



MEDICAL UNIVERSITY
of SOUTH CAROLINA

**Scientific Workshop on
Vitamin D**

March 23, 2017

8:00am to 3:00pm

Bioengineering Building 110

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Moving Vitamin D Research Into Practice Through Nutrient Field Trials

Carole Baggerly, Director, Grassroots Health

Sharon L. McDonnell, MPH, GrassrootsHealth; Jennifer L. Aliano, MS, GrassrootsHealth; Cedric F. Garland, DrPH, Dept. of Family and Preventive Medicine, University of California San Diego; Keith A. Baggerly, PhD, Dept. of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center; Christine B. French, MS, GrassrootsHealth; Leo L. Baggerly, PhD, GrassrootsHealth; Roger B. Newman, MD, Medical University of South Carolina; Carol L. Wagner, MD, Medical University of South Carolina

Background:

Moving research into public health and clinical practice is a notoriously slow and arduous process. The delay in the implementation of evidence-based vitamin D discoveries has allowed many diseases and adverse health effects to proliferate, where a substantial proportion could have been prevented by improving vitamin D status. To address this problem, we developed a new methodology for doing nutrient field trials with 8 steps, given an area of demonstrated science/benefit.

- (1) Identify a panel of experts and work with them to create a consensus direction statement to be used for education and evaluation.
- (2) Assemble the results of studies to develop scientific materials for education.
- (3) Create user guides for professionals and patients/consumers that are interactive and evidence-based.
- (4) Invite participation by large institutions (hospitals, community groups, individuals), local government and health agencies.
- (5) Present the educational materials via continuing medical education courses for practitioners, ongoing emails, and/or text messages to users.
- (6) Analyze and provide feedback to the participants of the data collected-from health outcomes to administrative information such as numbers of vitamin D tests done and serum concentrations of biomarkers of intake.
- (7) Create a publication with outcomes for submission to a scientific journal.
- (8) Present information to public health groups, insurers, and the public to enable broad action. This entire set of actions can be put together in an implementable package. It does not need to be done uniquely for every institution or group.

This methodology has been successfully implemented within a major hospital system and with over 10,000 individuals around the world. Given the methodology and the requisite materials, the implementation of major findings in vitamin D (or other nutrient) research can be implemented in large segments of the population in 2-4 years, not the 15-25 it currently takes. In addition, the use of the field trial approach allows extensive monitoring and data collection during the process so that adjustments can be made during the process as necessary. It represents a scientific approach to much faster and more effective implementation of vitamin D and other nutrient research.

The conference will afford an opportunity to explore methods of moving research into practice with other researchers and to further define their needs. And, to explore research areas with vitamin D that are ready for practice implementation.

Vitamin D and Human DNA Methylation: Global and Genome-Wide Association studies

Yanbin Dong, Georgia Prevention Institute, Department of Pediatrics, Medical College of Georgia, Augusta University. ydong@augusta.edu

Objectives:

We have previously shown that vitamin D3 supplementation reduces inflammation and improves arterial stiffness, endothelial dysfunction and increases telomerase activity. Understanding of the influence of low vitamin D on epigenome will provide novel insight into the pathogenesis of low vitamin D-related disorders. We tested our hypotheses that 1) suboptimal vitamin D is associated with DNA global hypomethylation and locus-specific methylation changes; and 2) maternal vitamin D supplementation affects neonatal epigenome regulation.

Methods:

Global DNA methylation level (percentage of 5-methylcytosine) was quantified using leukocyte DNA with the MethylFlash™ Methylated DNA Quantification kit (Epigentek). Two epigenome-wide DNA methylation studies (EWAS) were performed using the Illumina HumanMethylation 27K and 450K Beadchip, respectively.

Results:

In the 16-week RCT, a dose-response benefit of vitamin D3 supplementation on global DNA methylation in African American youth was observed, as indicated by a significant linear upward trend ($-0.01 \pm 0.01\%$, placebo; $0.11 \pm 0.01\%$, 600 IU/day; $0.30 \pm 0.01\%$, 2000 IU/day, and $0.65 \pm 0.01\%$, 4000 IU/day group, p -trend = 0.04). In the EWAS study comparing cases of vitamin D deficiency with controls in African American adolescents, 79 differential CpG sites were identified, which involve in cell differentiation, cellular defense response, T cell regulation and Wnt signaling pathways. In the cord blood EWAS, over 2,000 CpG sites were regulated by maternal vitamin D supplementation. The top 10 genes include GRIK2, GRIN2D, B3GNTL1, SLC2A1, PF4V1, TSPAN13, KLK5, PRICKLE1, PCNX, and AGBL5. Pathway analysis showed that the top 200 genes were enriched in neurogenesis, antigen processing, inflammation, cell proliferation, neuron differentiation, and transcription regulation.

Conclusions:

Low vitamin D status is associated with global hypomethylation. Vitamin D supplementation increases global DNA methylation and regulate locus-specific methylation.

A Survey of Placental Pathologic Findings Observed in 123 Singleton Placentas

Michael Caplan, MD, Suffolk County Office of the Medical Examiner

Objectives:

1. To summarize the gross and histopathologic observations of 123 singleton placentas that were enrolled in the Kellogg vitamin D study.
2. To identify any consistent pathologic patterns that potentially correlate with maternal 25-hydroxyvitamin D status.

Potential applications of maternal serum 25-hydroxyvitamin D include analyses of placental weight, vascular pathology, and correlations with gestational diabetes mellitus. We examined 122 singleton placentas as part of the Kellogg vitamin D placenta study. The placentas were all submitted in the fresh state and placed into a refrigerator in order to minimize the effects of autolysis; they were subsequently fixed into a specimen container of 10% neutral buffered formalin whose volume exceeded that of the placenta by several folds. The fixed weight of the placenta was subsequently multiplied by 0.95 in order to account for the artefactual weight gain in formalin. After the placenta was examined by either an attending pathologist or a pathology resident trained in gross placental examination, standard sections of umbilical cord, extraplacental membranes, and placental disc were submitted for microscopic examination. A study worksheet was created in order to record the gross and microscopic findings in a thorough and systematic fashion.

