January 2013

The Impact Of Continuous Prenatal And Early Postpartum Maternal Vitamin D Supplementation On The Vitamin D Status Of Exclusively Breastfed Infants

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THE IMPACT OF CONTINUOUS PRENATAL AND EARLY POSTPARTUM MATERNAL VITAMIN D SUPPLEMENTATION ON THE VITAMIN D STATUS OF EXCLUSIVELY BREASTFED INFANTS

by

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A Dissertation
Submitted to the Graduate Faculty

of the

University of North Dakota

In partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Grand Forks, North Dakota
August
2013

This dissertation, submitted by Doria K. Thiele in partial fulfillment of the requirements for the Degree of Doctor of Philosophy from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done, and is hereby approved.

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Title	The Impact of Continuous Prenatal and Early Postpartum Maternal Vitamin D Supplementation on the Vitamin D Status of Exclusively Breastfed Infants
Department	Department of Nursing, College of Nursing and Professional Disciplines
Degree	Doctor of Philosophy

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Doria K. Thiele
July 12th, 2013

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ACKNOWLEDGMENTS

With my deepest appreciation I want to thank my chairperson, Dr. Cindy Anderson, PhD, WHNP-BC, FAAN, for her unconditional support and mentorship. Dr. Anderson had confidence in my ability to complete this project, gave freely of her time and energy, and demonstrated a wonderful model of mentoring for me to emulate in the future. This dissertation would not have been possible without her continuously cheering me on and for that my family and I are forever grateful. I also want to thank each member of my committee for contributing their expertise and helping me grow as a nurse scientist.

I would like to thank Angela Scheett, MPH, RD, LuAnn Johnson, MS, and Bonita Hoverson, RD at the Grand Forks Human Nutrition Research Center for their support with evaluation of the Food Frequency Questionnaire data. Additionally, I would like to thank Dr. Holly Brown-Borg, PhD, Dr. Lalida Rojanathammanee, PhD, PT and Ms. Sharlene Rakoczy for their laboratory support.

With much love, I would like to thank my husband, Joe Thiele, for altering his life path to accommodate my educational endeavor. Thank you too, to our son, August Thiele, who has not known a life without mommy working on a dissertation. I look forward to a much overdue shift in focus.

ABSTRACT

In the United States, at least 50% of breastfeeding mothers are vitamin D deficient, increasing risk for vitamin D deficiency in exclusively breastfed infants. A gap in knowledge exists regarding best practices in maternal vitamin D supplementation during pregnancy and lactation that will yield adequate infant vitamin D levels. The objective of this study was to identify the combined effect of maternal prenatal and postnatal vitamin D supplementation on vitamin D transfer to exclusively breastfed infants. Additionally, due to the immune modulating effects of vitamin D, maternal pro- and anti-inflammatory cytokines were measured across pregnancy and the postpartum. A double-blind, randomized controlled trial design was used. A total of 16 pregnant women were enrolled in the study at 24-28 weeks gestation. The control group (N= 6) received a prenatal vitamin containing vitamin D 400 IU daily plus a placebo void of vitamin D. The experimental group (N=7) received the same prenatal vitamin plus a capsule containing 3400 IU vitamin D, for a total of 3800 IU daily. Participants continued their assigned supplements through 4-6 weeks of lactation. Pertinent pregnancy, delivery, and postnatal health data were collected on maternal and infant participants. Serum levels of 25-hydroxyvitamin D were measured in maternal participants at enrollment and in both maternal and infant participants at delivery and after 4-6 weeks of lactation. Maternal plasma TNF-alpha, IL-6, and IL-10 were measured at enrollment, delivery and 4-6 weeks of lactation. There was a significant

effect of maternal vitamin D supplementation on maternal 25-hydroxyvitamin D at delivery (p=0.044) and at 4-6 weeks of lactation (p=0.002). A significant difference in the infant participant groups at delivery was also found (p=0.017), however this was not significant at 4-6 weeks of lactation (p=0.256). Controlling for maternal baseline using repeated measures techniques, the overall effect of maternal vitamin D supplementation on infant 25-hydroxyvitamin D approached significance (p=0.065). There was no impact of vitamin D supplementation on maternal cytokine production. This study adds novel information regarding the impact of continuous prenatal to postpartum maternal vitamin D supplementation on the vitamin D status of exclusively breastfed infants.

CHAPTER I

INTRODUCTION

Background

The rapidly increasing incidence of vitamin D deficiency is now considered by most experts to be a global epidemic (Holick, 2005; Lappe, 2011; Saadi et al., 2009). Simultaneously, vitamin D deficiency is being linked with more diseases including cardiovascular disease (Barnard & Colon-Emeric, 2010; Feneis & Arora, 2010), diabetes (Eliades & Pittas, 2009), autoimmune disorders (Arnson & Amital, 2011; Kamen et al., 2006), as well as the general functioning of the innate immune system (Akbar & Zacharek, 2011; Di Rosa et al., 2012; Lagishetty, Liu, & Hewison, 2011; Thota, Farmer, Garfield, Menon, & Al-Hendy, 2013). Certain pregnancy specific diseases are connected with vitamin D deficiency as well, including gestational diabetes (Baker, Haeri, Camargo, Stuebe, & Boggess, 2012; Clifton-Bligh, McElduff, & McElduff, 2008; Maghbooli, Hossein-Nezhad, Karimi, Shafaei, & Larijani, 2008; Senti, Thiele, & Anderson, 2012; Soheilykhah, Mojibian, Rashidi, Rahimi-Saghand, & Jafari, 2010; Zhang et al., 2008) and preeclampsia (Baker, Haeri, Camargo, Espinola, & Stuebe, 2010; Bodnar, Catov et al., 2007; Bodnar & Simhan, 2010; Haugen et al., 2009; Robinson, Alanis, Wagner, Hollis, & Johnson, 2010). Manufactured in human skin when exposed to adequate sunlight, vitamin D is responsible for calcium homeostasis, plays a role in the functioning of almost all body tissues, and has receptors on at least 200 genes

(Mulligan, Felton, Riek, & Bernal-Mizrachi, 2010). When blood levels of vitamin D are being tested, it is the circulating 25-hydroxyvitamin D (25[OH]D) form that is being analyzed and this is what determines a person's vitamin D status as sufficient, insufficient, or deficient (Hollis, 2005; Hollis, 2008; Hollis, 2012). Although everyone is susceptible to vitamin D deficiency if they lack sunlight on the skin, pregnant women are at increased risk due to the calcium and vitamin D demands of the fetus. Additionally, the exclusively breastfed infant continues to be at risk of vitamin D deficiency similar to their mothers, compounding the risk of disease in these infants (Balasubramanian, 2011; Salama & El-Sakka, 2010; Thandrayen & Pettifor, 2012).

Vitamin D deficiency is not a new or novel health problem in the United States. During the Industrial Revolution in the 18th and 19th centuries, there was a rapid rise in rickets cases. Marked by bone deformities, particularly in the legs, rickets is caused by vitamin D deficiency (Wagner, Taylor, & Hollis, 2008). As adults and children spent more time indoors, out of natural sunlight, average vitamin D levels fell, leading to this epidemic of rickets (Wagner et al., 2008). A national campaign was initiated and foods such as milk and juice were fortified with vitamin D starting in 1933 (Committee on Use of Dietary Reference Intakes in Nutrition Labeling, 2004). This vitamin D supplementation all but rid the country of rickets (Thandrayen & Pettifor, 2012). However, starting in the 1970s and then more so in the 1980s, there was another spike in rickets in the U.S. (Cosgrove & Dietrich, 1985; Harrison, 1975). Those hardest hit by the rickets resurgence were African Americans and immigrants with darkly pigmented skin (Thandrayen & Pettifor, 2012). It is proposed that the cause of the increased prevalence of vitamin D deficiency is multifactorial, but two major health campaigns played a large

role; first, a successful campaign against skin cancer leading to minimal sun exposure and consistent sunscreen use, and second, a successful campaign encouraging exclusive breastfeeding (Bodnar, Simhan et al., 2007; Wagner et al., 2008). Exclusive breastfeeding led to increased rates of vitamin D deficiency in infants because lactating mothers were vitamin D deficient and unable to pass significant amounts of vitamin D to their infants via breast milk (Hollis & Wagner, 2011; Wagner et al., 2008). Although both of these campaigns were important for human health and their success should be encouraged, negative implications regarding vitamin D status are now being discovered.

Maternal transfer of both calcium and vitamin D are critical to the health and development of the fetus and infant (Kaludjerovic & Vieth, 2010; Mulligan et al., 2010). The fetal immune system and metabolic function begin to develop *in utero* and then continue refinement during infancy and childhood (Walker et al., 2011; Weiss & Litonjua, 2011). It is accepted that maternal nutritional status can influence the most fundamental early mechanisms of the fetal immune and metabolic functions. This occurs by means of fetal programming that carries over in to childhood and adult life, negatively impacting health status (Kaludjerovic & Vieth, 2010; Thandrayen & Pettifor, 2012; Weiss & Litonjua, 2011). The fetus may be affected and if the infant is then exclusively breastfed, the lack of maternal vitamin D stores leads to poor vitamin D content in breast milk, compounding infant vitamin D deficiency (Hollis & Wagner, 2011; Mulligan et al., 2010).

Vitamin D deficiency in childbearing women leads to deficiency in their infants, exacerbated among infants who exclusively breastfeed (Hollis, 2007). Breast milk is the ideal form of infant feeding, containing a full complement of nutrients perfectly suited to

meet infants' needs (Riordan & Countryman, 1980). The exception is vitamin D, which due to inadequate levels in the mother, is generally not transferred in sufficient amounts to meet infant needs (Basile, Taylor, Wagner, Horst, & Hollis, 2006; Hollis & Wagner, 2004a; Merewood et al., 2010; Saadi et al., 2009; Wagner, Hulsey, Fanning, Ebeling, & Hollis, 2006). Prevalence of vitamin D deficiency in childbearing women and newborns ranges from 5-100% and 9.7-90%. (Bodnar et al., 2007; Collins-Fulea, Klima, & Wegienka, 2012; Dror, King, Durand, & Allen, 2011a; Holmes, Barnes, Alexander, McFaul, & Wallace, 2009; Merewood et al., 2010; Thomson, Morley, Grover, & Zacharin, 2004). Variability exists based on definition of vitamin D deficiency, race and ethnicity, climate, and level of vitamin D supplementation.

Infantile rickets is the primary outcome of vitamin D deficiency, however there is a growing body of knowledge that links maternal and early infancy hypovitaminosis D to many other diseases. Currently, research in regards to the association of vitamin D deficiency and disease covers a wide array of topics including breast cancer, colon cancer, atherosclerosis, diabetes I and II, obesity, depression, schizophrenia, preeclampsia, innate immune response, and multiple autoimmune disorders (Akbar & Zacharek, 2011; Arnson & Amital, 2011; T. Barker et al., 2013; Di Rosa et al., 2012; Eliades & Pittas, 2009; Kamen et al., 2006; J. J. McGrath, Burne, Feron, Mackay-Sim, & Eyles, 2010; Munger, Levin, Hollis, Howard, & Ascherio, 2006). Because of the role vitamin D may play in the prevention of so many diseases, it is critical that infants receive adequate amounts. The extent to which vitamin D transfers through breast milk is still unclear, although emerging research indicates vitamin D sufficient women can pass adequate doses of vitamin D to their infants without the need for direct infant

supplementation (Basile et al., 2006; Hollis & Wagner, 2004b; Saadi et al., 2009; Wagner et al., 2006). Recommendations designed to improve adequate maternal vitamin D status will promote vitamin D transfer to the developing fetus *in utero* and to the infant during lactation, thus potentially improving the health of women and their infants. Adult onset vitamin D deficiency related disorders and diseases could be substantially reduced through optimal infant vitamin D status during the vulnerable periods of fetal and infant development (Brannon, 2012; Christesen, Elvander, Lamont, & Jorgensen, 2012; Pludowski et al., 2013).

Problem Statement

There is currently a lack of evidence to identify best practice in maternal dosing of vitamin D supplementation during pregnancy and lactation. Epidemiologic data supports the notion that a majority of pregnant women, their fetuses, and newborns are vitamin D deficient, yet health care practitioners are unable to offer evidence-based advice regarding supplementation. There is a significant need to address the gap in knowledge regarding the specific needs for vitamin D supplementation in pregnant and lactating women that would promote vitamin D transfer to meet the needs of the exclusively breastfed infant.

Purpose Statement

The objective of this study was to identify the combined effect of maternal prenatal and postnatal vitamin D supplementation on vitamin D transfer to infants during exclusive breastfeeding. A randomized controlled trial was designed to generate evidence that will add to what is known regarding the influence of maternal vitamin D supplementation in late pregnancy and lactation on vitamin D transfer via breast milk.

By identifying the outcome of the optimal dosage and timing of maternal vitamin D supplementation on infant vitamin D status, we have the potential to greatly improve maternal health and establish lifelong benefits for babies starting before birth.

Therefore, this study was conducted to test the central hypothesis that maternal supplementation with vitamin D during pregnancy and lactation will significantly increase circulating vitamin D levels during lactation in mothers and their exclusively breastfed infants. As vitamin D is a key regulator of immune response, a secondary hypothesis that increased vitamin D levels in the mother would result in decreased pro-inflammatory cytokines and an increase in an anti-inflammatory cytokine was tested. The rationale for this study is that establishment of conditions that support maternal delivery of adequate vitamin D to infants, along with other essential nutrients through breast milk, will reduce the need for infant vitamin D supplementation while optimizing infant health. A growing body of evidence suggests that sufficient vitamin D may be transferred to the infant through breast milk if the maternal vitamin D status is adequate (Basile et al., 2006; Hollis & Wagner, 2004b; Saadi et al., 2009; Wagner et al., 2006), however, the optimal timing and dosage of vitamin D supplementation during gestation and lactation to achieve maternal vitamin D adequacy and breast milk transfer is unknown. There is, therefore, a critical need to determine the dosage and timing of maternal vitamin D supplementation necessary to achieve maternal vitamin D adequacy and transfer via breast milk for vitamin D adequacy in infants.

Research Hypotheses

To achieve the objectives of this proposal, the following hypotheses were tested:

Central Hypothesis: Maternal supplementation with vitamin D during pregnancy and lactation will significantly increase circulating vitamin D levels during lactation in mothers and their exclusively breastfed infants;

1. Women who receive supplemental vitamin D starting in the early third trimester of pregnancy will have significantly higher 25[OH]D serum levels compared to control participants by delivery;
2. Women who receive supplemental vitamin D starting in the early third trimester of pregnancy and continue with this dosing during the early postpartum will maintain significantly higher serum 25[OH]D levels than control participants during lactation;
3. Infants born to women who receive supplemental vitamin D starting in the early third trimester of pregnancy will have significantly higher 25[OH]D levels at birth compared to control infants;
4. Infants who exclusively breastfeed from a mother receiving supplemental vitamin D will have significantly higher 25[OH]D levels than infants exclusively breastfeeding in the control group at 4-6 weeks of age.

Due to immune and inflammatory modulating effects of vitamin D, the following secondary hypotheses were tested:

1. Women with higher vitamin D levels will have decreased levels of pro-inflammatory cytokines (TNF-α and IL-6);
2. Women with higher vitamin D levels will have increased levels of an anti-inflammatory cytokine (IL-10).

The findings from this study are expected to add to current evidence regarding the recommended dosage and duration of vitamin D supplementation during pregnancy and lactation necessary to achieve adequate infant vitamin D status. This study is innovative in its approach to initiate maternal vitamin D supplementation during pregnancy and continue in to early lactation, which has not been previously studied.

Theoretical Framework

This study was conceptualized using the Developmental Origins of Health and Disease hypothesis (DOHaD), also known as the Barker Hypothesis (Cota & Allen, 2010). Dr. David Barker is currently a professor of clinical epidemiology at the University of Southampton, UK, and professor in the Department of Cardiovascular Medicine at Oregon Health and Science University. Dr. Barker was one of the first epidemiologists to widely disseminate research underscoring the importance of the maternal nutritional environment on infant health and development of later adult disease (D. J. Barker, 1994; D. J. Barker, 1997). The focus of his work has been on the development of disease in the adult whose mother experienced famine or malnutrition during pregnancy (D. J. Barker, 1995). In his early investigation of maternal under-nutrition in pregnancy and long-term health risks in offspring, he explored the connection between low birth weight and development of heart disease (D. J. Barker & Fall, 1993). In his 1997 work, Dr. Barker hypothesized that many adult diseases are set in motion or even programmed during fetal life (D. J. Barker, 1997).

The DOHaD hypothesis is founded on statements of causal relationships, namely that maternal nutriture has life-long effects on the infant. As a predictive theory, DOHaD researchers seek to test these causal relationships until agreement is reached that a true

relationship exists and predictions of disease risk can be made (McEwen & Wills, 2010). However, DOHaD researchers including Dr. Barker warn against oversimplification of findings and acknowledge that environmental influences beyond *in utero* exposures affect disease development (D. J. Barker, 1997). Gleaned from reading Dr. Barker's work along with other DOHaD researchers, the following theoretical statements influenced this project:

- A woman's nutritional status prior to and during pregnancy affects the lifelong health of her offspring;
- The intrauterine environment, including such variables as vitamin transfer, hormonal milieu, and physiologic mechanisms, has health impacts on the fetus that can last a lifetime;
- The fetus responds to under-nutrition, or vitamin deficiency, with permanent physiologic and metabolic adaptations;
- Poverty, poor living conditions, social disparities based on race and ethnicity, and continued nutritional deficits experienced during childhood increase the risk of adult onset disease later in life.

The underlying processes of DOHaD are two-fold: developmental plasticity and fetal programming. Developmental plasticity refers to the ability of the developing embryo or fetus to change phenotype (appearance) without change in genotype (genetic sequence) (Cota & Allen, 2010; Kaludjerovic & Vieth, 2010). There may be critical periods during embryogenesis and fetal development where particular organs, tissues, or mechanisms are more susceptible to outside influences and demonstrate physiologic

change as a means of adaptation (Cota & Allen, 2010). An example of physiologic adaptation occurring *in utero* is the increased growth of the placenta seen with under-nutrition in the fetus (D. J. Barker, 1997). When exposed to a lack of calories, the fetus limits cell division in the body and increases cell division in the placenta, theoretically to increase surface area from which to draw nutrition. This ability to change the course of development without changing the genome is an example of developmental plasticity. Fetal programming refers to the sometimes permanent nature of these phenotypic changes (Cota & Allen, 2010). When exposed to under-nutrition followed by an enlarged placenta the fetus develops hypertension, which appears to persist through adult life (D. J. Barker, 1997). The body is programmed based on the available nutrition, hormones, and physiologic mechanisms found *in utero* (Kaludjerovic & Vieth, 2010). Barker (1997) states that permanent changes caused by under-nutrition during fetal development include, "change in the distribution of cell types, hormonal feedback, metabolic activity, and organ structure" (p. 807). Several researchers exploring fetal development have stated that vitamin D deficiency may be the key feature of disease programming (Kaludjerovic & Vieth, 2010; J. McGrath, 2001; Thandrayen & Pettifor, 2012; Weiss & Litonjua, 2011).

