively to the suicidal questions will be escorted to MUSC's Emergency Department for further evaluation and treatment.

9. **Unknown Risks:** The experimental treatments may have unknown side-effects that previously have not been reported (unexpected and unanticipated adverse events or effects).

16.0 Potential Benefits to Subjects or Others

Mothers enrolled in the study who will be taking the 6,600 IU/day vitamin D_3 supplementation regimen are predicted to have improved calcium and vitamin D homeostasis. In addition, it is hypothesized that mothers taking 6,600 IU/day vitamin D will have higher circulating 25(OH)D concentrations, and thus, greater transfer of vitamin D in their milk, rendering their milk superior in its antirachitic activity compared to the concentration in their milk at baseline or compared to those mothers randomized to the 600 IU vitamin D_3/day regimen.

Another potential benefit of this study is that data will be generated that will translate into recommendations for optimal vitamin D status for the mother-infant breastfeeding dyad and postpartum women in a controlled fashion. As was mentioned earlier, increasing numbers of Americans are becoming aware of the risks of hypovitaminosis D, and have begun to purchase vitamin D through mail-order vendors; thus, higher dose vitamin D supplements are easily available. Without monitoring, the potential for overdosing—although small, exists. There needs to be a controlled trial that will serve as a guide for practitioners and health care policy makers when recommending the recommended dietary intake for vitamin D of lactating women who deliver premature infants.

On a separate issue, women who are suffering from postpartum depression will be identified early and referred appropriately for evaluation and treatment. Early identification carries a better prognosis than if the symptoms persist without intervention.

17.0 Sharing of Results with Subjects

At the end of the study we will share with each participant her vitamin D status at baseline, and if it has improved or worsened over the course of the study. We will do the same for their infants' vitamin D status. Infant body composition results will also be shared with the mother. Maternal milk calorie/oz, protein, carbs, and fats will also be shared.

18.0 Drugs

The vitamin D gummies (1000 IU/gummy) along with placebo gummies, vitamin D drops, and placebo drops will be dispensed by Investigational Drug Services (IDS). At the start of the trial, they will dispense 4 bottles of gummies, and 1 bottle of drops. At each of the 3 following visits, they will bring their opened bottles, which will be checked for compliance through pill counts. This will also be done at the end of the study. All leftover medication will be returned to Investigational Drug Services for confiscation. Cholecalciferol, vitamin D_3 has an IND 1571 held by Dr. Bruce Hollis and Dr. Carol Wagner.

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