The results of the study were not particularly surprising or illuminating: the majority of the placental weights were either within the 10th to 90th percentile for gestational age (51.3%) or less than the 10th percentile (42%) the remaining 6.7% were greater than the 90th percentile. The most common findings were: 1) laminar decidual necrosis (37.0% of cases); 2) pigment-laden macrophages (36.1%); 3) amniotic fluid infection (28.7%); 4) intervillous thrombi (16.7%); and 5) perivillous fibrin(oid) deposition (12.0%).

In summary, the following 3 major conclusions can be drawn from this study:

1. The overwhelming majority (93.3%) of the placental weights were either within the normal weight distribution for gestational age (GA) (10th-90th percentile) or were underweight (below the 10th percentile for GA).
2. The majority of the placental pathologic findings have not been demonstrated to be clinically relevant.
3. Villous maturation is by far the most subjective assessment that is made on microscopic examination of the placenta.

Nutrigenomic Profiling for Early Risk Stratification and Preventive Intervention to Decrease Disparities in Spontaneous Preterm Birth

John Finnell, ND, MPH, MS, Lac, Director of Research, AOMA Graduate School of Integrative Medicine

The leading causes of neonatal morbidity and mortality are complications of preterm birth, defined as <37 weeks' gestation 1,2. Spontaneous preterm birth (SPTB), represents a uniquely high-risk population, for which there are no clear predisposing medical conditions 3,4. Known risk factors may influence the balance between the nutritional status of the mother and embryo at the maternal-placental interface and are most associated with SPTB include infection and inflammation 5, maternal stress 6, smoking 7, nutritional status 8, and advanced maternal age 9. The early identification of women at high risk for SPTB and implementation of efficacious intervention are paramount in the prevention of SPTB; however, prenatal care does not, as of yet, provide safe and effective, evidence-based interventions in the prevention of SPTB 10,11.

Higher prevalence of SPTB is associated with the season of conception correlated with the seasonality of lower vitamin D levels 12. Vitamin D insufficiency and deficiency may also predispose preterm birth outcomes and sequelae, such as small for gestational age and preeclampsia 13,14. A recent study suggests that maintaining serum vitamin D levels at an optimal level of ≥ 40 ng/mL, especially in the third trimester, may reduce risk of preterm birth 15. Our published clinical trial validated the quality of three vitamin D supplements, established a safe and effective vitamin D3 supplementation strategy to correct vitamin D insufficiency 16.

Klotho is a novel gene, which encodes both a 130-kD trans-membrane Klotho protein (mKL) and a soluble extracellular domain (sKL), principally expressed in the distal-convoluted tubule of the kidney, placenta and the choroid plexus of the brain 17. Studies suggest Klotho plays a role in the maintenance of calcium (Ca), phosphate (Pi) and VitD homeostasis 18-20, endothelial function 21,22, normal energy metabolism 23, glutathione production 24, regulation of oxidative stress 25-27, inflammation 28, and suppression of the insulin/IGF-1 signaling 20. In addition, decreased Klotho levels may be associated with decreased risk of preeclampsia 29-31. Our study in humans showed that correction of vitamin D insufficiency 16, may increase circulating Klotho (data not published).

Circulating microparticle (CMP) biology offers a novel methodology to examine the nutrient profiles of the utero-placental interface, and have already been used to establish a proteomic profile to identify populations at risk for SPTB, defined as ≤ 34 weeks, as early as the end of the first term 32. We propose that circulating Klotho levels may act as a surrogate biomarker of vitamin D activity with respect to perinatal human physiology and normal maternity.

My research is intended to support a K23 career development grant application and aims to use nutrigenomic techniques to explore dosage strategies to correct vitamin D insufficiency so as to optimize expression and circulating levels of Klotho protein in the placenta and attenuate oxidative stress and inflammation, while promoting endothelial function at the utero-placental interface. I am interested in identifying collaborators with existing samples and data sets, for secondary analysis, or prospective studies.

Vitamin D and the Placenta: Two Sides to the Story

Martin Hewison, PhD, Professor, University of Birmingham, Birmingham, England

Keynote Address

Vitamin D-deficiency is common to many communities around world, but it is a particular health challenge to pregnant women where low serum levels of vitamin D may contribute to adverse events in pregnancy as well as fetal development. Despite the growing clinical evidence of a role for vitamin D in pregnancy, our understanding of how vitamin D influences maternal and fetal health across gestation remains poor. Current studies suggest that the placenta is a crucial component of vitamin D function in pregnancy, with proposed actions on both maternal (decidual) and fetal (trophoblast) tissues.

How key questions remain to be answered:

1. How does vitamin D access different tissues in the placenta?
2. Can the decidua and trophoblast metabolize vitamin D in functionally meaningful fashion?
3. What is the functional impact of vitamin D in the placenta, and does this vary within different tissues?

To answer these questions, we have used human maternal, placental and fetal tissues, including material from preeclampsia, a key adverse event in pregnancy. We have also used mouse models to investigate the placental/pregnancy impact of specific components of the vitamin D pathway, notably the vitamin D receptor (VDR), the vitamin D-activating enzyme CYP27B1, and the vitamin D binding protein (DBP). Mouse models have also enabled detailed analysis of the pregnancy effects of vitamin D-deficiency, highlighting changes maternal health (hypertension), pregnancy progression (fetal size), pregnancy outcome (fetal loss) and neonatal health.

The over-arching conclusion from these studies is that vitamin D plays a crucial role in healthy pregnancies by orchestrating a diverse array of responses at the fetal-maternal interface. Although we can view these as distinct facets of vitamin D biology in the decidua and trophoblast, it is becoming increasingly clear that vitamin D function in pregnancy requires integrated actions on both sides of the placenta.

The aim of this presentation will be to demonstrate how improved understanding of the basic biology of vitamin D during pregnancy is crucial to current and future studies of maternal and fetal health.