A plausible explanation for the developmental plasticity and fetal programming described by the DOHaD hypothesis is the theory of epigenetics. Epigenetics is the study of inherited changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence (Baccarelli & Bollati, 2009; Gravina & Vijg, 2010). The changes that do occur are in DNA strand structure through processes of histone modification and DNA methylation (Baccarelli & Bollati, 2009; Gravina & Vijg,

2010). Epigenetic expression can be altered by environmental toxins, heavy metals, and also nutrients and hormones found naturally occurring in the body (Baccarelli & Bollati, 2009). The period of fetal development is considered a critical time for epigenetic expression because of the rapid cell differentiation and proliferation as well as development of metabolic pathways. Epigenetic changes in gene expression in the fetus and infant may be set in motion by low vitamin D levels *in utero* and in breast milk, leading to future disease (Kaludjerovic & Vieth, 2010; J. McGrath, 2001; Thandrayen & Pettifor, 2012; Weiss & Litonjua, 2011). It is clear from current research that the placenta is altered by maternal vitamin D levels, increasing the risk for preeclampsia in the mother and therefore hypertension in her offspring (Baker et al., 2010; Haugen et al., 2009; Robinson et al., 2010). Concurrently, there is increasing evidence that hypovitaminosis D *in utero* and in early infancy can increase risk for asthma and respiratory infections (Camargo et al., 2011), juvenile arthritis (Ellis, Munro, & Ponsonby, 2010), allergic rhinitis (Erkkola et al., 2009; Erkkola, Nwaru, & Viljakainen, 2011), type 1 diabetes (Cooper et al., 2011; Marjamaki et al., 2010), eczema (Miyake, Sasaki, Tanaka, & Hirota, 2010), and poor innate immune response (Erkkola et al., 2011; Walker et al., 2011). Continued research in this field is necessary in order to determine threshold vitamin D requirements for the pregnant woman, the lactating woman, and her infant. By exposing the unborn fetus to adequate vitamin D through maternal supplementation, there is the potential to reduce future adult diseases.

Although the broad concepts of the DOHaD and epigenetics cover large areas of scientific research, particularly as it relates to maternal nutrition, this study took a narrower focus of maternal/newborn vitamin D supplementation. Researchers studying

the effects of vitamin D deficiency in pregnancy, the fetus, and the infant are finding that the current recommendation of 600 IU/d vitamin D for women is inadequate to reach or maintain adequate serum vitamin D levels (Hollis, 2009; Hollis & Wagner, 2011; Hollis, Johnson, Hulsey, Ebeling, & Wagner, 2011). This knowledge combined with the emerging data that vitamin D deficiency is linked to multiple disorders and diseases requires researchers to determine adequate supplementation doses. This study adds to this knowledge base regarding dosage and timing of vitamin D supplementation for pregnant and lactating women and the influence on vitamin D status in exclusively breastfed newborns.

Significance of the Study

There are a growing number of randomized controlled trials investigating appropriate dosing of vitamin D during pregnancy and lactation to achieve and maintain maternal and infant 25[OH]D levels in the sufficient range (Basile et al., 2006; Hollis & Wagner, 2004b; Hollis et al., 2011; Merewood et al., 2010; Saadi et al., 2009; Thiele, Senti, & Anderson, 2013; Wagner et al., 2006). Evidence is mounting that vitamin D deficiency plays a significant role in fetal programming of later disease including cancers, cardiovascular disease, susceptibility to infection, and metabolic disorders such as diabetes. Providing pregnant and lactating women with appropriate vitamin D will benefit both mother and infant. This study adds information regarding vitamin D dosing during pregnancy and lactation that results in adequate maternal 25[OH]D levels and adequate transfer of vitamin D to exclusively breastfed infants.

Assumptions

- Women will be willing to join the study as participants.
- Acquired sample size will be adequate.
- Participants will take their assigned supplements on a daily basis.
- Participants will fill out their questionnaires and tools accurately.
- Adequate numbers of participants will continue exclusively breastfeeding through 4-6 weeks postpartum.
- Supplementation with higher dose vitamin D will significantly increase 25[OH]D levels in maternal participants.
- Supplementation with higher dose vitamin D will significantly increase 25[OH]D levels in infant participants born to and breastfeeding from maternal participants receiving the supplementation.

Summary of Key Points

- Vitamin D is important in multiple body functions and overall health.
- A majority of pregnant women and newborns are vitamin D deficient.
- Vitamin D supplementation may be an easy intervention for improved health.
- A gap in the literature exists in regards to appropriate dosing of vitamin D supplementation for pregnant and lactating women.
- This study adds new information regarding vitamin D supplementation initiated in pregnancy and continued through early postpartum and its impact on newborn 25[OH]D levels in the exclusively breastfed infant.

- This study investigated the impact of supplemental vitamin D on *in vivo* cytokine production in pregnant women.

Operational Definitions

In order to lend clarity to the results of this study, the following definitions are used throughout:

- IU: International Unit
- Supplemental Vitamin D: vitamin D3, cholecalciferol
- 25-hydroxyvitamin D (25[OH]D, calcidiol): serum value used for evaluation of vitamin D status
- 1-25-dihydroxyvitamin D (1,25$[OH]_2$D, calcitriol): serum value of biologically active vitamin D
- Data Collection Time Points
    - Enrollment: baseline data collected on the maternal participant including FFQ data, and blood for analysis of 25[OH]D and cytokines
    - Delivery: blood collected within 24 hours of birth on maternal participant for evaluation of 25[OH]D and cytokines and on the infant for evaluation of 25[OH]D
    - Lactation: follow-up data collected 4-6 weeks after birth on the maternal participant including FFQ and blood for 25[OH]D and cytokines, and blood collected on the infant for evaluation of 25[OH]D

- A priori vitamin D status definitions
    - Sufficiency: 25[OH]D serum level > 32 ng/mL
    - Insufficiency: 25[OH]D serum level 20-32 ng/mL
    - Deficiency: 25[OH]D serum level < 20 ng/mL

CHAPTER II

LITERATURE REVIEW

Introduction

Discrepancies regarding the 25[OH]D serum status that provides maximal health benefits as well as appropriate supplementation doses for all age groups remain and are in fact currently highlighted due to the recent Institute of Medicine (IOM) vitamin D supplementation recommendations (Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, 2011). It is the purpose of this study to add to the body of scientific and nursing knowledge regarding vitamin D transfer from mother to infant during exclusive breastfeeding when the mother is supplemented with vitamin D. There is no more critical time than during pregnancy, lactation and fetal/neonatal development to insure appropriate nutritional status.

A review of the extant literature informs the background and methods for this study. Topics to be reviewed include: history of vitamin D deficiency, vitamin D status definitions and supplementation recommendations, current estimates of vitamin D deficiency prevalence, the functional role of vitamin D in the human body, impact of vitamin D on health outcomes, maternal health effects of vitamin D deficiency, infant health effects of maternal vitamin D deficiency during pregnancy and lactation, and evidence of vitamin D transfer through breast milk.

History of Vitamin D Deficiency

With the recent emphasis on vitamin D in the scientific literature, health care providers are left to wonder how one vitamin has become so important to researchers and how it could possibly have so many significant effects in the body. In fact, vitamin D is a steroid hormone precursor formed in the skin when the skin is exposed to ultraviolet B (UVB) wavelengths (Holick, 2011). The compound now recognized as vitamin D was historically noted to exist naturally in cod liver oil and to be responsible for preventing and curing rickets (Wagner et al., 2008). Because the nature of the compound was unknown, it was thought to be a vitamin and was thusly named. Cod liver oil is one of the few naturally occurring dietary sources of vitamin D. Other natural sources include fatty fish, dried mushrooms, and egg (Holick, 2011). These dietary sources contain very small amounts of vitamin D when compared to the physiologic amounts produced in the skin when exposed to UVB (Haddad, Matsuoka, Hollis, Hu, & Wortsman, 1993; Matsuoka, Wortsman, Haddad, & Hollis, 1989). It is believed that early humans evolved in order to respond to the amount of sunlight present in their environment (Yuen & Jablonski, 2010). For instance, humans living in much of Africa would be exposed to tremendous amounts of UVB when their skin was exposed to sunlight, conditions which increased heavier pigmentation in the skin for protection. As humans moved away from Africa and into areas further from the equator, it would be beneficial to lose skin pigment in order to convert adequate vitamin D in the skin in lower light conditions (Yuen & Jablonski, 2010). Therefore, if exposed to the same sunlight intensity and time, a white person would create more vitamin D in their skin compared to a darker skinned individual. This is exemplified in the literature by the consistently higher prevalence of

vitamin D deficiency amongst African Americans compared to individuals from other ethnic groups who typically have lighter pigmentation (Ginde, Liu, & Camargo, 2009).

Because humans evolved to spend much of their time outdoors, the transition from agrarian societies to industrialized societies played a significant role in the prevalence of vitamin D deficiency. First identified in the 1600s, rickets became a prominent childhood disease during the industrial revolution of the 18th and 19th centuries (Holick, 2006; Rajakumar, 2003). Because of the shadowing effect of buildings in the cities, long days spent working inside factories, and pollution blocking UVB radiation, children were not able to maintain adequate vitamin D levels to prevent rickets (Holick, 2006). By 1921 it was estimated that approximately 75% of the children in New York City had rickets, already identified as a nutritional disorder (Centers for Disease Control and Prevention (CDC), 1999). As a matter of national health, scientists worked to identify the nutritional deficit causing rickets and found that both sunlight and cod liver oil could cure rickets (McCollum, Simmonds, Becket, & Shipley, 1922). Once clearly established as the compound in cod liver oil that was affecting bone health, vitamin D fortification was enacted and foods including milk and juice were fortified in order to eradicate rickets on a national level. This campaign was very successful and rickets was quickly viewed as a disease of the past (Rajakumar, 2003).

Around the time that rickets was being effectively prevented on a national scale, fears of vitamin D toxicity began to surface (Wagner et al., 2008). These fears were based on cases in which young children were given hundreds of thousands or millions of international units (IUs) of vitamin D and in a few cases, these children died (Wagner et al., 2008). Several decades later, cases of children with hypercalcemia and elfin facies

were thought to be victims of vitamin D toxicity and this was additionally linked to risk of developing supravalvular aortic stenosis (SAS) (Wagner et al., 2008). In fact, in the 1960s it was believed that maternal supplementation with vitamin D during pregnancy was the most likely cause of SAS and the concomitant hypercalcemia and elfin facies (Wagner et al., 2008). This certainly put physicians and patients on alert to avoid excessive vitamin D during pregnancy and vitamin D gained a reputation for causing irreversible harm. It was not until the early 1990s that it was known that an underlying genetic disorder, Williams' syndrome, was causing the SAS and faulty vitamin D metabolism, which led to the hypercalcemia and toxicity (Wagner et al., 2008). In the 1980s and 1990s, there was a concurrent emphasis on sunscreen use, which led to further vitamin D deficiency as sunscreen blocks UVB rays from the skin, therefore limiting or completely inhibiting cutaneous vitamin D production (Thandrayen & Pettifor, 2012). All of these factors combined to move us toward the 21st century with an increasing prevalence of vitamin D deficiency.

Vitamin D Status Definitions and Supplementation Recommendations

Because vitamin D deficiency has such high prevalence, it was important for scientists to create efficient means for testing human blood levels of vitamin D. When testing an individual's functional vitamin D level the blood analysis used is 25-hydroxyvitamin D (25[OH]D). Also known as calcidiol, 25[OH]D is the major circulatory form of vitamin D found in the blood. There is inconsistency in the units of measure used to describe 25[OH]D levels and in the units of measure used for vitamin D dosing (Table 1).

Table 1. Commonly used units and conversions for 25[OH]D levels and vitamin D supplementation doses.

Unit of 25[OH]D Measure	Abbreviation	Conversion factor	Example
Nanograms per milliliter	ng/mL	1 ng/mL = 2.496 nmol/L	32 ng/mL = 80 nmol/L
Nanomoles per liter	nmol/L		
Dose measures			
International Units	IU	1 IU = 0.025 µg	40 IU = 1 µg
Microgram	µg		

Opinions as to what level of serum 25[OH]D is sufficient vary widely as well (Table 2).

Table 2. 25[OH]D level considered adequate by recommending agency.

Organization	Institute of Medicine[1]	Endocrine Society[2]	American Academy of Pediatrics[3]	American College of Obstetricians and Gynecologists[4]
Sufficient 25[OH]D Level	16-20 ng/mL All ages	30 ng/mL All ages	20 ng/mL Infants and children	20 ng/mL and 32 ng/mL noted as widely accepted in non-pregnant individuals

1 – Institute of Medicine (US) Committee to review dietary reference intakes for vitamin D and calcium (2011). The National Academies Press.

2 – Holick, et al. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism, 96(7),* 1911-1930.

3 – Wagner & Greer (2008). Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics, 122(5),* 1142-1152.

4 - ACOG Committee on Obstetric Practice. (2011). ACOG committee opinion no. 495: Vitamin D: Screening and supplementation during pregnancy. *Obstetrics and Gynecology, 118*(1), 197-198. doi:10.1097/AOG.0b013e318227f06b.

In addition there is inconsistency regarding agency recommendations for daily vitamin D intake (Table 3). It remains unclear if the same 25[OH]D level should be used

to define adequacy across the lifespan, in different geographic locations across the globe, between genders, ages and ethnicities.

Table 3. Recommendations for daily vitamin D intake by recommending agency.

Organization	Daily Intake: Infants to one year	Daily Intake: Pregnancy/ Lactation	Daily Intake: Adult (age 1-70 years)
Institute of Medicine[1]	400 IU	600 IU	600 IU
Endocrine Society[2]	At least 400 IU, may need 1000 IU	At least 600 IU, may need 1500-2000 IU	At least 600 IU, may need 1500-2000 IU
American Academy of Pediatrics[3]	400 IU	N/A	N/A
American College of Obstetricians and Gynecologists[4]	N/A	1000-2000 IU when deficiency during pregnancy is noted	N/A

1 – Institute of Medicine (US) Committee to review dietary reference intakes for vitamin D and calcium (2011). The National Academies Press.

2 – Holick, et al. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism, 96(7),* 1911-1930.

3 – Wagner & Greer (2008). Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics, 122(5),* 1142-1152.

4 - ACOG Committee on Obstetric Practice. (2011). ACOG committee opinion no. 495: Vitamin D: Screening and supplementation during pregnancy. *Obstetrics and Gynecology, 118*(1), 197-198. doi:10.1097/AOG.0b013e318227f06b

Researchers use several physiologic markers to explicate their support of specific levels set for vitamin D sufficiency. Vitamin D plays an important role in mineral homeostasis in the body, affecting parathyroid hormone (PTH) production in order to stimulate release of calcium from the bone when serum calcium levels fall (Holick, 2006). Researchers argue that the serum 25[OH]D level that corresponds to maximal suppression of PTH is a good benchmark for 25[OH]D sufficiency (Gloth, Tobin, Sherman, & Hollis, 1991; Gloth, Gundberg, Hollis, Haddad, & Tobin, 1995; Lips et al.,

1988; Vieth, Ladak, & Walfish, 2003). Several studies have investigated the 25[OH]D level that corresponds to development of secondary hyperparathyroidism and conclude that levels dropping below 15 to 20 ng/mL stimulate this response (Gloth et al., 1991; Gloth et al., 1995; Lips et al., 1988). Vieth, Ladak, and Walfish (2003) demonstrated that in older adults, PTH is maximally suppressed with 25[OH]D levels above 32 ng/mL and therefore, this level has become widely used as a cutoff for sufficiency.

Another measure that can be used to determine appropriate levels for vitamin D sufficiency is intestinal calcium absorption. Heaney, Dowell, Hale, and Bendich (2003) found that there is a continuum of calcium absorption across the 25[OH]D range and that individuals with levels ≤ 20 ng/mL absorbed less calcium through the intestine compared to individuals with levels ≥ 32 ng/mL. A third marker that can be used to determine 25[OH]D levels that correspond to sufficiency is bone mineral density (BMD). In 2004, Bischoff-Ferrari, Dietrich, Orav, and Dawson-Hughes found that in the adult population 25[OH]D levels have a positive correlation to BMD and that optimal BMD is found with 25[OH]D levels above 32 ng/mL. Vitamin D researcher, Dr. Bruce Hollis, reviewed the research regarding PTH, calcium absorption, and BMD as biomarkers of 25[OH]D sufficiency and concluded "nutritional vitamin D deficiency should be defined as <80 nmol (32 μg/L) circulating 25[OH]D…" (Hollis, 2005, p. 320). Another vitamin D researcher, Dr. Michael Holick (2007) stated that clinicians should strive for 25[OH]D levels of >30 ng/mL in children and adults in order to optimize physiologic processes. After reviewing data on BMD, lower-extremity function, dental health, and risk of falls, fractures, and colorectal cancer, Bischoff-Ferrari, Giovannucci, Willett, Dietrich, and Dawson-Hughes (2006) found that, "for all endpoints, the most advantageous serum

concentrations of 25[OH]D begin at 75 nmol/L (30 ng/mL), and the best are between 90 and 100 nmol/L (36-40 ng/mL)" (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006, p. 18). Audran and Briot (2010) noted that serum levels of vitamin D greater than 75 nmol/L (30 ng/mL) are needed for bone health, but for protection from other diseases even higher levels might be necessary. Cannell, Hollis, Zasloff, and Heaney (2008) set their recommended serum level of 25[OH]D at > 40 ng/mL (100 nmol/mL) as this corresponds to levels seen in people exposed to moderate amounts of UVB year round.

Similarly, recommendations for adequate intake or optimal supplementation of vitamin D for infants, children and adults also vary (Table 1). The IOM provides recommendations in terms of Adequate Intake (AI), which is used when a Recommended Dietary Allowance (RDA) cannot be determined and is an approximation of the amount assumed to be adequate for the population. The RDA is the dietary intake needed to meet the needs of 97.5% of a particular group (Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, 2011). The current RDA from the Food and Nutrition Board (FNB) of the IOM is 600 IU per day for ages 1 through 70 years (Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, 2011). The IOM report suggests a Tolerable Upper Intake Level (TUIL) of 2,500 IU daily for ages 1-3; 3,000 IU daily for ages 4-9, and 4,000 IU daily for ages 9-70+. The revised recommendations do not include any difference in RDA or TUIL during pregnancy or lactation separate from all other adult populations (Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, 2011). Interestingly, the FNB set an AI of 400 IU per day

for infants up to 12 months, but they do not give an RDA. The TUIL for 0 to 6 months is 1,000 IU daily and for 6 to 12 months it is 1,500 IU daily. The recent report states that vitamin D supplementation recommendations were set based on the desire to achieve 25[OH]D levels of 16 ng/mL and that this is "consistent with the intended nature of an average requirement, in that it reflects the desired level for a population median – it meets the needs of approximately half the population" (Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, 2011, p. 8). The American College of Obstetricians and Gynecologists (ACOG Committee on Obstetric Practice, 2011) does not recommend routine screening for vitamin D deficiency during pregnancy or lactation, but states that if found, it should be treated with 1000-2000 IU vitamin D per day.