Mouse models to characterize the impact of maternal vitamin D deficiency on offspring epigenetic outcomes

Folami Ideraabullah, PhD, Assistant Professor, University of North Carolina - Chapel Hill, Chapel Hill, North Carolina

Jing Xue, University of North Carolina at Chapel Hill Nutrition Research Institute; Raad Gharaibeh, University of North Carolina at Charlotte Bioinformatics Service Division, Corey Brouwer, University of North Carolina at Charlotte Bioinformatics Service Division; Lisa Tarantino, Will Valdar, University of North Carolina at Chapel Hill

Objectives: To understand the impact of maternal vitamin D inadequacy on offspring epigenetic outcomes and the role this plays in development and health.

Maternal nutrition plays an important role in fetal development and can impact offspring health throughout life. Epigenetic changes in response to excess or insufficient nutrients during gestation are implicated as a key mechanism in determining offspring phenotype. However, for most nutrient-phenotype relationships, evidence of direct epigenetic involvement remains unclear. As an essential nutrient, vitamin D inadequacy is linked to diseases such as colon and breast cancer, cardiovascular disease, bone disease, immune dysfunction and metabolic, neurological and reproductive disorders. Causal mechanisms and timing of insult remain unclear, however, gestation is a likely time of origin since this is a stage of rapid fetal development and requires adequate maternal sources. Our work is focused on characterizing offspring epigenetic response to maternal vitamin D inadequacy for the purpose of improving diagnoses of inadequacy and methods of intervention. Our mouse model data show that maternal 25(OH)D levels are dependent on diet and genetic background.

In addition, maternal dietary vitamin D depletion is associated with changes in body weight, body composition, metabolic profile and DNA methylation at developmentally relevant imprinting control regions in two generations of offspring. Genome-wide methylation analyses of offspring germline and soma reveal vitamin D deficiency-induced methylation changes in both somatic and germ cells in proximity to genes with key roles in disease, development, and epigenetic regulation. Our ongoing work incorporates the use of genetically diverse collaborative cross (CC) inbred mouse strains to fully investigate the role of genetic differences in influencing these outcomes. When combined with human studies we expect these studies will allow for better characterization of maternal vitamin D adequacy and potential epigenetic mechanisms of disease outcomes.

Adiponectin and Vitamin D-binding Protein are Independently Associated at Birth in Both Mothers and Neonates

Spyridon Karras, MD, PhD, Internal Medicine, Aristotle University, Thessaloniki, Greece

Stergios Polyzos; Danforth A. Newton; Carol L Wagner; Bruce W. Hollis; Jody van Ouweland; Erdinc Dusun; Diugy Gezen-Ak D; Declan P. Naughton

Introduction:

Pregnancy is a unique dynamic state where VDBP concentrations are known to increase throughout pregnancy. In addition, body weight and adipokine profile at birth, have been recently associated with increased fat mass in late childhood and adolescence respectively.

The primary aim of this study was to explore the association between adiponectin, irisin and VDBP levels in both mothers and neonates at birth. Secondary aims were to investigate the associations between neonatal anthropometric parameters, and maternal and neonatal adiponectin, irisin, VDBP, 25(OH)D and 25(OH)2D, in the context of a potential plurimetabolic interaction.

Methods:

This was a case-control study of pregnant women and their neonates, conducted from January 2014 until December 2015. Blood samples were obtained from mothers by antecubital venipuncture 30-60 minutes before delivery and were measured for corrected calcium (mg/dl), parathyroid hormone (PTH) (pg/ml), 25(OH)D (ng/ml), 1,25(OH)2D (pg/ml), VDBP (µg/ml) adiponectin (µg/ml) and irisin (ng/ml). At enrolment, demographic and social characteristics were recorded and Neonatal anthropometry measurements were undertaken by a trained nurse.

Results:

Seventy pairs of newly delivered neonates and their mothers were included in this study. As expected, the neonates had lower PTH and higher corrected calcium levels compared with mothers. On the other hand, 25(OH)D, 1,25(OH)2D and VDBP levels were not different between groups, with 1,25(OH)2D only marginally not reaching the level of statistical significance ($p=0.057$). Interestingly, adiponectin levels were higher, whereas irisin levels were lower in neonates than mothers. Notably, VDBP levels were positively correlated with adiponectin and irisin levels, whereas inversely with age. Adiponectin levels were inversely correlated with weight and BMI at term, and irisin levels positively with weight. After sequential adjustment for weeks of gestation (model 1), weeks of gestation and maternal age (model 2), weeks of gestation, maternal age and BMI (term) (model 3), maternal adiponectin and irisin levels remained significantly positively associated with VDBP levels. After sequential adjustment for weeks of gestation (model 1), weeks of gestation and neonate gender (model 2), weeks of gestation, neonatal gender and weight (model 3), neonatal adiponectin levels remained significantly positively associated with VDBP levels. Neonatal adiponectin and irisin remained non-significantly associated with maternal VDPB, after sequential adjustment for weeks of gestation (model 1), weeks of gestation and maternal age (model 2), weeks of gestation, maternal age and BMI (term) (model 3), weeks of gestation and neonatal gender (model 4), weeks of gestation, neonatal gender and neonatal weight

Conclusions:

This is the first study to investigate potential correlations between vitamin D metabolites, VDBP and the adipokines, adiponectin and irisin, in mother-neonate pairs and the interactions between neonatal anthropometry and maternal-neonatal vitamin D and adipokine homeostasis at birth. Main findings of this study, is the perspective of a potential independent interaction of VDBP and adiponectin in both mothers and neonates and the lack of a causative model effect of maternal or neonatal 25-(OH)D and adipokine profile on neonatal anthropometry at birth.

The results of this small cross sectional study come from a South European population of pregnant women with hypovitaminosis D.

Immune Mediators and Vitamin D Status in the Development of Comorbidities of Pregnancy

Rebecca Moore, MD, Neonatal-Perinatal Medicine Fellow, Pediatrics, Medical University of South Carolina, Charleston, SC

Jennifer K. Mulligan, PhD; Howell Harmon; Bruce W. Hollis, PhD; Carol L. Wagner, MD

Background:

Comorbidities of pregnancy (COP) lead to adverse neonatal outcomes. Improper maternal immune system regulation may be a contributing factor to the development of COP. Vitamin D is a known immune modulator of the innate and adaptive immune system. Prior studies confirm a decreased risk of COP with sufficient vitamin D [25(OH)D] levels > 40 ng/mL.

Objectives:

1. To examine the relationship between maternal immune mediators and vitamin D levels in relationship to the development of COP.
2. To further investigate the possible predictive value of maternal immune mediator alterations in women who subsequently develop preterm labor/birth.

Methods:

Hematologic samples were obtained monthly from subjects enrolled in a RCT supplementing either 400 IU or 4400 IU of vitamin D per day during pregnancy. Maternal 25(OH)D levels were assayed. Plasma cytokines and other immune regulatory product concentrations were measured by ELISA. COP were defined as preterm labor/birth <37 weeks' gestation, preeclampsia and chorioamnionitis. Associations of both 25(OH)D and immune mediator concentrations with COP were evaluated with Wilcoxon rank-sum tests or Spearman correlation, as appropriate.

Of 270 participants, 21 subjects developed preterm labor/birth, 10 preeclampsia and 4 chorioamnionitis, thus far. No significant correlations were found between maternal 25(OH)D levels and the development of COP. Subjects with preterm labor/birth had decreased TGF- β concentrations throughout pregnancy, significant in the 1st ($p < 0.01$) and 2nd trimesters ($p < 0.02$), increased TNF- α concentrations, significant in all trimesters ($p < 0.05$), and decreased IL-6 concentrations in the 2nd and 3rd trimesters ($p < 0.05$), compared to subjects without COP.

Mothers who developed preterm labor/birth were found to have alterations in their immune mediators, including decreased TGF- β and IL-6, in addition to increased TNF- α . This data suggests that these early immune mediator changes are possible predictors for preterm labor/birth. Low prevalence of COP in the study group did not allow for adequate power to detect a relationship between 25(OH)D levels and COP.

Further analyses are underway to more accurately delineate the relationship between maternal immune mediator concentrations and preterm labor/birth. Maternal hematologic samples collected nearer preterm birth/delivery will be analyzed and compared to age and race matched controls of the same gestation. This data will hopefully incite further investigation into maternal immune mediator concentration changes as a possible predictor of preterm labor/birth.

Improved Vitamin D3 Status is Associated with Increases in Circulating T-regulatory Cells during Pregnancy

Jennifer Mulligan, PhD, Assistant Professor, Otolaryngology, Medical University of South Carolina, Charleston, South Carolina

Aman Sumal, BS; Sarah Smith; Judy Shary, MS; Bruce W. Hollis, PhD; Carol L. Wagner, MD

Introduction:

The maternal immune system undergoes numerous changes during pregnancy. When a proper balance in these changes is not maintained a number of adverse outcomes such as preeclampsia, preterm birth and gestational diabetes may occur. The prohormone vitamin D is a well described regulator of a number of cell types involved in immunity.

Study Objective:

To determine the impact of 400 IU vs 4,400 IU vitamin D3/day on maternal circulating immunity.

Methods:

Women participating in a vitamin D supplementation trial (NCT01932788) were recruited for participation between 8-12 weeks of gestation, randomized into one of two treatment groups and followed prospectively. Peripheral blood mononuclear cells from 40 randomly selected patients were assayed for several immune cell types including T-cells, T-regulatory cells, B-cells, dendritic cells, macrophages and natural killer cells.

Results:

Of the patients examined, 18 received 400 IU and 22 received 4,400 IU/daily. Mothers receiving 4,400 IU had significantly higher numbers of T-regulatory cells than mothers receiving 400 IU daily in the 2nd trimester. A similar trend was observed in the 3rd trimester though was not statistically significant. There were no statistically significant differences between treatment arms for any other of the other immune cell populations examined.

Conclusions:

During pregnancy, higher doses of vitamin D3 are associated with increased numbers of circulating immune suppressive T-regulatory cells. These findings may provide mechanistic insight into prior reports that have shown high dose vitamin D3 supplementation is associated with a reduced risk of inflammatory complications of pregnancy.

Funding:

These studies were funded by a grant to CLW from the W.K. Kellogg Foundation

Preliminary Results from a Vitamin D Screening and Supplementation Field Trial to Prevent Preterm Birth

Roger Newman, MD, Professor, OB-GYN, Medical University of South Carolina, Charleston, South Carolina

CA Baggerly, GrassrootsHealth; SL McDonnell, GrassrootsHealth; KA Baggerly, Department of Bioinformatics and Computational Biology, The University of Texas, MD Anderson Cancer Center, Houston, TX; Jen Aliano, GrassrootsHealth; CB French, GrassrootsHealth; LL Baggerly, GrassrootsHealth; Bruce Hollis, Department of Pediatrics, Medical University of South Carolina; Carol Wagner, Department of Pediatrics, Medical University of South Carolina

25-Hydroxy Vitamin D [25(OH)D] ≥ 40 ng/ml has been associated with a 59% lower risk of preterm birth < 37 weeks' gestation compared to women with levels ≤ 20 ng/ml ($P=0.02$) in two pregnancy supplementation trials by Wagner et al.

The objective of this field trial was to determine if this inverse relationship between 25(OH)D levels and the rate of preterm birth (PTB) could be replicated at an urban medical center treating a large, diverse population of women.

A vitamin D screening and supplementation program was implemented in September 2015 at the Medical University of South Carolina. The program involved universal vitamin D screening for pregnant women at their first prenatal visit with follow-up testing for those < 40 ng/ml at approximately 28 weeks and prior to delivery. In addition, all obstetrical health care providers received education regarding the potential health benefits associated with sufficient vitamin D status and standard recommendations to improve that status using vitamin D supplements.