Although the recommendations for both intake and 25[OH]D levels have been set higher than the previous IOM recommendations from 1997, they have still sparked controversy. There is evidence that current RDAs, especially those outlined by the IOM, are inadequate and will not provide optimal protective effects against disease (Grant, 2011b; Heaney & Holick, 2011; Hollis & Wagner, 2011). Weaver and Fleet (2004) cite multiple reasons for the variation in recommendations including poor control over fortification of foods, concluding that research regarding calcium absorption indicates a need for vitamin D intake > 2000 IU/d. Matsuoka, Wortsman, Haddad, and Hollis (1999) and Haddad, Matsuoka, Hollis, Hu, and Wortsman (1993) found that a white person exposing their total body to sunlight in summer for 10 to 15 minutes will produce 10,000 to 20,000 IU of vitamin D cutaneously, indicating that these doses are physiologic and would not cause harm. Cannell et al. (2008) found that obese, elderly, and dark-skinned

individuals need to ingest 5000 IU per day to maintain an adequate serum level in the absence of adequate sunlight. In 2005, Hollis stated that further research was needed but that vitamin D intake exceeding 2000 IU/d was probably necessary to bring blood levels up to sufficiency for the average adult. Bischoff-Ferrari et al. (2008) conclude that intake of 1000 IU/d vitamin D is needed to bring at least 50% of the population up to sufficiency levels.

Safety and Efficacy of Supplementation

Non-Pregnant Adult Populations

Earlier work synthesizing the evidence regarding safe upper limits of vitamin D intake was completed by Vieth (1999) who concluded that adults receiving 10,000 IU daily may reach 25[OH]D levels of 140 nmol/L but that there were no corresponding markers of toxicity or adverse effects. Vieth, Chan, and MacFarlane (2001) conducted a trial of healthy adult men and women (N=61) randomized to receive either 1000 IU or 4000 IU daily for 2 to 5 months starting in the winter. They concluded that 4000 IU/d for adults did not cause toxicity even with extended dosing and that this dosing would be necessary to raise 25[OH]D levels to adequacy. The authors reported a dose-response increment of 0.56 nmol/L per microgram or 0.014 nmol/L per IU in the group receiving vitamin D 4000 IU daily (Vieth, Chan, & MacFarlane, 2001). A similar study by Heaney, Davies, Chen, Holick, and Barger-Lux (2003) randomized healthy men (N=67) to receive 0, 1000, 5000, or 10,000 IU per day of vitamin D. They found a similar dose-response relationship to Vieth et al. (2001) of 0.70 nmol/L per microgram, or 0.0175 nmol/L per IU (0.007 ng/mL per IU), across all dosage groups (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). In a response paper to the new IOM recommendations,

Heaney and Holick (2011) point to this evidence to support recommendations for increases in 25[OH]D resulting from vitamin D supplementation, stating that for every increase of 100 IU of vitamin D taken per day there will be a subsequent increase of approximately 1 ng/mL in the 25[OH]D level (Heaney & Holick, 2011).

Pregnant/Lactating Populations

In a recent article reviewing safety considerations for designing randomized controlled trials of vitamin D supplementation in pregnancy, Roth (2011) encourages researchers to use vitamin D doses that will be sufficient in raising maternal 25[OH]D levels to the normal range. Roth (2011) states that doses sufficient to bring the 25[OH]D level of the intervention group up to >80 nmol/L are necessary to achieve valid results, and that doses <10,000 IU daily can be considered as these are considered safe in non-pregnant adults (Roth, 2011).

Intervention studies of vitamin D supplementation have been conducted using almost exclusively adult non-pregnant participants. There is a lack of contemporary research in regards to efficacy and safety of vitamin D supplementation during pregnancy. In 2000, Mahomed and Gulmezoglu completed a meta-analysis for the Cochrane Review regarding vitamin D supplementation in pregnancy and were only able to include 4 studies (Brooke et al., 1980; Brooke, Butters, & Wood, 1981; Mallet et al., 1986; Maxwell, Ang, Brooke, & Brown, 1981) and excluded an additional 3 published studies (Ala-Houhala, Koskinen, Terho, Koivula, & Visakorpi, 1986; Delvin, Salle, Glorieux, Adeleine, & David, 1986; Marya, Rathee, Lata, & Mudgil, 1981) found from 1980 to 1986 (Mahomed & Gulmezoglu, 2000). After completing this review Mohamed and Gulmezoglu (2000) concluded that insufficient evidence was available to recommend

routine prenatal vitamin D supplementation. This review has since been withdrawn citing outdated content. In 2012, De-Regil, Palacios, Ansary, Kulier, and Pena-Rosas completed a new review of vitamin D supplementation for women during pregnancy. They were able to include 6 trials after excluding those that did not meet the criteria of being a randomized or quasi-randomized trial. The authors concluded that vitamin D supplementation during pregnancy raised 25[OH]D levels in the pregnant woman, but correlations between increased 25[OH]D and improved health outcomes were weak (De-Regil, Palacios, Ansary, Kulier, & Pena-Rosas, 2012). Outcomes of interest included rates of preeclampsia, gestational diabetes, cesarean section, and neonatal admission to the intensive care unit, among others. In conclusion they called for high quality, randomized controlled trials that evaluate the role of vitamin D supplementation in pregnancy. Unfortunately, after completion of the De-Regil et al. (2012) review, two high quality randomized controlled trials (reviewed later) evaluating effects of different doses of maternal vitamin D supplementation were published, and therefore were not included in the review (Hollis et al., 2011; Wagner, McNeil et al., 2013a).

Literature related to maternal vitamin D status during pregnancy dates back to the 1980s. Ala-houhala et al. (1986) demonstrated in a sample of 49 pregnant women that those who received 2000 IU daily of vitamin D during pregnancy had significantly higher vitamin D levels at 8 weeks than women who had taken 1000 IU vitamin D in pregnancy. Marya et al. (1981) randomized pregnant women to receive no vitamin D (N= 75), 1200 IU daily (N= 25), or 2 doses of 600,000 IU one month apart at the end of the third trimester (N= 20). Women receiving 1200 IU daily had lower serum alkaline phosphatase levels as did infant cord blood in this group, but calcium and phosphate were

similar to the no vitamin D group. The women in the group receiving 600,000 IU twice at the end of pregnancy had higher calcium and phosphate and lower alkaline phosphatase levels compared to controls and the 1200 IU daily group with no adverse outcomes. Delvin et al. (1986) conducted a study with 40 women randomized to receive either 1000 IU vitamin D daily (N= 20) in the third trimester or no vitamin D supplementation (N= 20). Both the women and the infants in the 1000 IU group had significantly higher 25[OH]D compared to the controls. The infants born to women who had been supplemented showed a decrease in serum calcium at 4 days after birth, but it was significantly less than infants born to none supplemented mothers. Of the studies that were included in the earlier meta-analysis (Brooke et al., 1980; Brooke et al., 1981; Mallet et al., 1986; Maxwell et al., 1981), Brooke, Butters, and Wood (1981) randomized 126 pregnant Asian women to receive either 1000 IU vitamin D daily (N=59) in the third trimester or no supplementation (N=67). They reported no differences between the two groups in terms of infant weight or measurements at birth, however the infants from the treated group were significantly heavier at each data point thereafter (3, 6, 9, and 12 months). Using the same data set, Brooke et al. (1980) reported that women receiving 1000 IU vitamin D daily gained more weight during pregnancy than the non-supplemented group participants. Five of the infants in the control group developed symptoms of hypocalcemia whereas none did in the 1000 IU group. Additionally, there were significantly more infants born small for gestational age and with large fontanelles in the control group suggesting that bone growth and ossification were impaired. Mallet et al. (1986) randomly assigned women to receive 1000 IU vitamin D daily in the third trimester (N=21), a one-time dose of 200,000 IU in the seventh month (N=27), or no

vitamin D supplement (N=29). There were no significant differences between the two treatment groups in terms of maternal or infant serum 25[OH]D or calcium, but both treatment groups had significantly higher serum 25[OH]D and serum calcium levels than controls. They reported no difference in birth weight between any of the groups. Maxwell, Ang, Brooke, and Brown (1981) randomized Asian pregnant women to receive 1000 IU vitamin D daily (N= 59) or no supplement (N= 67). Women in the supplemented group gained weight faster than those in the control group and infants born to women who were supplemented were significantly heavier at birth than infants in the control group.

In 2002, Datta et al. completed a prospective study of pregnant women of ethnic minority origins in South Wales. Women were from African, Afro-Caribbean, Asian, Far-Eastern, and Middle-Eastern ethnic groups. A total of 160 women had their 25[OH]D levels checked at their first antenatal visit and those with levels < 8 ng/mL were enrolled in the study (N= 80). All participants were started on 800 IU/d of calciferol (vitamin D_3). There was no control group used. The women were then retested for 25[OH]D levels at 36 weeks followed by an increase in vitamin D supplementation to 1600 IU/day if levels were still below 8 ng/mL. The vitamin D supplemented women had a final blood analysis to determine status at delivery. By delivery, the mean had almost doubled to 11.24 (± 6.34 SD) ng/mL. By current conservative IOM standards, the women would still be considered deficient. The authors did not find abnormal PTH, alkaline phosphatase, calcium, or phosphate levels in the intervention group (Datta et al., 2002). This study was limited by poor methodology including lack of a control group and extremely low definition of vitamin D deficiency.

Yu, Sykes, Sethit, Teoh, and Robinson (2009) completed an intervention study in the United Kingdom using a diverse group of pregnant women (N=180). Women were enrolled at 27 weeks gestation and randomized to receive 200,000 IU vitamin D as a one-time oral dose (N=60), 800 IU daily (N=60), or no treatment through delivery (N=60). Yu et al. reported a significant difference between the two intervention groups and the no treatment group in terms of 25[OH]D levels at delivery, fetal cord-blood 25[OH]D levels, and maternal secondary hyperparathyroidism. There was not a significant difference between the two intervention groups. Only 30% of women and 8% of newborns achieved adequate 25[OH]D (> 50 nmol/L) levels by delivery in the intervention groups (Yu, Sykes, Sethi, Teoh, & Robinson, 2009).

In 2011, Hollis et al. reported results from a randomized clinical trial of vitamin D supplementation in pregnancy. Participants were randomized to receive 400 (N= 166), 2000 (N= 167) or 4000 (N=169) IU vitamin D3 daily from 12-16 weeks through delivery. Vitamin D sufficiency was set at a 25[OH]D level of 80 nmol/L. The authors reported a significant increase in mean 25[OH]D levels achieved between the group that received vitamin D 2000 IU/day versus 400 IU/day and in the group given vitamin D 4000 IU/day versus those who received 400 IU/day, but not between the 2000 IU and 4000 IU groups although the 4000 IU group did have the highest mean 25[OH]D level. By the end of the study, 82% of the women in the 4000 IU vitamin D group had reached vitamin D adequacy, demonstrating the need for long term supplementation before levels rise sufficiently. This was a large study with over 300 women participating from early pregnancy through delivery. The authors reported no adverse outcomes or abnormal biomarkers. Further, 25[OH]D levels of 30 ng/mL were necessary to normalize calcium

excretion in the urine and reduce PTH levels and levels of 40 ng/mL were necessary to support maximum $1,25(OH)_2D$ production, which is the hormonally active form of vitamin D responsible for increasing calcium absorption from the gut. In summary, there are very few studies examining vitamin D supplementation during pregnancy, but in those that exist, doses of 4000 IU daily over extended time periods demonstrate efficacy and safety, whereas doses less than 2000 IU daily are minimally effective.

Using an almost identical protocol to that of Hollis et al. (2011), Wagner et al. (2013) reported the findings of a randomized trial of 257 pregnant women. Women were enrolled into the study at 12-16 weeks gestation and all were provided with 2000 IU vitamin D daily for one month. They were then randomized to receive 2000 IU vitamin D daily (N=130) or 4000 IU vitamin D daily (N=127). This study design did not include a control group as it was deemed by the researchers and review board to be unethical to treat a group with 400 IU only as most of them would remain vitamin D deficient through the trial. The researchers defined vitamin D deficiency as < 20 ng/mL, insufficiency as ≥ 20-32 ng/mL and sufficiency as > 32 ng/mL, but only include women achieving a 25[OH]D of > 40 ng/mL when discussing rates of sufficiency in particular groups. The overall mean 25[OH]D level at enrollment was 22.7 (± 9.7) ng/mL. Both intervention groups saw significant increases in mean 25[OH]D level by delivery, but there was no statistically significant difference between the two intervention groups in terms of mean 25[OH]D. There were no incidences of vitamin D toxicity as evidenced by urine and serum calcium levels staying in the normal range. The neonatal cord blood mean 25[OH]D level was significantly higher in the infants born to women in the 4000 IU daily group than the 2000 IU daily group. Additionally, the researchers found a decline in

pregnancy complications with increasing serum 25[OH]D level, but this was not statistically significant between groups.

A review of the current literature reveals 4 contemporary articles that explore the efficacy and safety of vitamin D supplementation during lactation for both mother and infant (Basile et al., 2006; Hollis & Wagner, 2004b; Saadi et al., 2009; Wagner et al., 2006). When vitamin D passes through breast milk it is referred to as the antirachitic effect of the milk because the main clinical outcome of vitamin D is rickets prevention. In each of the 4 studies, women were recruited and randomized at one month postpartum. Basile et al. (2006) randomized 64 lactating women to receive either 2000 or 4000 IU daily. Of this original sample, 25 continued exclusive breastfeeding through the study period of 3 months. Basile et al. reported that serum calcium remained normal in both mothers and infants in all groups and mothers did not have increased urine calcium. Wagner et al. (2006) enrolled 19 women to receive either 400 (N= 10) or 6400 (N= 9) IU vitamin D daily during lactation. There was no difference in maternal or infant serum calcium or phosphorus levels and no difference in urinary calcium to creatinine ratios between the two groups. Hollis and Wagner (2004) randomized 64 lactating women to receive either 2000 or 4000 IU daily of vitamin D. Of those, 18 women, 9 in each group, completed the study. Hollis and Wagner reported no adverse outcomes and 4000 IU had great efficacy in increasing maternal and infant 25[OH]D levels over the study period. In 2009, Saadi et al. assigned 90 women to receive either 2000 IU daily vitamin D or 60,000 IU monthly oral dose for 3 months. Only one participant was lost to follow-up due to breastfeeding cessation. Saadi et al. reported no adverse events related to vitamin D exposure and the daily and monthly regimens were equally effective in raising maternal

and infant 25[OH]D levels. To summarize the safety and efficacy data from these studies, there were no reports of any adverse outcomes or effects and biomarkers of toxicity such as serum calcium remained in the normal range for mothers and infants. In terms of efficacy, doses above 4000 IU daily were most effective at bringing a majority of women into the sufficient 25[OH]D range along with exclusively breastfed infants (Thiele et al., 2013).

Current Estimates of Vitamin D Deficiency Prevalence

Adults

The National Health and Nutrition Examination Surveys (NHANES) have provided good data in regards to vitamin D and health status. In a recent National Center for Health Statistics (NCHS) Data Brief, researchers Looker et al. (2011) explored vitamin D status in populations living in the United States. Using the designations of sufficiency (>50 nmol/L), inadequacy (30-49 nmol/L), and deficiency (< 30 nmol/L) set by the IOM, they reported overall population rates of 67%, 24%, and 8%, respectively. Females, across the lifespan, are at greater risk of vitamin D deficiency with 12% being deficient versus 8% of males. However, women who were pregnant or lactating were at lower risk of deficiency compared to other adult women, which differs from findings of most studies. Those categorized as non-Hispanic black and Mexican American persons were more likely than whites to be in the deficient category, with 73% of black Americans in the combined inadequate and deficient group. Certainly the rates of insufficiency and deficiency would be higher if the 25[OH]D levels used for these status definitions were reflective of those used by most researchers, namely >80 nmol/L for sufficiency.

Pregnant and lactating women and their infants

The World Health Organization (FAO/WHO, 2004) described risks for vitamin D deficiency in their report, "Vitamin and Mineral Requirements in Human Nutrition". Infants are described as an at-risk group due to their rapid growth and reliance on maternal stores of vitamin D during fetal and newborn development. In a French study, Zeghoud et al. (1997) found that 64% of infants had 25[OH]D levels below 30 nmol/L at birth. Studies based in several different states in the U.S. document the extent of vitamin D deficiency among newborns. Dror, King, Durand, and Allen (2011) from Oakland, California found 90% of infants and 54% of mothers had 25[OH]D levels <75 nmol/L. Basile et al. (2007) in South Carolina report 65.5% of African American infants and 24% of white infants had cord blood 25[OH]D levels <11 ng/mL. In Massachusetts in 2010, Merewood et al. found that 58% of infants and 35.8% of mothers had 25[OH]D levels < 20 ng/mL and 38% of infants and 23.1 % of mothers had 25[OH]D levels < 15 ng/mL. In the relatively sun rich area of Sacramento, California, Liang, Chantry, Styne, and Stephensen (2010) found that 28.3% of infants had 25[OH]D levels < 75 nmol/L with exclusive breastfeeding being a significant risk factor for vitamin D deficiency. Collins-Fulea, Klima, and Wegienka (2012) found that amongst 2839 pregnant women in Detroit, 92.5% had 25[OH]D levels <30 ng/mL and 71.7% had levels <20 ng/mL. This was one of few studies that included a relatively large percentage of non-white participants. The authors found that significant risk factors for vitamin D deficiency included being Middle Eastern, African American, or Asian and wearing the hijab as part of their cultural dress. In British Columbia, Li et al. (2011) found that 65% of pregnant women between 20 and 35 weeks gestation had 25[OH]D levels < 30 ng/mL and 24% had 25[OH]D levels < 20

ng/mL. Participants in the study regularly took supplements containing ≥ 400 IU daily (80%), however, deficiency was still prevalent. In contrast, Bendall, de Costa, Woods, and Howat (2012) from Australia found that out of 116 women, only 6.9% had 25[OH]D levels <30 ng/mL, thus they questioned the need for routine screening or supplementation in Australia. In New Orleans, Gangat, Ponnapakkam, Bradford, Katikaneni, and Gensure (2012) found that African American infants (N=26) had significantly lower mean cord blood 25[OH]D levels than Caucasian infants (43 ± 2.8 nmol/L vs 69.2 ± 4.2 nmol/L, P < .001). Infants across the globe are at risk for deficiency as Agarwal, Faridi, Aggarwal, and Singh (2010) reported. 70% of mothers and 55.67% of infants at 10 weeks postpartum had 25[OH]D levels < 11ng/mL and that at 6 months 16.49% of infants developed rickets as defined by the authors as serum alkaline phosphatase > 420 IU/L. Infants with darker skin pigmentation, who were kept out of sunlight, born in the winter and exclusively breastfed by a vitamin D deficient mother were at greatest risk for vitamin D deficiency (Greer, 2008; Thandrayen & Pettifor, 2012).