Between September 2015 and January 2017, delivery information was available for 1387 women with a live, singleton birth and at least one 25(OH)D test result during pregnancy. Mean age was 30 years and 52% were white, 32% black, 8% Hispanic, and 9% other race. Overall, 90% of pregnancies had 25(OH)D levels < 40 ng/ml and 97% of black women had levels < 40 ng/ml. There were 207 PTBs < 37 weeks' gestation (15%), of which 50 were very preterm (< 32 weeks), 23 were moderately preterm (32 to < 34 weeks), and 134 were late preterm (34 to < 37 weeks). Mean 25(OH)D closest to delivery was 18.3 ng/ml for very preterm, 30.7 ng/ml for moderately preterm, 31.6 ng/ml for late preterm, and 34.3 ng/ml for term birth. Preterm birth (< 37 weeks) rates were 21% in women with 25(OH)D < 20 ng/ml ($N=65$), 15% in women with 25(OH)D 20-39 ng/ml ($N=98$), and 11% in women with 25(OH)D ≥ 40 ng/ml ($N=44$). There was a 56% lower risk for PTB < 37 weeks' gestation for pregnant women with 25(OH)D levels > 40 ng/ml compared to those with levels < 20 ng/ml ($p<0.0001$). The fitted LOESS curve shows gestation week at birth steadily rising with increasing 25(OH)D, following a similar pattern as the RCTs by Wagner et al. (Figure 1).

These preliminary field trial results show a trend towards higher 25(OH)D serum levels being associated with a lower risk of PTB. As more data becomes available, we will have sufficient power to provide more extensive and precise results overall and by race/ethnicity.

Association of Bacterial Vaginosis and Vitamin D Deficiency in Pregnancy

13Anna M Powell MD¹, Judy Shary⁴, Vishwanathan Ramakrishnan³, Carol Wagner MD⁴

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Objective:

To investigate the association between bacterial vaginosis (BV) and vitamin D deficiency in a prospective, randomized cohort of pregnant women.

Materials and Methods:

This was a randomized controlled trial of vitamin D supplementation to a cohort of women with and without medical comorbidities enrolled between 2012-2015 (W. K. Kellogg Foundation, NIH/NCAT UL1 TR000062). 257 women were randomized to 400 IU versus 4400 IU of cholecalciferol starting at 8-12 weeks of gestation and followed with monthly visits. Vaginal swabs were collected each trimester prior to delivery and submitted for Gram staining and Nugent score calculation. Continuous variables were analyzed using Student's t test and Wilcoxon rank sum tests; categorical variables were analyzed by chi-square and Fisher's exact test using SAS 9.4 (Cary, NC). Pregnancy outcomes of interest were defined as gestational hypertension, pre-eclampsia, HELLP syndrome, chorioamnionitis and preterm birth.

Results:

There were 45 women diagnosed with BV at study entry. Compared to women without BV initially, these women were more likely to be African American ($p < 0.0001$) and have lower vitamin D levels at visit 1 and 4 ($p = 0.05$ and 0.03 , respectively). There were no differences in pregnancy outcomes of interest among this group. In mixed regression modeling to account for repeated measurements, BV was significantly related to time ($p = 0.27$), race (0.0021) and BMI ($p < 0.0001$), but not with vitamin D level ($p = 0.79$) or treatment group ($p = 0.83$). African American race was associated with an OR 3.1 (95%CI 1.9 -5.02) of having BV.

Conclusions:

Vitamin D supplementation in pregnancy does not appear to significantly decrease incidence of bacterial vaginosis.

Maternal Vitamin D3 Supplementation during Pregnancy and Pediatric Dental Enamel Hypoplasia

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Objective 1:

To investigate the impact of maternal factors during pregnancy and at birth on developmental defects of the maxillary central incisor teeth that develop in utero.

Objective 2:

To assess the study's design and research methodology for the conduct of a future multi-site study that focuses on pregnancy and infancy factors for developmental defects of enamel.

Enamel hypoplasia (EH), is a developmental defect of tooth enamel that affects both the primary and secondary dentition. The etiology of EH is unknown. We conducted a secondary analyses of existing data from a randomized and controlled clinical trial of maternal vitamin D3 supplementation during pregnancy to study the impact of maternal vitamin D (by treatment group, serum circulating total 25(OH)D and 1,25(OH)2D); serum parathyroid hormone, calcium, and phosphorus; urinary calcium/creatinine ratio; acute infections and medications; anti-reflux medications; and birth outcomes as related to development of enamel hypoplasia in the maxillary central incisor teeth that develop in utero. Enamel hypoplasia was scored using the Enamel Defects Index from digital images made using 3x magnification. The statistical models developed are hierarchical Bayesian longitudinal mixed effect models. Informative drop-out is handled via survival models jointly fitted with the longitudinal data. OpenBUGS version 3.2.3 rev 1012 and R version 3.2.3 are used. Results of our ongoing analysis will provide evidence for maternal 25(OH)D concentrations during pregnancy necessary to correlate with development of EH during pregnancy. Study results will inform future studies of prenatal nutrient exposure for prevention of EH.

We plan to achieve the two objectives as listed above through presentation of our study to date, addressing retreat participants' questions, and by incorporating retreat participants' suggestions into our future multi-site study.

Technology Assisted Medication Adherence in Pregnancy

Elizabeth V. Schultz, MD, Neonatology, Medical University of South Carolina, Charleston, SC

Myla Ebeling; Wei Wei, MD, PhD; Sachin Patel, MSc; Frank A Trieber, PhD; David J Annibale, MD; Carol Wagner, MD

Objective:

- 1) Review the utility of cell phone technology as an intervention to target medication compliance during pregnancy.
- 2) Highlight the importance of changing the role of these technologies to meet the demands of a diverse target population.

Purpose of Study:

We examined the utility of cell phone technology, in the form of text messaging, to promote medication adherence in a current study evaluating the role of Vitamin D during pregnancy.

Methods Used:

In an ongoing RCT with 374 participants, women were randomized to receive 400 IU/day (n=184) vs an additional 4000 IU/day (n=189) of vitamin D3. We performed an a priori designed analysis to compare the difference in those who opted to receive text message medication reminders vs those who did not throughout the course of pregnancy and the % change in maternal 25(OH)D.