Researchers in New England recently found that of infants at 4 months of age (N= 177), 11.9% had 25[OH]D levels < 20 ng/mL (Merewood et al., 2012). They did not report findings using any biologically based definition of vitamin D sufficiency, namely > 32 ng/mL. The strongest predictor of vitamin D deficiency in the infants was lack of appropriate supplementation. This underscores the necessity of all infants to receive appropriate external vitamin D, whether from infant formulas, sufficient breast milk vitamin D content, or a vitamin D supplement. Of infants who were exclusively breastfed without vitamin D supplementation, 40% had 25[OH]D levels < 20 ng/mL, compared to 6% of formula fed infants. Researchers in Turkey also examined a group of

4-month-old exclusively breastfed infants (Halicioglu et al., 2012). Mothers had been instructed to give 400 IU of oral vitamin D to their infants and were supplied with the supplement. Despite "regular use" of the supplement (> 6 times per week), 28% of infants had 25[OH]D levels < 20 ng/mL and in infants with "irregular use" of the supplement (<6 but >3 times per week) 38.5% had vitamin D deficiency.

Ethnicity certainly plays a significant role in vitamin D deficiency as well. In a recent study, cord blood was collected at birth from black (N=75) and white (N= 38) male infants (Eichholzer et al., 2013). The mean 25[OH]D level in black infants was significantly lower than the mean 25[OH]D level in white infants (11.44 vs. 18.24 ng/mL). Of the black infants 84% had 25[OH]D levels < 20 ng/mL compared to 63% of white infants, which demonstrates the high rate of vitamin D deficiency in both groups.

Children

In 2009, Mansbach, Ginde, and Camargo reviewed the 25[OH]D levels obtained on children aged 1 to 11 years through the National Health and Nutrition Examination Survey (NHANES). A total of 4558 children had a 25[OH]D level checked between 2001 and 2006. Of those children, 69% had serum 25[OH]D levels less than 75 nmol/L (30 ng/mL). Analysis by ethnicity demonstrated that 92% of non-Hispanic black and 80% of Hispanic children fell into this category. Because this is a nationally representative sample, the authors conclude that millions of children may have insufficient vitamin D levels. They reiterate that further research is necessary for improved understanding of how this affects health outcomes and what supplementation doses might be used to correct this widespread problem.

Madden et al. (2012) collected blood samples on 511 critically ill children admitted to the Pediatric Intensive Care Unit. Of these participants, 40.1% had 25[OH]D levels < 20 ng/mL and the mean 25[OH]D level was only 22.5 ng/mL. Due to the role vitamin D seems to play in the immune response, the authors recommended screening critically ill children for vitamin D deficiency.

The Functional Role of Vitamin D in the Human Body

Although the compound known as vitamin D has been studied for almost a century, the mechanisms by which it has an impact on human health have only recently become clearer. Because of the observations that serum 25[OH]D seems to impact many disease processes, biological researchers have set out to determine specific actions that vitamin D has on human genes, cells, tissues, and systems. It is now understood that, like all endocrine functions, there is an exquisite balancing of vitamin D production and feedback regulation in the body. When our skin is exposed to UVB radiation from the sun in the 290-315 nm wavelength range, the provitamin D3, a cholesterol precursor 7-dehydrocholesterol, is converted to previtamin D3, which is then further transformed into cholecalciferol or vitamin D3, see Figure 1 (Lappe, 2011). The cholecalciferol compound is able to bind to vitamin D binding protein (DBP) and is then carried into the blood stream (Holick, 2006; Mulligan et al., 2010). Once present in the blood stream, cholecalciferol is either stored in fat or converted in the liver by a 25-hydroxylation process completed by a cytochrome P-450 enzyme (25-hydroxylase) forming 25[OH]D. Whether from a dietary source such as fatty fish, from a supplement, or from the skin as vitamin D3 (cholecalciferol), the precursor compounds will be metabolized in to the circulating 25[OH]D form. The conversion of previtamin D to 25[OH]D is directly

proportional to vitamin D synthesis or intake, therefore 25[OH]D is used as the blood marker of overall vitamin D status.

When acting as an endocrine prohormone, circulating 25[OH]D is further metabolized by 1-α-hydroxylase, typically present in the kidney. The vitamin D is then in its active hormonal form 1,25– dihydroxyvitamin D ($1,25[OH]_2D$, calcitriol), which has high affinity with vitamin D receptors (VDR) throughout the body (Lappe, 2011). One of its primary functions is to affect calcium absorption in the intestine relative to

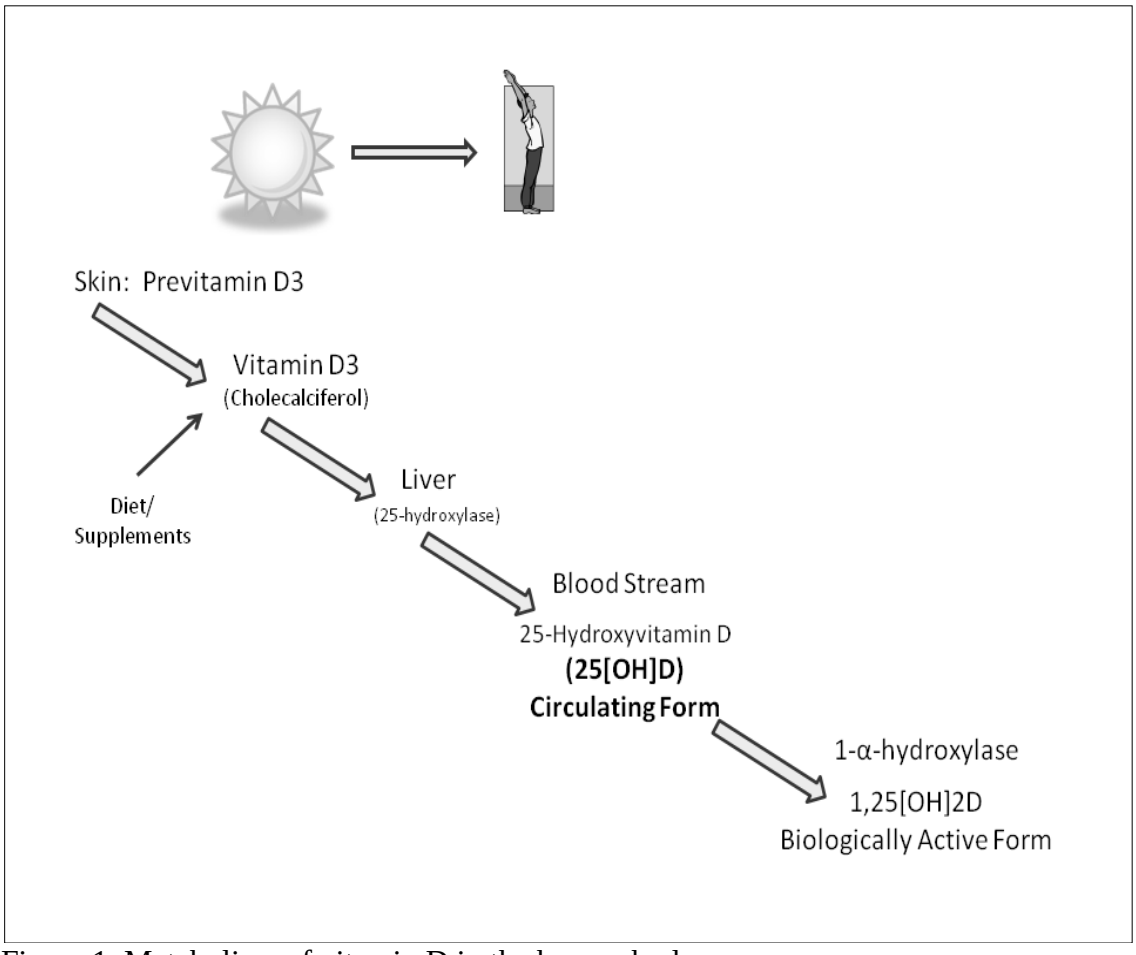


Figure 1. Metabolism of vitamin D in the human body.

calcium intake. The unbound, free 1,25$[OH]_2$D is able to cross the cell membrane in the intestine, bind to the VDR and increase expression of the epithelial calcium channel, which leads to increased calcium absorption (Holick, 2011).

It is now understood that cells outside the kidney are able to convert 25[OH]D to 1,25$[OH]_2$D, which then has an autocrine or paracrine effect (Lappe, 2011). The 1-α-hydroxylase needed for this conversion has been found in cells of the skin, placenta, colon, prostate, brain, lungs, monocytes and macrophages (Holick, 2011). In these cells 1,25$[OH]_2$D induces transcription of proteins or other molecules the cell has been signaled to produce. This allows for cell and tissue specific conversion of 25[OH]D to its biologically active form (Lappe, 2011). The action of the 1,25$[OH]_2$D can also be slowed or stopped inside the cell by the action of vitamin D 24-hydroxylase, providing a homeostatic balance inside the cell itself (Lappe, 2011).

The historically understood role of vitamin D in the body is calcium and phosphorus homeostasis (Holick, 2006). The biologically active 1,25$[OH]_2$D encourages calcium and phosphorus absorption in the intestine as well as inducing osteoclasts to promote bone resorption when dietary calcium is lacking (Holick, 2006). Calcium homeostasis is imperative to the functioning of muscle, including the heart, bone formation, growth and strength, and many metabolic functions (Lappe, 2011). Because vitamin D is primarily formed in the skin and only minimally acquired through diet, anything that affects skin exposure to UVB wavelengths will affect 25[OH]D production. These factors can include skin pigmentation, geographic latitude, use of sunscreen, aging, cloud cover or pollution, winter season, skin covering (clothing), and time spent indoors. If someone has minimal UVB exposure for any of these reasons and their 25[OH]D level

falls, calcium absorption is then diminished and the subsequent calcium deficiency leads to increased production of parathyroid hormone (PTH) (Figure 2). PTH causes increased reabsorption of calcium in kidney tubules in an effort to maintain tight control of serum calcium levels. The PTH also signals the kidney to increase production of $1,25[OH]_2D$, which results in increased calcium and phosphorus absorption in the gut as well as

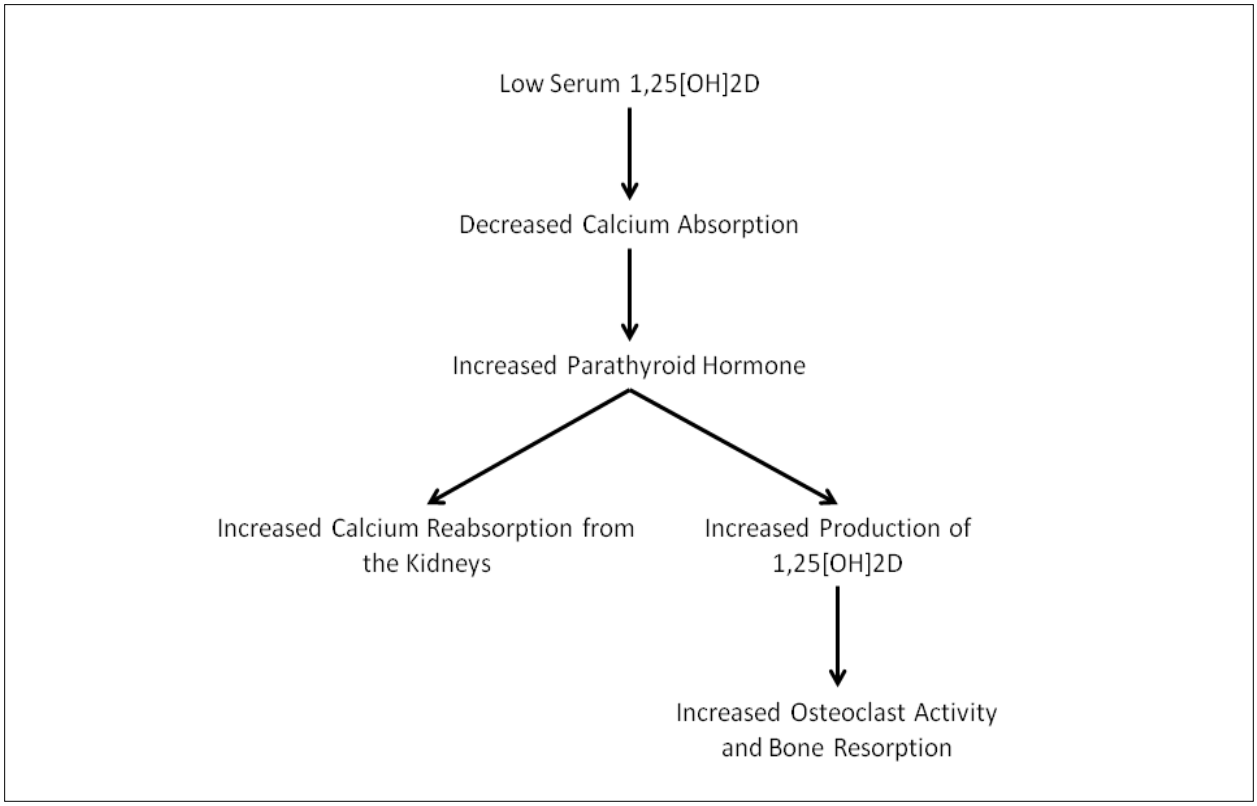


Figure 2. Vitamin D deficiency and the compensatory parathyroid hormone mechanism.

increased bone turnover and loss in order to maintain serum calcium. Although this is a functional homeostatic reaction of the endocrine system, it is meant to be short term, perhaps needed only briefly in the winter, and not a long term consequence of vitamin D deficiency. This process can lead to rickets, osteomalacia, and osteoporosis as the body needs to use more and more bone to maintain serum calcium levels.

Impact of Vitamin D on Health Outcomes

There has been rapid dissemination of vitamin D research as it relates to disease prevention and treatment (Grant, 2011a). Although not all studies find a correlation between 25[OH]D levels or vitamin D intake and disease, there is growing evidence that vitamin D deficiency may be an important modifiable risk in global health. The areas of inquiry reviewed here are bone health, cardiovascular disease, diabetes, cancer, and immune function.

Bone

The most widely accepted consequence of vitamin D deficiency is rachitic deformities in children (Thandrayen & Pettifor, 2012), and osteoporosis in adults (Epstein, 2006). Rickets is the consequence of extremely low vitamin D levels experienced in infancy (Thandrayen & Pettifor, 2012), whereas osteoporosis is a long-latency disease found most often in the elderly and signals both vitamin D and calcium deficiencies (Epstein, 2006). Biochemical markers of rickets disease can be noted before the visible physical changes occur. These markers include high alkaline phosphatase, low 25[OH]D levels with paradoxically normal 1,25-OHD levels, secondary hyperparathyroidism, low serum phosphorus, and hypocalcemia (Ponnapakkam, Bradford, & Gensure, 2010). Other physical symptoms that may be noted are splaying of the growth plates in the wrists and knees noted on X-ray (Ponnapakkam et al., 2010). On the other end of the age spectrum, vitamin D deficiency results in osteoporosis with 50% of those > 50 years having either diagnosed osteoporosis or low bone mineral density (BMD) (Epstein, 2006). Osteoporosis can result in bone fractures and significant morbidity and mortality (Epstein, 2006). When vitamin D levels are insufficient to signal

for increased calcium absorption from the intestine over long periods of time there is a compensatory mechanism that pulls calcium from the bone to maintain serum calcium homeostasis (Figure 2). This leads to decreased BMD and osteoporosis, a significant cost to health and well-being (Epstein, 2006). In summary, there is significant evidence that vitamin D deficiency can have subtle effects on bone development and maintenance and if severe enough can lead to rickets.

Cardiovascular Disease

Cardiovascular disease can have many components, but one common underlying dysfunction is hypertension. Vaidya and Forman (2010) completed an analysis of the existing data regarding vitamin D effects on blood pressure. Most studies are observational and have varying results regarding the magnitude of vitamin D's effect (Vaidya & Forman, 2010). There is some convincing evidence that vitamin D has an effect on the renin-angiotensin system leading to decreased hypertension rates, with some studies completed with mice (Y. C. Li, 2003) and others with humans (Vaidya & Williams, 2012; Vaidya, Sun, Larson, Forman, & Williams, 2012). In a meta-analysis, Feneis and Arora (2010) found that 8 of the 10 reviewed observational studies describe an inverse relationship between vitamin D and blood pressure. Grant (2011a) compiled existing literature regarding vitamin D levels and multiple disease outcomes. Part of this analysis included describing a hazard ratio for serum 25[OH]D and mortality from cardiovascular disease and concludes that the hazard ratio drops by 18% with an increase in serum 25[OH]D from 54 to 110 nmol/L. Certainly there is sufficient evidence of a relationship to warrant support for RCTs of vitamin D and cardiovascular risks.

Diabetes

The mechanisms by which vitamin D may have an effect on type 2 diabetes include impact on beta cell function, insulin action, and general inflammation (Eliades & Pittas, 2009). Much like studies regarding vitamin D and cardiovascular outcomes, studies examining vitamin D and diabetes are mostly observational. Although they offer evidence of the effect and the mechanism of action, conclusions regarding widespread supplementation cannot be made from these studies (Eliades & Pittas, 2009). Intervention studies on humans are limited as they have been part of larger studies not investigating the relationship between vitamin D and diabetes (Orwoll, Riddle, & Prince, 1994). Alemzadeh, Kichler, Babar, and Calhoun (2007) found a positive correlation between 25[OH]D and insulin sensitivity and a negative correlation between 25[OH]D and hemoglobin A1C in obese children, indicating that earlier intervention of vitamin D supplementation may help prevent development of impaired glucose metabolism.

Cancer

Early prospective studies noted that people with higher circulating serum vitamin D had a significantly decreased risk of several cancers (C. F. Garland et al., 1989; F. C. Garland, Garland, Gorham, & Young, 1990). This was more recently explicated in a larger review (C. F. Garland et al., 2009). Newhouser et al. (2008) examined the relationship between vitamin D insufficiency and breast cancer survivors. When controlling for mediating variables, the stage of disease independently predicted serum vitamin D levels with more advanced disease being associated with lower levels of vitamin D. Overall, 75.6% of participants (N= 790) had low serum vitamin D levels. A meta-analysis of the relationship among vitamin D, calcium, and breast cancer prevention

identified a significant relationship between serum vitamin D and breast cancer (Chen et al., 2010). Women in the highest quartile of circulating 25[OH]D had a 45% decreased risk of breast cancer and those in the highest quartile of circulating calcium had a 19% decreased risk of breast cancer. Studies investigating colorectal cancer found that higher circulating vitamin D status was associated with decreased risk of colon cancer (Touvier et al., 2011) and resulted in decreased mortality rates from colorectal cancer after that diagnosis had been made (Ng et al., 2009).

Immune Function

Vitamin D plays a role in both the innate immune response (Lagishetty et al., 2011) and in modulation of autoimmune diseases (Waterhouse, Perez, & Albert, 2009). Waterhouse et al. (2009) report that some of the diseases showing a favorable response to vitamin D supplementation are systemic lupus erythematosis, rheumatoid arthritis, scleroderma, sarcoidosis, psoriasis, and autoimmune thyroid disease. Kamen et al. (2006) reported significantly lower mean 25[OH]D levels in Caucasian patients with lupus versus healthy controls. In an in-depth description of the mechanism by which vitamin D affects the innate immune response, Lagishetty et al. (2011) state that T-cells, B-cells and macrophages express the vitamin D receptor (VDR) and are able to synthesize the biologically active $1,25[OH]_2D$ form. Lagishetty et al. also report that antibacterial action of monocytes is linked with their ability to metabolize vitamin D, which then induces the innate immune response. Because of the correlation between vitamin D deficiency and poor immune response, immunologists have been researching the direct impact that vitamin D has on inflammation and cytokine production. Vitamin D's impact seems to be directly aimed at monocytes and macrophages and their

production of cytokines (Di Rosa et al., 2012; Tiosano et al., 2013). Several studies have found that vitamin D will inhibit production of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), which are both pro-inflammatory (Di Rosa et al., 2012; Thota et al., 2013). It has also been found that vitamin D can increase production of the anti-inflammatory cytokine interleukin 10 (IL-10) (Thota et al., 2013; Tiosano et al., 2013). Looking specifically at vitamin D deficient adults, researchers have observed increased TNF-α, IL-6 and decreased levels of IL-10 (T. Barker et al., 2013). A recent randomized controlled trial investigated the impact of 4000 IU vitamin D daily for 5 days on the production of inflammatory markers following myocardial infarction (Arnson et al., 2013). The researchers found that vascular cell adhesion molecules, C-reactive protein, and IL-6 were produced at a significantly lower rate amongst the participants receiving the vitamin D compared to those who did not. They conclude that even modest doses of vitamin D can have immediate benefit on the inflammatory response (Arnson et al., 2013).

Maternal Health Effects of Vitamin D Deficiency

Preeclampsia

Contemporary research demonstrates widespread vitamin D deficiency amongst pregnant women (Basile, Taylor, Wagner, Quinones, & Hollis, 2007; Collins-Fulea et al., 2012; Hollis & Wagner, 2006; Hollis, 2009; Lee et al., 2007; Merewood et al., 2010; Mulligan et al., 2010; Robinson et al., 2010). Merewood et al. (2010) explored the physiologic underpinnings of vitamin D in the human body and how it affects many body tissues and functions. They describe preeclampsia and hypertensive disorders as the most well documented effects of vitamin D deficiency in pregnancy. Robinson et al. (2010)

completed a case-control investigation of women diagnosed with early-onset severe preeclampsia (EOSPE) (N= 50). They found a significant relationship between vitamin D serum levels and EOSPE and concluded that a 10 ng/mL increase in serum 25[OH]D yielded a 63% decrease in the odds of developing EOSPE.

Haugen et al. (2008) correlated data regarding vitamin D intake from the Norwegian Mother and Child Cohort Study with subsequent risk of preeclampsia development (N= 23,423). Participants reported general health and diet factors at 15, 22 and 30 weeks gestation. Haugen et al. report an odds ratio of 0.76 for preeclampsia for women ingesting 600-800 IU daily compared with those ingesting less than 200 IU daily. When vitamin D supplement intake was separated from dietary intake the researchers found a 27% reduction in risk for preeclampsia in women taking 400-600 IU daily compared to no supplementation.

In 2010 Baker et al. reported the association of midgestation 25[OH]D level and later development of severe preeclampsia. Participants who developed severe preeclampsia (N= 51) had lower 25[OH]D levels at midgestation than those who remained healthy (N= 204). Further, a midgestation 25[OH]D level of < 50 nmol/L was associated with an almost 4 fold increase in development of severe preeclampsia. Similarly, Wei, et al. (2012) found that midgestation (24-26 week) 25[OH]D levels < 50 nmol/L was accompanied by a 3.24 fold risk of preeclampsia development.

Gestational Diabetes

There is a growing body of evidence that vitamin D affects glucose homeostasis and that deficiency in vitamin D may increase risk for gestational diabetes (Senti et al., 2012). In another case-controlled investigation of 204 women, Soheilykhah et al. (2010)

found that women diagnosed with gestational diabetes mellitus (GDM) were 2.66 times more likely to have a deficient vitamin D level compared to pregnant women without GDM. Additionally, women with one abnormal glucose test were significantly more likely to have vitamin D deficiency than women with no abnormal glucose tolerance.

Maghbooli et al. (2008) investigated the correlation between gestational diabetes and vitamin D in 741 pregnant women in Tehran, Iran. They found an overall 25[OH]D deficiency rate of 70.6% with a cut-off of < 25 nmol/L. A positive correlation between 25[OH]D levels and insulin sensitivity was found as well as a negative correlation between 25[OH]D and GDM. In a nested case-control study of 57 women diagnosed with GDM and 114 healthy controls, Zhang et al. (2008) found a 2.66 fold increased risk of developing GDM when vitamin D deficient, defined as < 20 ng/mL. Clifton-Bligh et al. (2008) used midgestation blood samples from women (N= 264) at high risk of GDM and report an inverse relationship between 25[OH]D level and PTH, fasting glucose, fasting insulin, and insulin resistance. However, not all studies find significant relationships. Farrant et al. (2009) found that 66% of participants (N= 559) had 25[OH]D levels less than 50 nmol/L and 31% less than 28 nmol/L, but there was no association between 25[OH]D levels and GDM. Women with higher 25[OH]D levels did have significantly lower ($p = 0.03$) 30 minute glucose concentrations during their glucose tolerance test.

Parlea (2012) compared women with GDM (N= 116) to those without (N= 219) and found that women with GDM had significantly lower 25[OH]D levels. Women who had 25[OH]D levels that fell below the top quartile (< 73.5 nmol/L) had a 2.21 fold increased risk for developing GDM. Similarly, Burris et al. (2012) found a linear

relationship between 25[OH]D level and glucose tolerance at 26-28 weeks gestation. Overall, women with 25[OH]D level < 25 nmol/L had a 2.2 fold increased risk of developing GDM. However, not all studies support a relationship between 25[OH]D level and GDM. Baker, Haeri, Camargo, Stuebe, and Boggess (2012) compared a population of 60 women with GDM with 120 controls. There was not a significant relationship between 25[OH]D level and GDM. Their population had relatively high 25[OH]D levels with 73% of them having levels > 75 nmol/L.

Immune Modulation

During normal pregnancy, there seems to be only moderate changes to the expression of immune response as evidenced by changes in cytokine production. There are differing results in the literature examining normal cytokine production in pregnancy. Palm, Axelsson, Wernroth, Larsson, and Basu (2013) found that, among 37 women experiencing normal pregnancy and delivery, IL-6 does steadily increase over the course of a pregnancy, whereas there seems to be no significant change in TNF-α (Palm, Axelsson, Wernroth, Larsson, & Basu, 2013). This is different than the findings of Denney et al. (2011) who found that amongst 45 pregnancies, TNF-α, IL-6, and IL-10 response steadily declined throughout pregnancy, with the change in IL-6 and IL-10 being significant (Denney et al., 2011). It seems that IL-10 plays a critical role in allowing pregnancy to continue without the maternal inflammatory response causing a rejection, or miscarriage, to occur (Denney et al., 2011; Palm et al., 2013). Simultaneously, increased levels of TNF-α and IL-6 are associated with early miscarriage, especially in the presence of infections (Denney et al., 2011). There seems to be a significant role for these cytokines in process of preeclampsia as well (Sharma,

Satyam, & Sharma, 2007). It has been demonstrated that there is a significant increase in the pro-inflammatory cytokines TNF-α and IL-6 accompanied by a decrease in the anti-inflammatory cytokine IL-10 when a woman is experiencing preeclampsia (Sharma et al., 2007). Few studies exist investigating the impact of vitamin D supplementation on human cytokine production during pregnancy (Barrera et al., 2012; Thota et al., 2013). Barrera et al. (2012) noted that both IL-10 and calcitriol ($1,25[OH]_2D$) independently inhibit production of pro-inflammatory cytokines in the placenta (Barrera et al., 2012). They exposed human trophoblast cells, obtained from normal and preeclamptic pregnancies, to calcitriol and TNF-α, expecting to see increased IL-10 production in both instances. However, they found that calcitriol actually suppressed IL-10 production and TNF-α stimulated increased IL-10 production, in the normal and preeclamptic cells. They hypothesize that calcitriol suppresses all cytokine production and the anti-inflammatory impacts of calcitriol negate the need for increased IL-10 production. Thota, Farmer, Garfield, Menon, and Al-Hendy (2013) used a similar method with human myometrial cells stimulated with bacterial endotoxin to simulate infection (Thota et al., 2013). They found that then exposing the cells to vitamin D resulted in decreased TNF-α production and increased IL-10 production, supporting their hypothesis that vitamin D would have anti-inflammatory impacts in a uterine infection model (Thota et al., 2013).

Infant Health Effects of Maternal Vitamin D Deficiency During Pregnancy and Lactation

Health consequences for the fetus and infant from exposure to maternal vitamin D deficiency can be found in the immediate neonatal period or later in childhood. Problems from the neonatal period reviewed here include hypocalcemic seizure and rickets, and

later onset diseases include asthma, upper respiratory infection (URI), atopic dermatitis, and type 1 diabetes.

Rickets

Although easily prevented with low doses of vitamin D and thought to be vanished from the United States, rickets reemerged as a public health concern in the 1980s (Thandrayen & Pettifor, 2012). Most cases of rickets seen in the 1980s involved dark-skinned infants who were exclusively breastfed and from families who had immigrated to the U.S. (Thandrayen & Pettifor, 2012; Wagner et al., 2008). Rickets has now been identified as a significant problem globally because of the rapid industrialization of developing countries leading to indoor work environments and increased pollution (Thandrayen & Pettifor, 2012). The calcium-vitamin D relationship between mother and fetus or newborn plays a role in the development of rickets. If a pregnant woman is calcium or vitamin D deficient this process will be altered and the fetus may have disordered bone development. Likewise, 25[OH]D crosses the placenta readily but 1,25$[OH]_2$D does not cross the placenta easily, leading to 25[OH]D deficiency in the fetus or infant born to a 25[OH]D deficient mother (Thandrayen & Pettifor, 2012). This generational vitamin D deficiency can continue if the vitamin D deficient mother exclusively breastfeeds her infant who is undergoing rapid bone growth. The end result can be nutritional rickets, seen rarely as a congenital disorder and more often during the early lactation stage of infancy (Thandrayen & Pettifor, 2012).

Hypocalcemic Seizures

Another consequence of severe vitamin D deficiency in infants is hypocalcemic seizures (Balasubramanian, Shivbalan, & Kumar, 2006; Balasubramanian & Ganesh,

2008; Balasubramanian, 2011; Camadoo, Tibbott, & Isaza, 2007; Teaema & Al Ansari, 2010). Again, this is most often seen in infants with dark skin who are exclusively breastfed (Salama & El-Sakka, 2010). In 2010, Salama and El-Sakka reported maternal 25[OH]D status in lactating women whose infants developed rickets with hypocalcemic seizures. Of the 32 infants who were diagnosed with rickets, 9 developed hypocalcemic seizures. Salama and El-Sakka found that 69% of the mothers and 72% of the infants had 25[OH]D levels < 20 ng/mL. They conclude that maternal supplementation with vitamin D might prevent some of the hypocalcemic seizures in these infants (Salama & El-Sakka, 2010).

Wheeze, Asthma, and Respiratory Infection

Interesting research is emerging in regards to long latency disease and vitamin D deficiency during fetal and neonatal development. Camargo et al. (2011) correlated cord blood 25[OH]D levels obtained at birth (N= 823) with later development of wheeze or asthma up to age 5 and with respiratory infection before the age of 3 months. Results included an inverse relationship between fetal 25[OH]D levels and risk of respiratory infection by age 3 months, risk of wheezing by 15 months, 3, and 5 years of age. There was not a correlation between fetal 25[OH]D status at birth and development of asthma (Camargo et al., 2011). In a study that compared 25[OH]D levels in newborns admitted to the neonatal intensive care unit with acute lower respiratory infection with a group of healthy newborns, Karatekin, Kaya, Salihoglu, Balci, and Nuhoglu (2009) found that the ill newborns (N= 25) had significantly lower 25[OH]D levels compared to healthy controls (N= 15). This study also reports that 87.5% of all the newborns had serum 25[OH]D levels less than 20 ng/mL.

Atopic Dermatitis

In 2011, Peroni, Piacentini, Cametti, Chinellato, and Boner reported their findings regarding atopic dermatitis severity in children (N= 37) aged 8 months to 12 years and its correlation to 25[OH]D status. In this study, children with mild disease had significantly higher 25[OH]D levels compared to children with moderate or severe atopic dermatitis. Additionally, the children were tested for specific IgE to *Staphylococcus aureus* and *Malassezia furfur* revealing an inverse correlation between vitamin D deficiency and prevalence of the IgE.

Type 1 Diabetes

In a study that spanned 30 years and included 10,821 infants in Finland, researchers found that infants who regularly received 2000 IU daily of vitamin D had an 80% decreased risk of developing type 1 diabetes over the course of their first year compared to infants who did not receive regular supplementation (Hypponen, Laara, Reunanen, Jarvelin, & Virtanen, 2001). Additionally, children who had been suspected of having rickets during their first year of life had a relative risk of 3.0 of developing type 1 diabetes compared to those who never had a suspicion of rickets (Hypponen et al., 2001). In 2011, Bin-Abbas, Jabari, Issa, Al-Fares, and Am-Muhsen completed a study in Saudi Arabia designed to examine the prevalence of vitamin D deficiency amongst children with type 1 diabetes. Among the 100 children diagnosed with type 1 diabetes compared with 100 healthy controls, those children with type 1 diabetes had significantly lower 25[OH]D levels, with 84% being defined as vitamin D deficient versus 59% vitamin D deficiency among the healthy controls. In summary, there is evidence that

deficient 25[OH]D levels are associated with increased risk of developing type 1 diabetes.

Evidence of Vitamin D Transfer Through Breast Milk

Seminal research from the 1980's began exploring maternal supplementation of vitamin D and its effect on infant (birth through age 10 months) vitamin D levels (Ala-Houhala, 1985; Ala-Houhala et al., 1986; Greer, Hollis, Cripps, & Tsang, 1984; Greer, Hollis, & Napoli, 1984; Hollis, 1983; Kunz, Niesen, von Lilienfeld-Toal, & Burmeister, 1984). It was noted in these studies that vitamin D did pass through breast milk and was the main antirachitic factor in breast milk, with average amounts of 20-70 IU per liter of breast milk passing from mother to infant (Hollis, Roos, Draper, & Lambert, 1981; Hollis, Roos, & Lambert, 1982; Hollis, Pittard, & Reinhardt, 1986). Additionally, researchers revealed that the vitamin D content of breast milk increased when lactating mothers were either supplemented with vitamin D or exposed to ultraviolet light (Greer et al., 1984; Greer et al., 1984). It was acknowledged that skin color played a role in breast milk vitamin D content with African American women having lower vitamin D levels in their milk than white women (Specker, Tsang, & Hollis, 1985). Kunz, Niesen, von Lilienfeld-Toal, and Burmeister (1984) reviewed the vitamin D content of breast milk, cow's milk, and infant formulas. Participants took a prenatal vitamin containing 400 IU D2, but it is unclear if similar supplementation was continued postpartum. Interestingly, the authors found over the course of lactation the level of vitamin D in breast milk steadily dropped, demonstrating that this low dose was not adequate to maintain breast milk vitamin D content.

As there have been advances in our understanding of vitamin D physiology in general, there have been specific advances in our understanding of vitamin D physiology during pregnancy and lactation. The physiologic adaptations that occur during pregnancy and lactation coincide with the increased calcium demands of the growing fetus and infant. Fetal serum calcium levels are maintained at slightly higher than maternal levels by active transport of calcium by the placenta (Thandrayen & Pettifor, 2012). Maternal calcium absorption increases during pregnancy, especially in the third trimester as the fetal skeleton is being developed (Thandrayen & Pettifor, 2012). There is an increase in maternal 1,25$[OH]_2$D that promotes increased intestinal absorption of calcium, along with other factors (Wagner et al., 2008). If a pregnant woman is calcium or vitamin D deficient, this process will be altered and the fetus may have disordered bone development. Likewise, 25[OH]D crosses the placenta readily but 1,25$[OH]_2$D does not cross the placenta easily, leading to 25[OH]D deficiency in the fetus or infant born to a 25[OH]D deficient mother (Thandrayen & Pettifor, 2012). The calcium needs of the infant far exceed those of the fetus with approximately 300 mg of maternal calcium being transferred to the infant daily via breast milk (Thandrayen & Pettifor, 2012). During pregnancy, the increased calcium need is achieved from increased maternal intestinal calcium absorption whereas during lactation, the increased calcium need comes from maternal bone turnover (Thandrayen & Pettifor, 2012). The transfer of vitamin D from mother to infant is much different in lactation than during pregnancy. The infant receives vitamin D from breast milk in its parent form before it is converted by the liver to 25[OH]D, the form received via placental transfer by the fetus (Thandrayen & Pettifor, 2012; Wagner et al., 2008). Therefore, it is critical that maternal serum vitamin D levels

remain adequate despite the fact that vitamin D has a short half-life and is quickly converted to 25[OH]D (Wagner et al., 2008).

An exhaustive review of the literature revealed 3 contemporary randomized controlled trials in which lactating mothers were supplemented with vitamin D and then followed for measurement of their 25[OH]D levels along with those of their infants (Thiele et al., 2013). There were no studies found in which participants started vitamin D supplementation during pregnancy and continued through lactation (Hollis & Wagner, 2004b; Saadi et al., 2009; Wagner et al., 2006). Researchers begin their intervention during the lactation period in order to minimize the high attrition from breastfeeding in the first month postpartum and because of the relative difficulty in doing intervention studies with pregnant participants. However, this method allows for a majority of pregnant women and infants to be vitamin D deficient through the critical time in pregnancy in which the mother transfers a large amount of calcium to her fetus and then again through the first month of lactation when significant maternal bone turnover occurs in order to support the rapidly growing infant skeleton (Thandrayen & Pettifor, 2012). Because of the significant role that vitamin D sufficiency plays in bone stability and mineral homeostasis in the body, it is critical that this time frame of the life cycle be examined in terms of vitamin D needs.

In 2004, Hollis and Wagner enrolled lactating women (N= 18) at one month postpartum into a study giving them 2000 IU (1600 IU as D2 and 400 IU as D3) or 4000 IU (3600 IU as D2 and 400 IU as D3) daily for a 3 month period. Enrollment mean 25[OH]D level was 27.6 ± 3.3 ng/mL in the 2000 IU group and 32.9 ± 2.4 ng/mL in the 4000 IU group. Both the 2000 IU group and the 4000 IU group had significant increase

in mean 25[OH]D levels to 36.1 ± 2.3 ng/mL and 44.5 ± 3.9 ng/mL, respectively. Infants of mothers in both groups had significant increases in circulating 25[OH]D. Infants of mothers in the 2000 IU group went from a mean 25[OH]D level of 7.9 ± 1.1 ng/mL to 27.8 ± 3.9 ng/mL after 3 months of maternal supplementation. Infants of mothers in the 4000 IU group also had significant change from a mean 25[OH]D of 13.4 ± 3.3 ng/mL to 30.8 ± 5.0 ng/mL. The group taking 4000 IU daily made much more progress toward adequate vitamin D amounts in breast milk to result in adequate serum levels for their infants. This study was limited in scope due to a small sample size, however, despite the small number of participants the study still had adequate power to demonstrate statistical significance. This underscores the large effect size of maternal vitamin D supplementation on transfer to the exclusively breastfed infant.