Summary of Results:

We found a statistically significant difference between those who opted to receive medication reminders and % change of maternal 25(OH)D concentrations.

Conclusions:

Our findings are consistent with the emerging evidence that technology assistance may play a critical role in improving maternal, and thus fetal, health. This study additionally highlights the importance of multilingual applications appropriate for the enrolled population to assess the generalizability of findings to a more diverse population.

How this Research plans to achieve the retreat objectives:

Future collaborative work may improve technologies to play a pivotal role in maternal and fetal health outcomes with vitamin

Maternal Obesity, 25(OH)D Concentration and Bone Density in Breastfeeding Dyads

Sarbattama Sen, MD, Assistant Professor, Pediatrics, Harvard Medical School, Cambridge, Massachusetts

Annie Penfield-Cyr; Bruce W. Hollis; Myla Ebeling; Cindy A. Howard; Ruth A Lawrence; Harry Blanke; Carol L. Wagner

Objective:

To examine the association between maternal BMI and vitamin 25(OH)D concentration and bone density in exclusively breastfeeding mother-infant pairs.

Study Design:

We conducted a secondary analysis of a cohort of 278 exclusively breastfeeding mother-infant dyads who were recruited in the first post-partum month for a randomized controlled trial of maternal vitamin D supplementation at two sites. We compared mean 25(OH)D concentrations and bone mineral density by BMI group and also examined the adjusted association between maternal BMI with 25(OH)D concentrations and bone mineral density at one, four and seven months postpartum. 25(OH)D concentrations were measured using RIA and bone mineral density using DXA.

Results:

At baseline, 7.5% of Lean women, 16.7% of overweight women and 21.7% of obese women were vitamin D deficient ($p < 0.05$.) Breastfeeding women who were overweight or obese had lower 25(OH)D concentrations and higher bone mineral density than lean women at all three time points ($p < 0.01$). Higher maternal BMI was associated with lower maternal serum levels of 25(OH)D (adjusted $\beta = -0.49$ ng/ml per kg/m² increase in BMI, 95% CI $-0.78, -0.19$ at one month and -0.63 ng/ml; $-0.98, -0.27$ at 4 months) and higher bone mineral density at all time points ($\beta = 0.006$ BMD Z-score; 95% CI $0.003, 0.01$ at one month). Infants born to overweight and obese mothers had lower 25(OH)D concentrations than infants of lean mothers ($p < 0.01$), with higher maternal BMI associated with lower 25(OH)D concentrations at four months ($\beta = -0.65$; 95% CI $-1.10, -0.20$).

By presenting this research in this forum, I hope to develop collaborations to further the understanding of the role of vitamin D in pregnancy and postpartum health of obese women.

Functional Indicators of Vitamin D Adequacy for Very Low Birth Weight Infants

Sarah Taylor, MD, MSCR, Associate Professor, Pediatrics, Medical University of South Carolina, Charleston, SC

Amy Wahlquist, School of Public Health, MUSC; Carol L. Wagner, Department of Pediatrics, College of Medicine, MUSC; Viswanathan Ramakrishnan, School of Public Health, MUSC; Myla Ebeling, Department of Pediatrics, College of Medicine, MUSC; Bruce Hollis, Department of Pediatrics, College of Medicine, MUSC

Background:

Healthy infant vitamin D status has been defined as the vitamin D required to avoid rickets. Identifying the vitamin D status to optimize calcium and bone health would benefit preterm infants who are at high risk of metabolic bone disease.

Methods:

Very low birth weight infants had measurement of 25-hydroxyvitamin D [25(OH)D] status and markers of calcium and bone health at birth, one month, and term age. Piecewise linear regression modeling was performed to identify a threshold of 25(OH)D status associated with stable parathyroid hormone (PTH) concentration and bone mineralization at term age.

Results:

Femur bone mineral content (BMC) and density (BMD) were significantly associated with 25(OH)D status but spine bone mineral measurements were not. In addition, as 25(OH)D increased, femur BMC and BMD increased until reaching a 25(OH)D threshold of 120.4 nmol/L and 116.4 nmol/L, respectively. PTH status decreased with increasing 25(OH)D until reaching a plateau at 25(OH)D of 107.3 nmol/L.

Conclusion:

Preterm infant vitamin D status is significantly associated with markers of calcium health, such as PTH concentration, and bone health. Furthermore, early data suggests a threshold of 25(OH)D status below which these health indicators improve incrementally with escalating 25(OH)D concentrations.

Vitamin D and Asthma Health Disparities

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Augusto Litonjua, MD, MPH

Asthma is both more common and more severe in African Americans than in Caucasians. Vitamin D levels are lower in African Americans and although reduced sun exposure may be one of the reasons for this there are other possibilities as well, related to differences in diet, the gut microbiome, genetics or biochemistry. Existing data on vitamin D and asthma health disparities is scant but, what data that does exist, suggest lower levels of vitamin D and more severe asthma in African Americans.

In VDAART, a randomized clinical trial of vitamin D supplementation in pregnancy to prevent asthma in the offspring, we had the largest African American enrollment of any major NHLBI clinical trial. Although African Americans had the lowest levels of vitamin D on entry to the trial they also had the greatest increase in levels in response to supplementation, and we found no differential racial effect on asthma outcomes. There was greater noncompliance with treatment among the African American participants.

In summary, much is still needed to be learned about the impact of low vitamin D on asthma health disparities.

Posters:

Vitamin D (VitD) and other factors' effects on bone mineral density (BMD) in lactating women

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Judy R. Shary, MS, MUSC Div of Neonatology; Myla Ebeling, MS, MUSC Dept of Pediatrics

Objectives:

- 1) Assess the effects of vitD, body mass index (BMI) and physical activity on BMD in lactating women by ethnicity.
- 2) Develop a clinical guideline to guide women in the postpartum period.