In 2006, Wagner et al. enrolled exclusively breastfeeding women at 1 month postpartum for a 6 month trial in which they either received 400 IU vitamin D3 or 6400 IU vitamin D3 daily (N= 10 after loss to follow-up and breastfeeding attrition). Women in group 1 receiving 400 IU vitamin D3 daily were assigned to give their infant 300 IU vitamin D3 by liquid drop each day, and women in group 2 gave one drop of liquid placebo to their infant each day. This regimen was continued for 6 months after enrollment. The two groups did not differ in demographics or adherence to protocol, with about 80% compliance with maternal vitamin supplementation and 61% compliance with infant vitamin supplementation. Results showed that women taking vitamin D3 400 IU per day had 25[OH]D levels slowly decrease over the first 5 months of the study and then increase slightly as increased sun exposure was experienced for the last 2 months. Women in group 2 had dramatic increases in 25[OH]D levels (mean of 34.0 ng/mL at

enrollment to mean of 58.8 ng/mL 6 months later) that stabilized around month 3 and were maintained over the course of the study. Women receiving only the vitamin D 400 IU daily had the vitamin D content of their milk slowly decrease over the study period but with little variation. The women receiving vitamin D3 6400 IU daily had a 10 fold increase in the amount of vitamin D in their breast milk (82 to 873 IU per liter). This resulted in their infants having a steady rise in circulating 25[OH]D levels nearly equivalent to the infants receiving vitamin D 300 IU directly via supplementation. This study demonstrates that infants can receive doses of vitamin D through breast milk that are equivalent to oral dosing of 300 IU per day. This study was limited by very small sample size, but regardless was able to demonstrate a tremendous improvement in breast milk antirachitic activity with high dose maternal supplementation. This improvement would only be enhanced by earlier maternal supplementation in order to reach appropriate serum 25[OH]D levels in the mother earlier in the breastfeeding relationship.

In 2006, Basile, Taylor, Wagner, Horst, and Hollis randomized lactating mothers at 1 month postpartum to receive vitamin D3 2000 IU (N= 12) or 4000 IU (N= 14) daily for 3 months. Because one of the early signs of hypervitaminosis D can be hypercalcemia, the authors sought to determine if high dose vitamin D could possibly cause excessive calcium to be transferred to breast milk. Outcome measures included serum 25[OH]D in mother and baby as well as serum calcium and breast milk calcium levels. There were no incidences of hypercalcemia in mothers or infants and no episodes of hypercalciuria in mothers. The authors found that both groups had increase in serum 25 [OH] D levels in both mother and baby, and there was no significant difference in milk calcium levels or serum calcium levels. Enrollment maternal mean 25[OH]D level

was 22.4 ± 8.8 ng/mL in the 2000 IU group and 28.5 ± 8.6 ng/mL in the 4000 IU group. Both the 2000 IU group and the 4000 IU group had significant increase in mean 25[OH]D levels to 33.9 ± 6.5 ng/mL and 43.0 ± 11.6 ng/mL, respectively. Infants of mothers in both groups had significant increases in circulating 25[OH]D. Infants of mothers in the 2000 IU group went from a mean 25[OH]D level of 7.8 ± 1.1 ng/mL to 27.8 ± 3.9 ng/mL after 3 months of maternal supplementation. Infants of mothers in the 4000 IU group also had significant change from a mean 25[OH]D of 13.4 ± 3.3 ng/mL to 30.8 ± 5.0 ng/mL. The findings from this study demonstrate that over a 3 month period of time, doses of 2000 IU and 4000 IU daily do not raise 25[OH]D serum levels high enough to produce any markers of toxicity. The mothers in the vitamin D 4000 IU per day group increased their serum and milk 25[OH]D levels significantly more than the women in the vitamin D 2000 IU per day group and the infants of mothers receiving vitamin D 4000 IU per day displayed significantly higher 25[OH]D serum levels by the end of the study.

In 2009, Saadi et al. undertook a larger study involving 90 breastfeeding women in the United Arab Emirates (UAE). Women were randomly assigned to receive vitamin D2 2000 IU daily (N= 45) or 60,000 IU monthly (N= 45). All infants were supplemented with vitamin D2 400 IU daily for three months. Because of the difference in group supplementation schedule, this study was not blinded to researchers or participants. The mothers and their babies had enrollment 25[OH]D levels measured at entry to the study and monthly before administration of their next monthly dose, with a final 25[OH]D level measured after the 3 month period. Infants had a second 25[OH]D measurement at the end of the 3 month study. The authors set the vitamin D deficiency level at ≤ 15 ng/mL

(≤ 37.5 nmol/L) based on two older reports regarding physiologic measures. Enrollment 25[OH]D levels were available on 92 infants (two sets of twins) and revealed 95% deficiency. Mothers had an 88% deficiency, even with the extremely low cut off value mentioned above. After 3 months of their assigned regimens, the infants had drastically increased their mean serum 25[OH]D levels, however 23% in the daily regimen group and 38% in the monthly regimen group still met the definition of deficiency. Circulating vitamin D levels in the mothers had shown improvement as well, but 36% of the daily regimen group and 50% of the monthly regimen group continued to meet the definition of deficiency. The researchers were also able to analyze vitamin D levels in the breast milk of 8 women. Levels were undetectable at the beginning of the study and increased to a median of 50.9 IU/L. Although this study concluded that significant increases were seen in both maternal and infant serum vitamin D levels, the increases were not sufficient over the 3 month study period to correct a majority of the deficiency seen, especially if more contemporary deficiency cutoffs were used. This study was limited by the use of vitamin D2 for supplementation, which is less effective at raising serum 25[OH]D levels (Heaney, Recker, Grote, Horst, & Armas, 2011).

It is clear from these randomized trials that lactating women receiving what are considered high doses of vitamin D have an increase in their serum 25[OH]D levels, increases in the vitamin D content of their breast milk, and their infants have subsequent increases in serum 25[OH]D levels. In a recent letter to the editor of Public Health Nutrition, Hollis and Wagner (2011) speak to the issue of vitamin D supplementation efficacy during lactation (Hollis & Wagner, 2011). They extrapolate from the existing data that for every 25 µg (1000 IU) of vitamin D ingested by the mother she will pass 2.5

µg (100 IU) of vitamin D to her breastfeeding infant. The linear relationship between maternal vitamin D intake and vitamin D transferred through breast milk in a 10 to 1 IU ratio was also shared by Dr. B.W. Hollis during a study design consultation (personal communication, September 21, 2010). Therefore, they argued if a woman receives 150 µg (6000 IU) daily of vitamin D, she will be able to replete herself and provide about 12.5 µg/L (500 IU per liter) of vitamin D to her infant. One liter would be a typical amount of breast milk for an infant to ingest in 24 hours. Hollis and Wagner (2011) reiterate that these doses have been used in several studies and there have been no adverse events. This speaks to the efficacy and safety of high dose vitamin D in lactating women and their exclusively breastfed infants. The reviewed studies are somewhat limited by sample size or lack of diversity amongst participants, however no adverse events or markers of toxicity were seen, which does bolster confidence in the safety of these doses. Studies with more participants and over longer periods of time are necessary to make further conclusions about efficacy and safety. It will also be critical to investigate the outcomes seen with vitamin D supplementation started during pregnancy and continued through lactation.

Gap in Knowledge Regarding Vitamin D Transfer Through Breast Milk

This study was designed to generate novel evidence regarding the effect of maternal supplementation during pregnancy and lactation on the resultant maternal and infant serum vitamin D levels. As described above, prior research has focused either on supplementation with vitamin D during pregnancy or during lactation, but studies spanning these two stages could not be found. This research study is innovative in its approach to start supplementation during pregnancy with the hypothesis that both

pregnant women and newborns will benefit from this longer term supplementation. Additionally, due to the extent of vitamin D deficiency, more time is needed for serum levels to reach adequacy before participants will be able to transfer adequate vitamin D through breast milk. Many of the studies presented above enrolled fewer than 20 participants and were able to document statistically significant relationships. Additionally, no studies investigating the impact of vitamin D supplementation on inflammatory cytokine production *in vivo* were identified. This study provides novel evidence of the effect of maternal vitamin D status on maternal inflammatory response. This study helps develop the growing body of knowledge regarding treatment of vitamin D deficiency and has the potential to generate evidence to support changes to the current recommendations for maternal and infant supplementation with vitamin D.

CHAPTER III

METHOD

Introduction

A gap in knowledge exists regarding adequate doses of maternal vitamin D supplementation during pregnancy and lactation that yield adequate infant serum 25[OH]D levels. The objective of this study is to identify the effect of continuous maternal prenatal and postnatal vitamin D supplementation on maternal 25[OH]D status and vitamin D transfer to exclusively breastfed infants through breast milk as evidenced by infant 25[OH]D status. The methodology used for this study including research design, study intervention, population and sampling, protection of human subjects, data collection, laboratory methods, statistical methods and analytic plan are described in this chapter.

Objectives and Hypotheses

The primary objectives of this study were to evaluate the effect of maternal vitamin D supplementation initiated during the third trimester and continued through early lactation on: 1. maternal 25[OH]D levels at delivery, 2. infant 25[OH]D levels at birth, and 3. both maternal and infant 25[OH]D levels at 4-6 weeks of lactation in exclusively breastfeeding dyads. To meet this objective, the following hypotheses were tested:

Central Hypothesis: Maternal supplementation with vitamin D during pregnancy and lactation will significantly increase circulating vitamin D levels during lactation in mothers and their exclusively breastfed infants;

1. Women who receive supplemental vitamin D starting in the early third trimester of pregnancy will have significantly higher 25[OH]D serum levels compared to control participants by delivery;
2. Women who receive supplemental vitamin D starting in the early third trimester of pregnancy and continue with this dosing during the early postpartum will maintain significantly higher serum 25[OH]D levels than control participants during lactation;
3. Infants born to women who receive supplemental vitamin D starting in the early third trimester of pregnancy will have significantly higher 25[OH]D levels at birth compared to control infants;
4. Infants who exclusively breastfeed from a mother receiving supplemental vitamin D will have significantly higher 25[OH]D levels than infants exclusively breastfeeding in the control group at 4-6 weeks of age.

Due to immune and inflammatory modulating effects of vitamin D, the following secondary hypotheses were tested:

1. Women with higher vitamin D levels will have decreased levels of pro-inflammatory cytokines (TNF-α and IL-6);
2. Women with higher vitamin D levels will have increased levels of an anti-inflammatory cytokine (IL-10).

Research Design

This study used a double blinded randomized controlled trial design. This design was chosen in order to test the effect of vitamin D supplementation on maternal and neonatal vitamin D status during pregnancy and lactation. A randomization scheme was used in order to generate two comparison groups (control and experimental) of approximately the same size in a 1:1 ratio (Moher et al., 2010). Randomization was achieved with the use of computer software provided by statistical consultant Dr. David Roth and monitored by Dr. Jody Ralph, a member of the dissertation committee and data safety monitoring board. Allocation concealment was used during the recruitment process. Prior to initiation of participant enrollment, Dr. Jody Ralph created enrollment packets that contained the necessary paperwork, blood tubes, and study pills corresponding to the random assignment, all labeled with participant numbers. After agreeing to participate and providing informed consent, participants were linked to the group assignment based on order of recruitment. The list of corresponding participant codes associated with predetermined group allocations was kept in a locked cabinet with Dr. Jody Ralph at the University of North Dakota in Grand Forks, North Dakota. All members of the research team, as well as the participants, were blinded to participant group assignment.

Protection of Human Subjects

This study received Institutional Review Board (IRB) approval through the University of North Dakota (UND) and Altru Health System (Appendix A). Several changes to the study protocol were made and implemented after approval by both IRB

entities. An IRB Authorization Agreement was provided between UND and Oregon Health and Science University, the PI's employer (Appendix A).

Participants received both verbal and written study information. This included reassurance that they were under no obligation to participate and lack of participation would not change the course of their medical care. Support for the study was provided by the clinic where recruitment took place (Appendix B).

A Data Safety Monitoring Board (DSMB) was created and included Dr. Elizabeth Tyree, Dr. Jody Ralph, Dr. Leah Whigham, and Dr. Edward Sauter. Dr. Jody Ralph reviewed 25[OH]D results as a member of the DSMB in order to implement steps to assure participant safety. The DSMB received a quarterly report from the PI, which included an update on participant recruitment and study procedures. The DSMB was notified immediately upon knowledge of any adverse event. In the case of potential adverse events, both IRB entities were also officially notified and provided with the DSMB's report. Enrollment maternal 25[OH]D serum levels were reviewed by the PI and data from subsequent collection periods were reviewed by Dr. Jody Ralph thereafter to preserve PI blinding. Any 25[OH]D serum level equal to or exceeding 90 ng/mL was reported to the DSMB. At that time, the participant would be asked to exit the study with follow-up evaluation of serum 25[OH]D level in one month.

Population and Sampling Procedures

Subjects for this study were recruited from an obstetrical practice serving local and surrounding communities of Grand Forks, North Dakota. All patients receiving care

from this practice planned to deliver at Altru Health System in Grand Forks, North Dakota, which has about 1500 deliveries per year, with a majority coming from this obstetrical practice. Prior studies completed with participants engaged in care through this practice demonstrated the sample population would be comprised of 81% Caucasian, 7% Native American, 4% African American, 2% Asian, 3% Latina, and 3% multi-racial women (C.M. Anderson, personal communication, June 1, 2012). All participants continued to receive routine care with their physicians or nurse practitioners during their pregnancy, intrapartum and postpartum periods.

Advertisements for the study were placed in several locations in the obstetrical clinic (Appendix C). Women who wished to participate were able to contact the PI directly to be evaluated for meeting inclusion and exclusion criteria. Additionally, key clinic staff were contacted every weekday morning to determine if there were potentially eligible patients who were scheduled for prenatal visits that day. The clinic personnel were able to determine if clients met the basic requirements of gestational timing in pregnancy and parity status of 1 or greater. When a potential participant was identified, she was met at her prenatal visit and introduced to the study to determine interest. If the participant was interested, a full discussion was initiated regarding requirements of the study, rights of the participant, and evaluation for meeting inclusion and exclusion criteria. The advertisement notified women that they were eligible to receive up to 40 dollars in incentive gift cards (10 dollars at delivery and 30 dollars at the final lactation visit) upon completion of the study. Incentive payment information was reiterated in verbal communications prior to informed consent.

Inclusion criteria were: pregnancy between 24 and 28 weeks, history of breastfeeding for at least 4 weeks with a prior infant, intent to breastfeed for at least 4-6 weeks, and maternal age greater than 18. Starting the intervention in the early third trimester allowed for enough time to elicit a full effect in the mean 25[OH]D levels of the intervention group by the time participants delivered and simultaneously kept the length of the study feasible (Heaney & Holick, 2011; Hollis & Wagner, 2011). The study was limited to participants who have previously breastfed to diminish the high rate of attrition due to failure to breastfeed, which can exceed 50% in women who have never breastfed before. Additionally, women who were recruited planned to breastfeed exclusively for 4-6 weeks as this was essential to determining the transfer of vitamin D through breast milk only without outside sources of vitamin D in the newborn's diet. Participants were at least18 years old in order to negate any differences in calcium and vitamin D physiology inherent in adolescent woman (Jamali et al., 2013). Exclusion criteria include: preexisting type 1 or type 2 diabetes, preexisting hypertension, parathyroid disease, uncontrolled thyroid disease, and use of vitamin D supplements beyond a prenatal vitamin in the last 6 months (Hollis & Wagner, 2004b; Wagner et al., 2006). Women with underlying metabolic disorders as listed above were excluded due to confounding effects of medications they may be taking along with possible underlying disruptions in their ability to metabolize vitamin D. Women who might be medically fragile, such as those with hypertension, were excluded to limit the possibility that vitamin D supplementation would interfere with medical care. In order to properly monitor and protect participants from vitamin D toxicity and to compile a typical population sample, women who were already taking vitamin D supplementation beyond

that found in a typical prenatal vitamin were excluded. Exclusion criteria for the infants, which would have excluded an infant from continuing in the study, included birth prior to 37 weeks gestation, admission to the neonatal intensive care unit for any reason, or congenital anomaly that prevented exclusive breastfeeding.

Based on data from previously published studies for the primary outcome of infant 25[OH]D at 4-6 weeks lactation, a sample size of 14 participants, equally divided between the intervention and control groups, was needed to detect a mean difference of 8 ng/mL with a standard deviation of 5, using alpha = 0.05 and assuming 80% power (Basile et al., 2006; Hollis & Wagner, 2004b; Hollis et al., 2011; Saadi et al., 2009; Wagner et al., 2006). A total of 8 women were recruited in each arm of the study in order to achieve a total sample size of 16, adequate to offset loss to follow-up.

Study Intervention

The study intervention included two groups, the control and experimental groups, as illustrated in Figure 3. Participants in both groups received a prenatal vitamin (Target brand, Minneapolis, MN) containing a complement of vitamin and mineral supplements including 400 IU of vitamin D3 (cholecalciferol). In addition to the prenatal vitamin, control group participants received a compounded microcrystalline vegetable cellulose filled placebo capsule. Experimental group participants received the same prenatal vitamin with an additional compounded capsule containing microcrystalline vegetable cellulose plus 3400 IU vitamin D3. The experimental group therefore received a total of 3800 IU vitamin D/day. Both the prenatal vitamin and the study capsule were taken daily

from initiation at 24-28 weeks gestation through 4-6 weeks postpartum. All compounding was provided by InHealth Specialty Pharmacy, Fargo, ND. InHealth is licensed and accredited by the North Dakota Board of Pharmacy.