Design/Methods:

A dual-site, RCT was conducted (n=273 women) with singleton pregnancies who began supplementing vitD at 1-month postpartum (baseline). Sixty women were non-lactating at baseline and were set as a postpartum control, while the remaining 213 lactating women were divided by ethnicity (African-American=A, Hispanic=H, and Caucasian=C); all were followed until 7-months postpartum. BMD loss was measured by DXA scans at 1, 4 and 7 month postpartum visits (V1, V4, V7). Maternal 25-hydroxyvitamin D was measured by RIA; BMI was calculated, and mothers completed surveys indicating their level of physical activity at these same time intervals.

Conclusion(s):

Higher BMI and less physical activity protected BMD during lactation. This study supports previous studies indicating BMD ethnic variation during lactation, regardless of vitD or other factors. Overall, BMD loss during lactation was less significant than previously suggested. Our results suggest breastfeeding Hispanic women lose more BMD than Caucasian women, who lose more than African-American women.

Effects of Maternal Vitamin D (VitD) Supplementation on Bone Mineral Content (BMC)/Bone Mineral Density (BMD) in the First Year of Life

Cody-Aaron Gathers, Medical Student, Pediatrics, Medical University of South Carolina, Charleston, South Carolina

Judy Shary; Myla Ebeling; Nina Forestieri; Carol Wagner, MD

Objectives:

1. Examine the role of vitD supplementation in pregnant women and its effects on infant BMC and BMD
2. Determine how race/ethnicity effects infant BMC and BMD

Vitamin D (VitD) serves a critical role in bone development during infant growth. While the role of VitD in infants is well understood, the effects of maternal VitD supplementation are not well studied. This study served to examine the role of maternal VitD on infant BMC and BMD. This is the first study of this nature to have a large group of infants from different racial/ethnic backgrounds. Therefore, this study proposes to be the baseline for all future studies involving different racial/ethnic groups. The effects of infant VitD on BMC and BMD were also analyzed in this study. Pregnant women in Charleston, SC were part of a double-blind, randomized controlled trial of vitamin D supplementation (400, 2000, or 4000 IU vitD/day) beginning at 12-16 weeks' gestational age. Maternal blood was assayed for 25-hydroxyvitamin D (25OHD). BMD and BMC were measured in 257 infants at 2 weeks and at 6 months; 218 at 6 months and at 12 months; 230 at 2 weeks and at 12 months. DXA scans were obtained via Hologic Discovery A densitometer. Data were analyzed using SAS 9.4 (Cary, NC). Baseline characteristics between groups showed no significant differences between the treatment groups.

When controlling for gender, ethnicity, feeding type and season, changes in BMD and BMC showed expected growth over the 1st year of life. While results of the multivariable regression analysis showed no association with vitD intake and BMD/BMC at 2 weeks, 6-, or 12 months; from 6- to 12 months there was a statistically significant increase in BMD positively associated with maternal baseline vitD (Parameter Estimate [PE]=0.0004 g/cm² p=0.03). Black infants had a larger increase in BMC in the first year of life (PE=16.8 g p=0.02) as compared to Hispanic infants (PE=8.9 g p=0.09). There was a 17.8 g decrease in BMC from 2 weeks to 12 months in infants who were vitD deficient (<20 ng/ml) compared to infants who had vitD ≥20 ng/ml at 12 months. While the findings suggest that vitD supplementation per se during pregnancy has little-to-no effect on infant BMD/BMC in the first year of life, vitD status was important in utero and during the infant period as maternal baseline vitD status during pregnancy predicted later infant BMD at 6- and 12 months. Additionally, vitD deficient infants had less BMC in the first year than vitD sufficient infants. There also was greater BMC in Blacks as compared to Hispanics, impacting future studies. VitD supplementation of offspring would reduce the risk of fracture and rickets later in life.

This randomized controlled trial highlights the effects on infants of vitamin D supplementation in pregnant women. The benefits of maternal vitamin D status were well established in this trial and will facilitate future studies on the importance of baseline vitamin D in pregnant women.

The Relationship Between Physical Activity and Vitamin D Status in Lactating Women

Jordan Hall, BS, Pediatrics, Medical University of South Carolina, Charleston, South Carolina

Myla Ebeling; Judy Shary; Nina Foresteri; Carol Wagner, MD

Background:

Existing research shows an association between physical activity (PA) levels and vitamin D (VitD) status in the elderly, men, women, and children and adolescent populations. This association has not yet been investigated in fully lactating women.

Objective:

Investigate the relationship between 25(OH)D levels and PA in lactating women. It was hypothesized that based on the relationship between VitD and PA found in other populations, greater PA levels in lactating woman will be associated with greater serum 25(OH)D levels.

Design/Methods:

A post hoc analysis of 286 lactating women with self-reported PA data from the America on the Move survey, and measured circulating serum 25(OH)D (measured by RIA) as an indicator of VitD status at baseline (4-6 weeks postpartum), month 4, and month 7. The data were analyzed using SAS 9.4 (Cary, NC).

Results:

36% of women at visit 1 (baseline), 49% of women at visit 4 (month 4), and 53% of women at visit 7 (month 7) were meeting the national recommendation of 150 minutes of moderate intensity (3-6 METs) PA per week. Significant differences were seen in PA by race ($p=0.043$). Caucasians were more likely to meet the standard recommendation than African Americans or Hispanics. Using multiple regression models to examine associations between duration of PA and 25(OH)D concentration, controlling for race, treatment, sun exposure, BMI, feeding type, and METs, it was found that at visit 1 achieving at least 2.5 hrs/wk of PA was associated with an increase in 25(OH)D of 3.68 ng/mL ($p=.02$). At visit 4, achieving at least 1.5 hrs/wk of PA was associated with an increase in 25(OH)D of 4.86 ng/mL ($p=.03$). By visit 7, no association between PA and maternal 25(OH)D was observed. In repeated measures, mixed model analysis predicting maternal 25(OH)D during the study, achieving >2.5 hours of PA per week was not significantly associated with VitD status (p NS).

Conclusion:

While no definitive conclusions can be drawn regarding precise levels of PA influencing 25(OH)D levels in lactating women, the data suggest that increased activity during the first 4 months of lactation is associated with improved VitD status. Additional research is needed because of the inconsistency seen at visit 7.