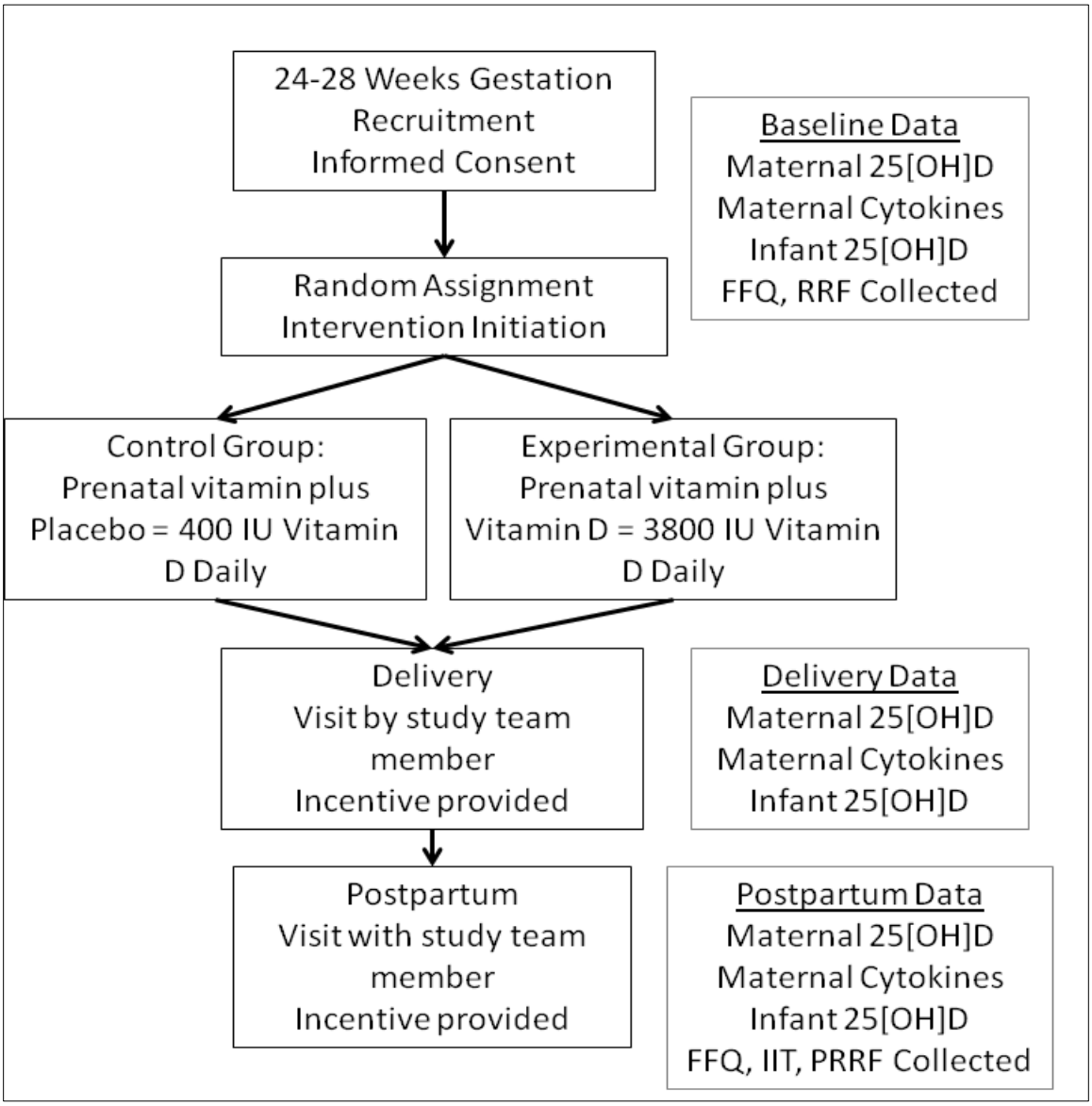


Figure 3. Study intervention time line and data points.

Although an intake of 600 IU daily is recommended by the Office of Dietary Supplements of the National Institutes of Health for all pregnant women, the study team could not locate a prenatal vitamin that contained this dose, therefore a supplement containing 400 IU was used (Table 4).

Table 4. Nutrient content of the prenatal vitamin received by all participants.

Nutrient	**Amount**	**Percent of Daily Allowance**
Vitamin A (100% as Beta Carotene)	4000 IU	50
Vitamin C	120 mg	200
Vitamin D	400 IU	100
Vitamin E	30 IU	100
Thiamin	1.8 mg	106
Riboflavin	1.7 mg	85
Niacin	20 mg	100
Vitamin B6	2.6 mg	104
Folic Acid	800 mcg	100
Vitamin B12	8 mcg	100
Calcium	200 mg	15
Iron	28 mg	156
Zinc	25 mg	167

The placebo pill used in the control group was indistinguishable in appearance from the additional vitamin D capsule used in the experimental group (Figure 4). This allowed for continuous blinding of study team members and participants.

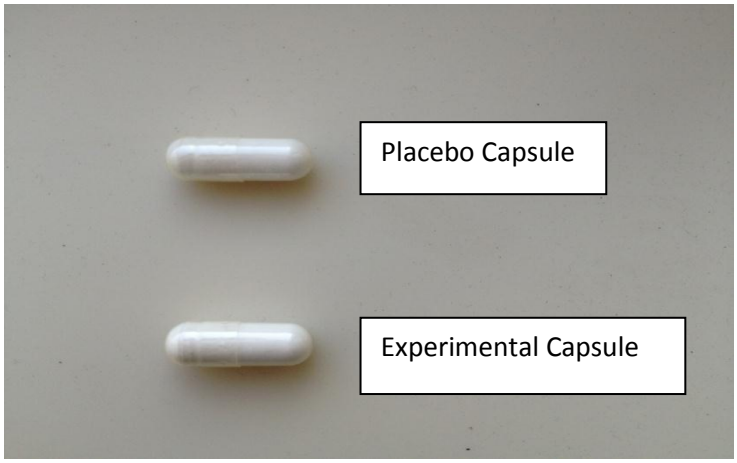


Figure 4. Photo demonstrating similarity of capsules used for the control and experimental groups.

Study Procedures

After being identified as a potential participant by the clinic personnel, women were approached by a study team member and the study explained. The study team consisted of Doria K. Thiele, MN, CNM, IBCLC and PI on the study, Dr. Cindy M. Anderson, PhD, WHNP-BC, FAAN and chair to the PI's dissertation committee, and Michelle Wright, RN, PhD Candidate. All study team members completed human subjects training through University of North Dakota. When a woman indicated interest in participation, full informed consent was provided verbally and the participant was given time to read the Consent to Participate and Authorization for Access to Personal Health Information documents (Appendix D). Participants had the opportunity to ask

questions and discuss the study procedures and timing. Both the Consent to Participate and Authorization for Access to Personal Health Information documents were then signed in duplicate to provide one set for the participant to keep and one to be retained for study records. Participants were encouraged to call the PI at any time with concerns or questions and contact information was provided on the Consent to Participate.

All participants were recruited during the same season, summer 2012, thus removing season as a confounding factor in the study. Season can have a significant effect on maternal and newborn vitamin D status due to variation in sun exposure and angle of UVB light (Eichholzer et al., 2013; Halicioglu et al., 2012; Seckmeyer et al., 2013; Utrillas et al., 2013). The timing of participant recruitment was coordinated with routine blood sample collection for screening of gestational diabetes. Women who consented to participate were accompanied to the laboratory where further discussion took place. The gestational diabetes screening test includes an hour of waiting time and allowed for the time needed to complete a Food Frequency Questionnaire (FFQ, Appendix E) to assess for dietary vitamin D intake, as well as answer questions and establish rapport. Random assignments were predetermined so that the correct study pills and blood collection tubes were used at the recruitment visit with the participant. Participants were shown their study pills and prenatal vitamins and their proper use was explained. When maternal venipuncture for the routine screening tests was underway, additional blood was collected in study-provided blood tubes. Please see section *Blood Collection* for details on these methods and procedures.

The PI contacted participants 2 weeks after recruitment to assess for compliance with the study intervention including daily ingestion of a prenatal vitamin and assigned study capsule and to be available for questions to be answered. Prenatal vitamins were provided in 90 day supply and study pills were provided in a 30 day supply. Participants were met at their prenatal visits at approximately 32 weeks, 36 weeks, and at delivery in order to exchange their previous study pill bottle for a new study pill supply. Participants were told to bring their study pill bottle with any remaining pills at each meeting so that compliance could be monitored. After each in-person meeting, the participant was contacted by the PI about 2 weeks later for another assessment of intervention compliance and to answer questions. This scheme allowed for every 2 week contact with participants, which supported participant compliance and intervention fidelity.

Participants were provided with a small slip of paper to take to the hospital when they were in labor that allowed hospital staff to identify them as a participant in the study. This slip of paper had a sunshine printed on it, as well as study team contact information, but no other identifying information to maintain privacy and confidentiality. The hospital staff notified the researcher that the participant was in labor. It was then arranged for the participant and her infant to have their blood draws completed approximately 24 hours after delivery. The participant was visited by a study team member while still in the hospital and provided another 30-45 day supply of study pills and prenatal vitamins. The number of pills provided was determined by when the final visit was scheduled. If the participant was finishing her study pills prior to the 4-6 week postpartum visit extra pills were mailed to her with her permission. Participants were instructed to continue to take

their assigned supplements daily through 4-6 weeks postpartum. The participant was also provided with the Infant Intake Tool (IIT, Appendix F) along with instructions as to how to record any formula or vitamin D supplement given directly to the infant for the next 4-6 weeks.

Participants were contacted by the PI two weeks after delivery to assess for both compliance with the study intervention and exclusivity of breastfeeding. During this conversation, the PI and participant discussed scheduling for final data collection visit at 4-6 weeks after delivery. At the time of the meeting for final data collection at the Grand Forks Human Nutrition Research Center (GFHNRC), maternal and infant blood samples were collected and the participant completed a second FFQ.

Data collected included maternal dietary intake via a FFQ at enrollment and lactation, maternal prenatal health parameters via the Record Retrieval Form (RRF, Appendix G), intrapartum and postpartum health parameters for mother and infant via the Postpartum Record Retrieval Form (PRRF, Appendix H), and infant supplementation with formula and vitamin D via the IIT. In addition, blood was collected to assess for maternal 25[OH]D, and cytokine (TNF-α, IL6, and IL10) levels at enrollment, delivery, and lactation, while infant blood was collected to assess 25[OH]D levels at delivery and lactation (Figure 3). Specific approaches to data collection including tools used are outlined in proceeding sections.

Instrumentation

Maternal Prenatal and Postnatal Dietary Intake

A FFQ was completed by each participant at entry to the study and at 4-6 weeks postpartum in order to assess maternal intake of foods containing vitamin D. The FFQ was developed by GFHNRC staff and patterned after the Harvard Service FFQ format (Suitor, Gardner, & Willett, 1989). Reliability and validity testing have not been completed on the FFQ, however other published studies have used the same form for quantification of maternal dietary intake (Swensen, Harnack, & Ross, 2001; Tande et al., 2012). The FFQ includes individually described food items. The participant indicated how often she ate that food in the previous 3 months. Serving sizes were indicated such as "milk, 8 fluid ounces" or "whole wheat bread or rolls, 1 slice". All food items on the FFQ are matched to food codes from Release 24 of the USDA Nutrient Database for Standard Reference (U.S. department of agriculture, agricultural research service. 2011. USDA national nutrient database for standard reference, release 24.2011) or the USDA Food and Nutrient Database for Dietary Studies 5.0, which are linked with the GFHNRC GRAND nutrient database (Ahuja et al., 2012). The GRAND nutrient database allowed for conversion of foods consumed on a monthly or weekly basis to an average daily consumption and then analyzed for nutrient content.

Maternal and Newborn Health Parameters

An RRF was used to collect data on maternal health status from the medical record began at enrollment to the study. The PI arranged access to participant health records with the administration of the Altru Health System Medical Records Department.

A designated computer was used to access health records, which had the necessary software and safety features required by Altru. Access to participant health records was limited to only information that pertained to this study and was limited in time to extend from time of enrollment to just after delivery. Data collected with the RRF included obstetric history, history of medical conditions, current medications and supplements, typical parameters from prenatal visits such as blood pressure and weight, and results from screening tests such as initial urine and blood screens. The RRF also included sections for recording additional laboratory tests that would be completed by the participant's obstetric provider if the participant was diagnosed with complications of pregnancy, as well as areas to record fetal surveillance if that were necessary.

After delivery, a PRRF was used to gather pertinent data about maternal and infant health. PRRF data were collected by the PI using the electronic access described above. The PRRF included data regarding labor and delivery outcomes for participant and infant, maternal diagnoses since delivery, infant diagnoses since delivery, infant anthropomorphic measures, and breastfeeding status while in the hospital.

The IIT was designed to record newborn dietary intake other than breast milk to include formula and supplements. Participants were asked to document a daily recording of their infant's formula or vitamin D intake from birth through 4-6 weeks of age. Because formulas contain vitamin D and participants were encouraged to follow the AAP guideline regarding infant supplementation with daily vitamin D, this instrument provided data to quantify infant vitamin D intake from non-breast milk sources.

Blood Collection and Processing

Blood samples were collected from the mother via venipuncture at enrollment, delivery, and lactation. Whenever possible, venipuncture samples were collected in conjunction with other routine laboratory analyses that were a typical part of prenatal and postpartum care. The enrollment blood samples were collected by staff at the clinic laboratory utilized by the obstetric practice in conjunction with laboratory tests routinely ordered at 24-28 weeks of gestation. After signing the Consent to Participate documentation and discussing the study, participants were accompanied to the laboratory for their blood draw. The laboratory technician was provided with four additional 6mL blood tubes (Fisher Scientific, New Hampshire). These additional tubes included two ethylenediaminetetraacetic acid (EDTA) coated purple top vacutainer tubes and two serum separator red top vacutainer tubes. The EDTA coating on the purple top tubes stops the coagulation process. Samples collected in EDTA coated vacutainer tubes were put on ice until sample preparation. The serum separator red top tubes are used to form a clotted sample and these samples are stable at room temperature. Samples were allowed to separate prior to sample preparation. Appropriate universal precautions were used for blood handling and transport. The blood collection tubes were taken to the GFHNRC for processing. Blood samples were centrifuged at 3000rpm for 10 minutes at 4°C. Centrifugation allowed for separation of the serum and plasma from the whole blood. A range of 0.5-1.0mL of serum was removed from the red top tubes and placed into 8 individual 2mL Eppendorf cryotubes. Plasma was removed in 1mL volumes from the EDTA purple top tubes and aliquoted into 2mL labeled Eppendorf cryotubes. The Eppendorf cryotubes were labeled with the participant number and sample source and stored at -80°C.

At delivery, blood collection was completed by Altru Hospital staff. The maternal samples were collected via venipuncture using study supplied blood tubes and processed as described previously. Infant blood samples were collected via heel stick. The heel was warmed using study supplied infant heel warmers (VWR Scientific, Bridgeport, NJ). Once the heel was warmed, a newborn lancet (BD, Franklin Lakes, NJ) was used to puncture the skin at no more than 1.0mm depth. Hospital staff was supplied with pediatric EDTA purple top and serum separator red top tubes, each holding a maximum of 500µL (Fisher Scientific, New Hampshire). Each infant had blood samples collected in 1 EDTA purple top pediatric tube and 1 to 2 serum separator red top pediatric tubes at the time of routine metabolic screen, when possible. The purple top pediatric tubes were put on ice and all samples were immediately transported to the GFHNRC for processing and storage. All pediatric tubes were spun at 3000rpm for 10 minutes at 4°C. Volumes of 0.5mL serum were aliquoted into 2mL Eppendorf cryotubes. All samples were appropriately labeled with participant code and stored at -80°C. The final blood collection at lactation for both maternal participants and infants took place at the GFHNRC. Maternal participants completed the second FFQ and returned the IIT. Maternal and infant participants had blood collected for serum and plasma collection as previously described. Blood samples were collected, processed and stored as outlined previously.

Laboratory Analyses

Maternal and Infant 25[OH]D Laboratory Methods

Maternal and infant blood samples were processed and stored at the GFHNRC as described previously. Maternal samples were batch analyzed at regular intervals using the Immunodiagnostic Systems Ltd (IDS) 25-Hydroxyvitamin D enzymeimmunoassay (EIA) kit. Frequent analysis allowed for data safety monitoring regarding serum 25[OH]D status.

The IDS-EIA kit system was analyzed on the NexGen platform by Dr. Holly Brown-Borg's lab in the department of Pharmacology, Physiology & Therapeutics, School of Medicine and Health Sciences at the University of North Dakota (Dr. Holly Brown-Borg, Dr. Lalida Rojanathammanee, and Ms. Sharlene Rakoczy). For each sample, 25 µl of serum was added to 1 mL of a biotin solution, which acts to dissociate the vitamin D from its binding proteins. Samples were then placed in an antibody coated plate and incubated for 2 hours. The NexGen automated platform washed the samples with a peroxidase solution for binding with the biotin complex. Color was developed using a chromogenic substrate and the absorbance of each well measured at 450 nm. IDS reports a correlation coefficient (r) of 0.9 when compared to a recognized radioimmunoassay. This method is considered comparable in sensitivity and accuracy to radioimmunoassay, but without the technical expertise and use of radioactive solutions needed to run the radioimmunoassay (Kimball & Vieth, 2007; Wallace, Gibson, de la Hunty, Lamberg-Allardt, & Ashwell, 2010). This method is also approved and monitored by the Federal Drug Administration (FDA).

Maternal Inflammatory Markers

Maternal samples were also analyzed for inflammatory markers including TNF-α, IL6, and IL10. All inflammatory markers were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. Analysis included use of the Quantikine High Sensitivity ELISA for IL-10, IL-6 and Quantikine ELISA for TNF-α (R & D Systems, Minnesota). All sample values are reported in picograms/mL (pg/mL). Plasma was batch analyzed for cytokines at the end of the study to minimize variability. The specific assay procedures for the three analyses were identical except for variation in amounts of solution added and the wavelength used for determination of optical density as the last step in the process; therefore, the procedures will be described here as representative of all three assays. The assay procedures include first creating a normal curve using the standards provided by the manufacturer. This allows for laboratory standardization and calibration for result analysis. For all three assays a normal curve was created to compare samples against. Plates were prepared and included a diagram for recording placement of specific participant samples. Each well in the plate is prepared with the appropriate assay diluents to which either standard, control, or sample is added. The plates are then incubated at room temperature for 2 hours. After the incubation period, the wells are aspirated of fluid and precisely washed with the wash buffer. Each well is then applied with the corresponding conjugate (TNF-α, IL-10, or IL-6) and again incubated for 2 hours. At the end of the incubation period, substrate solution was added followed by a 1 hour incubation, and addition of amplifier solution (IL-10 and IL-6 only). Each well is then exposed to a stop solution that stops the reaction and causes a visible color change indicating appropriate chemical processing. The plates are then read on the NextGen Bioplex using 490 nm wavelength for IL-10 and IL-6 and 450 nm wavelength for TNF-α.

Most samples were run in triplicate, although for IL-10 some samples were run in duplicate and in single due to lack of well space in the plate.

Data Analysis

The data gathered on the FFQ, RRF, PRRF, IIT, and blood analysis data were entered into IBM SPSS Statistics version 21 by the PI. Data collected from instruments including, but not limited to, baseline maternal characteristics, characteristics of labor and delivery, dietary intake, and newborn characteristics were tested for normality. The intention-to-treat model was used throughout. Mean values for maternal characteristics at enrollment, including 25[OH]D level, were compared between the control and intervention groups to analyze for selection bias. Descriptive statistics including mean and variance measures (standard error of the mean/standard deviation), were computed for maternal and infant 25[OH]D level at enrollment, delivery, and lactation, and for maternal inflammatory markers at enrollment, delivery, and lactation. Interaction and treatment effects between group allocation and 25[OH]D level were determined by independent samples t-tests and Analysis of Covariance (ANCOVA) while adjusting for maternal enrollment 25[OH]D level. ANCOVA included estimated marginal means and standard error of the mean. The estimated marginal means describe what the means would have been if a covariate had not had an influence on the outcome. In this case, the enrollment values were controlled for as the covariate. Repeated measures ANCOVA was used to determine the impact of the intervention across the entire study period while controlling for maternal enrollment 25[OH]D level. Cytokine data were analyzed by ANCOVA, controlling for the influence of enrollment cytokine value by removing it as a

covariate. Additionally, likelihood of participants and infants to achieve a significant 25[OH]D level at delivery and lactation was calculated as a relative benefit increase. Significance was established at $p \leq 0.05$.

CHAPTER IV

RESULTS

Introduction

This study aimed to quantify the transfer of vitamin D from mother to exclusively breastfed infant by evaluating the relationship between maternal and infant 25[OH]D serum levels, using a randomized controlled trial design. Participants were assigned to either the experimental or control group, receiving a total of 3800 IU of vitamin D or 400 IU of vitamin D, respectively. This chapter includes the findings of statistical analyses completed on the study data and is organized in terms of the four research hypotheses and two secondary hypotheses. The results of enrollment demographic data are presented, followed by results related to individual hypotheses.