The Effects of Vitamin D Binding Protein Genotypes on Circulating 25(OH)D and VDBP Levels During Pregnancy

Danforth Newton, PhD, Staff Scientist, Pediatrics, Medical University of South Carolina, Charleston, South Carolina

Sean K. Brady, Danforth A. Newton, Judith R. Shary, John E. Baatz, Bruce W. Hollis, Carol L. Wagner; Dept. of Pediatrics, MUSC

RATIONALE:

Vitamin D deficiency is widespread and is particularly problematic during pregnancy, because vitD plays a major role in the development of the fetus and is associated with greater birth weights and fewer adverse outcomes. There are common polymorphisms in the vitD binding protein (VDBP) gene which appear to be linked to 25(OH)D concentrations in the blood (this is the major circulating vitD metabolite). We hypothesize that these VDBP genotypes may be important factors to consider in interpretation of data in vitD supplementation studies of pregnant women, including any racial/ethnic differences in response. **METHODS:** Subjects were pregnant women enrolled in a large, randomized trial of vitD supplementation (400 or 4400 IU/day) at our institution (C.L. Wagner, PI; W.K. Kellogg Foundation, sponsor). Genotypes were determined by RFLP analyses for 3 VDBP polymorphisms (Gc1S, Gc1F, Gc2) using DNA extracted from blood of 122 trial subjects. Plasma VDBP concentrations were determined by ELISA. The genotypes were compared by their associations with mean plasma 25(OH)D and VDBP concentrations using Student's t-test (significant two-tailed p-value <0.05).

RESULTS:

There was a wide variation of VDBP alleles found in this population, with distinct racial/ethnic associations. Pregnant women homozygous for the VDBP Gc1S allele had significantly higher circulating 25(OH)D levels, while the Gc1F-1F genotype, particularly common in African-Americans, was associated with the lowest concentrations. However, plasma VDBP concentrations were not statistically correlated with genotype. **CONCLUSIONS:** VDBP polymorphisms affect circulating 25(OH)D concentrations in pregnant women (i.e., their "vitamin D status"). Certain genotypes were more common in different races, likely contributing to the known racial/ethnic disparities of vitD deficiency. These results strongly suggest that the VDBP genotype, which is relatively easy to analyze, is a valuable factor in interpreting data in vitD supplementation studies.

Preventing Health Disparities During Pregnancy Through Vitamin D (VITD) Supplementation: Preliminary Results of an RCT

Carol Wagner, MD, Professor, Pediatrics, Medical University of South Carolina, Charleston, South Carolina

Wei, W, Shary JR, Ebeling M, Garrett-Mayer E, Taylor SE, Reed SG, Baatz JE, Newton D, Powell A, Newman R, Mulligan J, and Hollis BW

Vitamin D deficiency is associated with adverse pregnancy outcomes in certain populations, particularly preeclampsia and preterm birth. The association of adverse outcomes in a diverse group of healthy women was previously linked to circulating 25(OH)D but not treatment due to a high rate of nonadherence. The objective was to determine, in a group of healthy women adherent to treatment using a gummy formulation, if higher dose vitamin D supplementation (4400 IU/day) was effective in reducing comorbidities of pregnancy compared with a control group (400 IU/day). Beginning in 2012-2015, 257 healthy women were randomized to receive 1 of 2 vitamin D daily regimens (400 vs 4400 IU VitaFusion™ gummies) beginning at 8-12 weeks' gestation until delivery. Circulating 25OHD and urinary calcium/creatinine (Ca/Cr) ratio were measured monthly. Utilizing standardized questionnaires, maternal and fetal health status were assessed monthly. The following comorbidities of pregnancy were collected for each maternal/fetal/neonatal pair: hypertension (HTN), diabetes, preeclampsia, preterm delivery, chorioamnionitis, miscarriage, intrauterine fetal death or bacterial vaginosis (BV). Data were analyzed using R (Vienna, Austria). The groups had similar baseline characteristics (Table 1). By the 2nd month, 25OHD remained statistically significantly higher in the 4400 IU group compared to the 400 IU group through delivery ($p < 0.001$). BV at months 4 and 7 was the most common pregnancy morbidity among African Americans (25.7% and 14.9%, respectively) and Hispanics (13.4% and 14.6%); whereas among Caucasians, diabetes (6%) and chorioamnionitis (6%) were the most common. No differences in serum calcium and urinary Ca/Cr ratios between groups were noted. After adjusting for effect of age, BMI, ethnicity, 25(OH)D at baseline or prior to delivery, higher dose vitamin D supplementation (4400IU) was not associated with any single pregnancy morbidity (HTN, diabetes, preeclampsia, preterm delivery, chorioamnionitis, miscarriage, intrauterine fetal death or BV); however, the higher dose was associated with lower risk of combined pregnancy comorbidities among African Americans (OR=0.26, $p=0.034$) but not Hispanics and Caucasians. Vitamin D supplementation impacted African American women and their pregnancy outcomes and may explain the higher rates of adverse events during pregnancy in this group. Such findings may inform future public health policy surrounding pregnancy interventions for these most at-risk women.

TABLE 1:

Maternal Characteristics and Vitamin D Status and Safety Parameters by Treatment Group

Maternal Characteristic	400 IU Group (N=120)	4400 IU Group (N=137)	p-value
Maternal Age, years (Mean \pm SD)	28.7 \pm 5.1	28.9 \pm 4.6	0.74
Maternal Race/Ethnicity, N (%)			
African American	34 (45.9%)	40 (54.1%)	0.57
Hispanic	42 (51.2%)	40 (48.8%)	
Caucasian	44 (43.6%)	57 (56.4%)	
Maternal Baseline 25(OH)D, ng/mL (Mean \pm SD)	26.9 \pm 10.4	26.0 \pm 9.1	0.49
Maternal 25(OH)D one month prior to delivery, ng/mL (Mean \pm SD)	34.4 \pm 13.7	51.9 \pm 18.5	<0.001