Trial Profile and Participant Progression

The study protocol dictated the course of the study and participants were tracked to determine adherence to the study protocol and desire to continue the study. As described by Moher et al. (2010), consistency in reporting findings from randomized controlled trials will improve understanding of such studies and improve future use of this study design (Moher et al., 2010). In order to provide consistent reporting of

findings, the recommendations from the Consolidated Standards of Reporting Trials (CONSORT) have been followed (Figure 5). Of the 20 women identified by clinic staff as eligible, 2 declined to participate and 2 did not meet inclusion criteria. The remaining 16 eligible participants who elected to participate were randomly assigned to either the experimental or control group (N=8/group). After enrollment, one participant in the control group was taking daily cod liver oil. Cod liver oil can contain over 1000 IU of vitamin D per teaspoon, which is in excess of that found in a prenatal vitamin thus disqualifying this individual from participating in the study. The reason for exclusion was discussed with the participant and she exited the study. Additionally, one participant from each group exited the study prior to initiating the intervention; one was moving out of state and the other did not give a reason. Therefore, 6 participants in the control group were followed through delivery and 7 participants in the experimental group were followed through delivery. Three participants in the control group failed to appear for their final visit. Follow up visits were scheduled at the convenience of participants. The participants that failed to keep appointments for final visits did not respond to attempts to reschedule. All 7 participants in the experimental group attended the final visit. Therefore, a total of 3 women in the control group and 7 women in the experimental group completed the intervention by remaining in the study through lactation.

For all participants completing data collection at delivery and lactation, blood was collected on maternal and infant participants. Using the intention to treat method, final analysis was completed on 6 maternal and infant participants in the control group, and 7 maternal and infant participants in the experimental group.

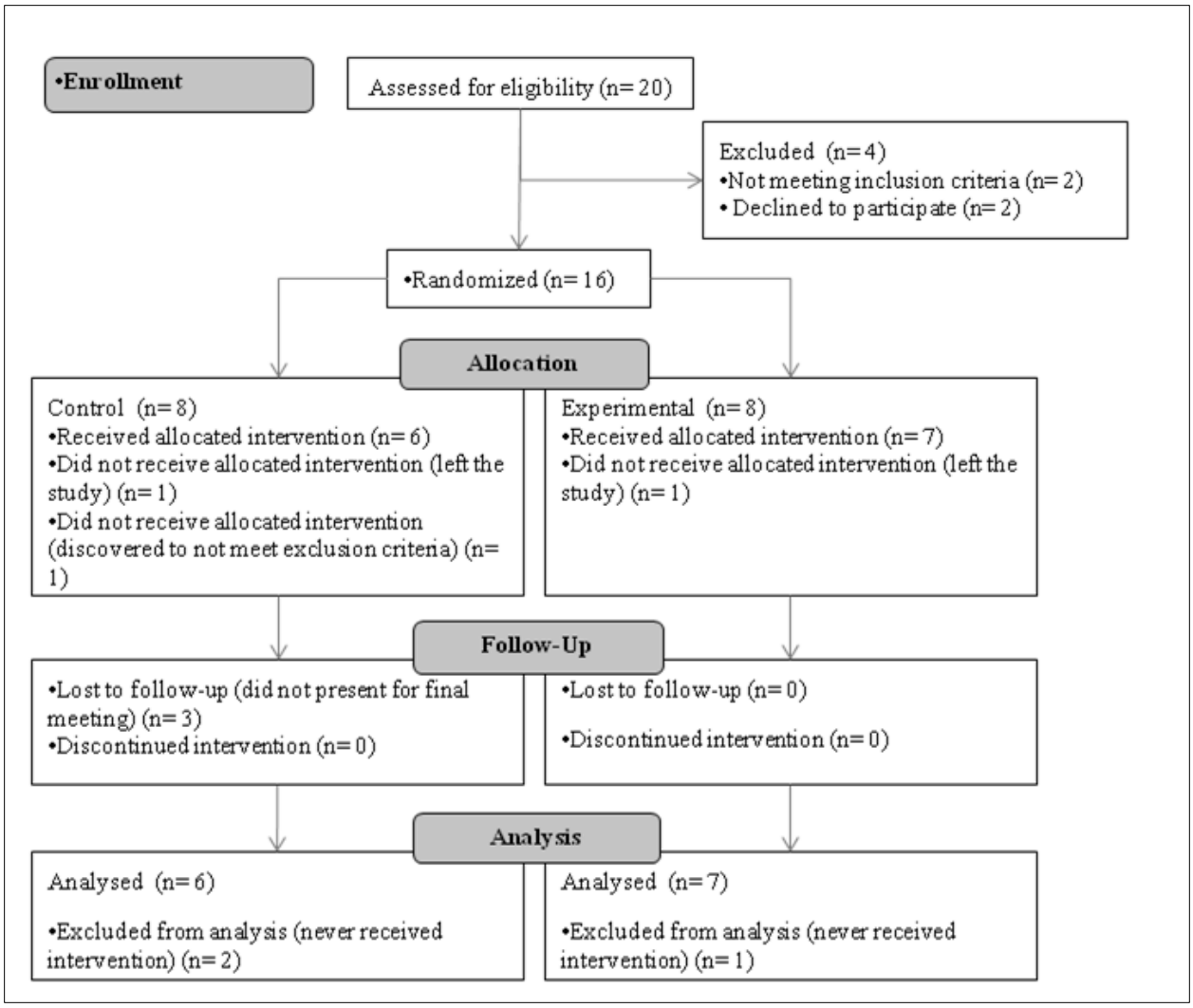


Figure 5. CONSORT statement for trial profile and participant retention. The diagram demonstrates flow of participants and participant contact through the study as recommended by Moher et al. (2010).

Safety Considerations

During the course of the study, one participant experienced development of kidney stones at 33 weeks gestation that required a 24 hour hospitalization, which was investigated as an adverse event. Dr. Jody Ralph, unblinded member of the DSMB, was notified and was able to determine the participant's enrollment 25[OH]D level, which was low-normal. During the participant's hospital stay, her urine and blood were not

analyzed for calcium levels. The participant notified the primary care physician of her participation in the study. Upon discussion between a research team member and the participant's primary practitioner, it was determined that the incident was not related to her study participation. Adverse event forms were sent to Altru Health System and UND IRB entities and the DSMB was notified and provided with as much information as was available (Appendix I). It was determined that this event was unrelated to study participation. No participants experienced a 25[OH]D level in excess of 90 ng/mL at any of the three data points and there were no other adverse event reports to the DSMB.

Description of Sample

Random sampling and random assignment were implemented to control for confounders, which are assumed to occur equally in the two groups. All participants were recruited during the same season, summer 2012, thus removing season as a confounding factor in the study. Analysis of normality was completed on all 25[OH]D data including evaluation of skewness and kurtosis. Data fell on a normal curve and were evenly distributed. Because values fell on a normal curve, the data were able to be analyzed using standard statistical methods.

Demographic data are shown in Table 5. Enrollment characteristics are reported as mean (±SD) for the 13 participants. Enrollment variables include ethnicity, serum 25[OH]D, daily dietary vitamin D intake, season of enrollment, age, gestation at enrollment, parity, and body mass index (BMI) at enrollment. Enrollment characteristics were compared between the groups using independent samples t-tests. There were no statistically significant differences in enrollment characteristics between the experimental

and control groups at enrollment. The absence of group differences demonstrates successful randomization in that the two groups are homogeneous allowing for comparison and evaluation of the impact of the intervention.

All participants self-identified as white ethnicity and all were recruited in the summer season; therefore these two characteristics were not analyzed. At enrollment, participants were an average age of 29 (± 5.6) years and 28 (±0.9) weeks gestation. The

Table 5. Enrollment maternal characteristics.

Variable	Total Sample	Control Group	Experimental Group	P value
25[OH]D Serum Level (ng/mL)	31.87 ± 3.82	32.39 ± 3.48	31.43 ± 4.31	0.664
Dietary Vitamin D (IU/day)	529 ± 186	587 ± 234	479 ± 131	0.348
Age (years)	29 ± 5.6	27 ± 5.5	30 ± 6	0.231
Parity	1.7 ± 1.4	1 ± .4	2 ± 1.8	0.202
Gestation at Enrollment (weeks)	28 ± 0.9	29 ± 1.22	28 ± 0.70	0.292
Body Mass Index* at Enrollment	30.5 ± 7.3	28.65 ± 7.6	32.1 ± 7.2	0.426

Descriptive statistics include mean ± standard deviation.
*Body mass index is defined as body mass in kilograms divided by height in meters squared.
Significant differences between experimental and control groups were determined at $p \leq 0.05$.

mean BMI of all participants was 30.5 (± 7.3). Overall, participants had a mean 25[OH]D level of 31.87 (± 3.82) ng/mL and average daily dietary intake of vitamin D of 529 (± 186) IU. At enrollment, 2 of the 6 control group participants had 25[OH]D levels ≥ 32 ng/mL, defined as sufficient. Three of the 7 participants in the experimental group at enrollment had 25[OH]D levels ≥ 32 ng/mL.

Maternal and infant characteristics at delivery were analyzed (Table 6) to

Table 6. Maternal and infant characteristics at delivery.

Variable	Total	Control Group	Experimental Group	P Value
Gestation at Delivery (weeks)	39.2 ± 0.76	39.5 ± 0.66	39 ± 0.8	0.221
Maternal Body Mass Index* at Delivery	32.37 ± 7.3	30.68 ± 7.12	33.81 ± 7.7	0.465
Infant Birth Weight (grams)	3588 ± 471	3643 ± 507	3541 ± 473	0.716
Infant Birth Length (inches)	20.8 ± 0.89	20.8 ± 1.2	20.75 ± 0.7	0.875
Infant Head Circumference (centimeters)	34.75 ± 1.6	34.7 ± 1.6	34.8 ± 1.7	0.875

Descriptive statistics include mean ± standard deviation.*Body mass index is defined as body mass in kilograms divided by height in meters squared. Significant differences between experimental and control groups were determined at $p \leq 0.05$.

determine differences between groups. Mean maternal delivery BMI was 32.37 (± 7.3) and mean gestation at delivery was 39.2 (0.76) weeks. Infant characteristics showed a mean birth weight of 3588 (± 471) grams, birth length of 20.8 (± 0.89) inches, and head circumference of 34.75 (± 1.6) centimeters. There were no statistical differences between the experimental and control groups on any of these outcomes.

Impact of Vitamin D Supplementation on Maternal 25[OH]D at Delivery

Hypothesis 1: *Women who receive supplemental vitamin D starting in the early third trimester of pregnancy will have significantly higher 25[OH]D serum levels compared to control participants by delivery.*

In order to test this hypothesis, a t-test of independent variables was used comparing experimental and control group means for 25[OH]D levels (Figure 6).

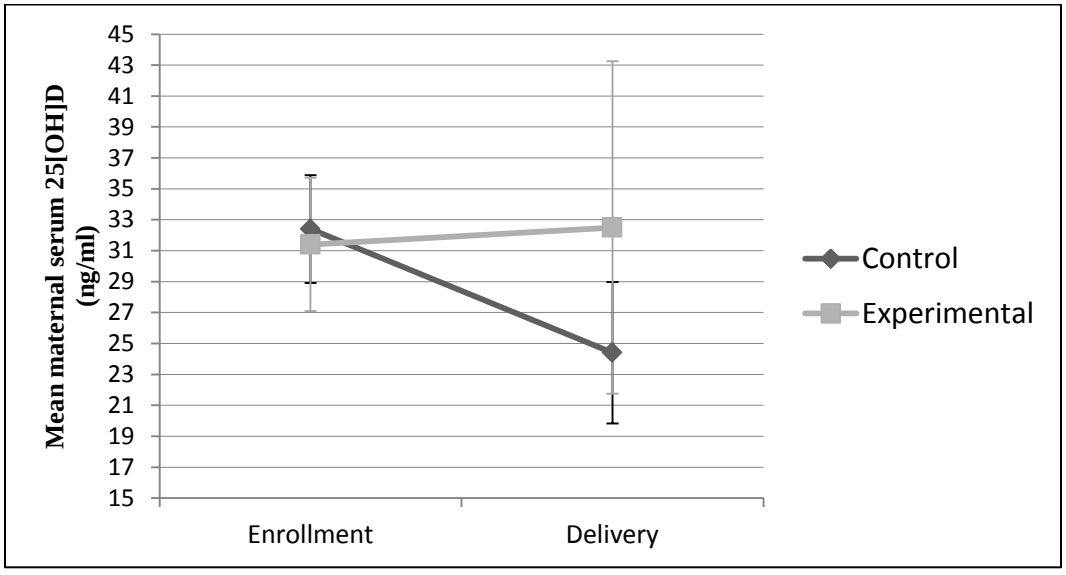


Figure 6. Influence of maternal supplementation on maternal 25[OH]D levels at delivery. Descriptive statistics include mean ± standard deviation.
Significant differences between experimental and control groups were determined at $p \leq 0.05$.

Results indicate differences between groups based on group means (±SD). T-test analysis demonstrates no statistically significant difference in maternal serum 25[OH]D levels between the experimental and control groups (32.48 ± 10.75 vs. 24.44 ± 4.57 ng/mL, p=0.108). The difference in group means was 8.04 ng/mL, which may have clinical implications, but was not a statistically significant finding.

However, further analysis was necessary because maternal mean 25[OH]D for a given group is dependent on the enrollment 25[OH]D values for the participants in that group (Table 7). In order to separate out the influence of the intervention from the influence of enrollment 25[OH]D level, a univariate Analysis of Covariance (ANCOVA) test was used. The ANCOVA allowed for comparison of means while controlling for the influence of a covariate, in this case enrollment 25[OH]D level. This analysis also produced estimates of effect size (partial eta squared) and power (observed power).

Table 7. Observed and estimated means for maternal 25[OH]D at delivery.

Variable	Control Group	Experimental Group	P Value	Estimate of Effect Size	Observed Power
Observed Mean (ng/mL)	24.44 ± 4.57	32.48 ± 10.75	0.108	N/A	N/A
Estimated Mean (ng/mL)	23.78 ± 2.94	33.05 ± 2.72	0.044	0.346	0.548

Observed mean (± SD) and estimated mean (± SEM) for maternal 25[OH]D at delivery. Significant differences between experimental and control groups were determined at $p \leq 0.05$.

Additionally, ANCOVA analysis produced estimated marginal means, also called corrected means, which are the means that would have been achieved in the two groups had the covariate (enrollment 25[OH]D level) not had an influence. Using this technique with the maternal data at delivery estimated means of 33.05 ± 2.72 and 23.78 ± 2.94 ng/mL were generated in the experimental and control groups, respectively, which were statistically significant (p= 0.044).

The 2 participants who had had sufficient 25[OH]D levels at enrollment in the control group dropped below 32 ng/mL at delivery. One of the 6 maternal participants in the control group increased from 31 ng/mL to 32 ng/mL, thus putting her in the sufficient category. Three of the 7 participants in the experimental group had sufficient 25[OH]D status at delivery; two were previously sufficient and increased their 25[OH]D levels, and one was newly sufficient at delivery. This demonstrates a 157% benefit increase of the intervention on achieving sufficiency in maternal participants by delivery.

Impact of Vitamin D Supplementation on Maternal 25[OH]D at Lactation

Hypothesis 2: Women who receive supplemental vitamin D starting in the early third trimester of pregnancy and continue with this dosing during the early postpartum will maintain significantly higher serum 25[OH]D levels than control participants during lactation.

Three of the 6 participants in the control group failed to attend the final visit for data collection. In order to manage missing data, the last observation carried forward method was used. Data collected on the maternal and infant participants at delivery were

carried forward to the lactation data point for the 3 maternal and infant participants with missing data. In order to test the hypothesis, an independent samples t-test was used comparing the experimental and control group means for maternal lactation 25[OH]D. There was a significant difference in means between the experimental group and the control group at lactation (35.57 ± 8.87 vs. 22.38 ± 2.82 ng/mL, p=0.007) (Figure 7).

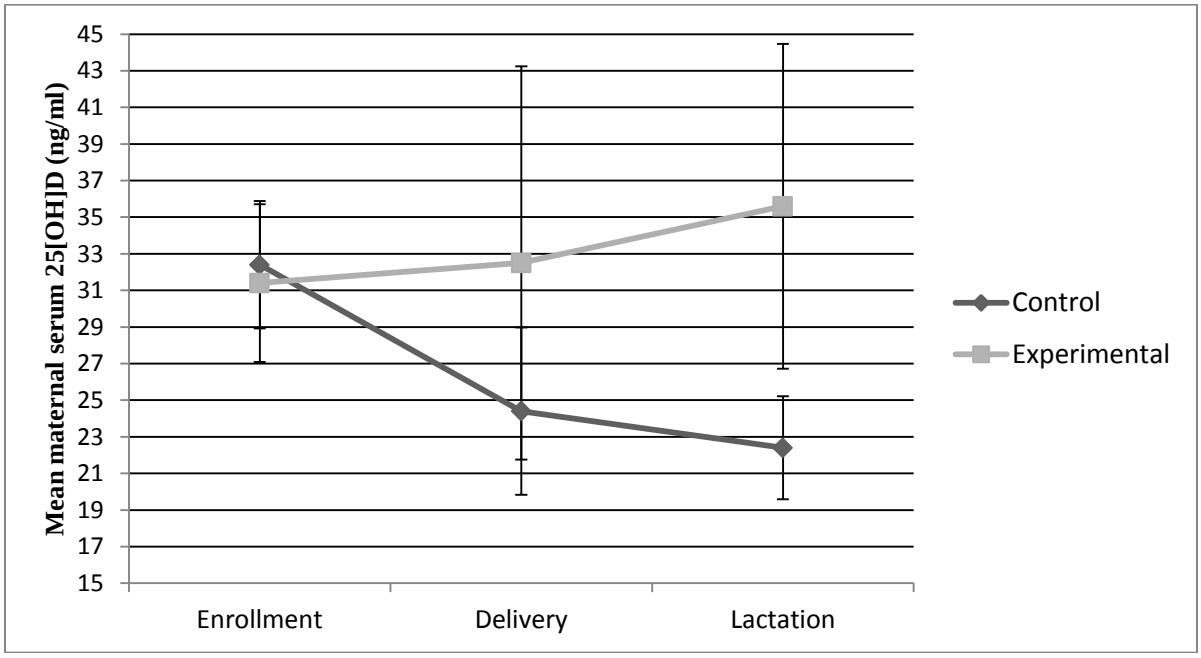


Figure 7. Influence of maternal supplementation on maternal 25[OH]D level at enrollment, delivery, and lactation.
Descriptive statistics include mean ± standard deviation. Significant differences between experimental and control groups were determined at $p \leq 0.05$. Mean maternal 25[OH]D levels increased in the experimental group and declined in the control group at lactation (p=0.007).

Using the univariate ANCOVA method outlined previously, the data for mean maternal 25[OH]D at lactation was analyzed. This analysis produced estimated marginal means, which describe what the means for the two groups would have been at lactation had enrollment 25[OH]D level not had an influence. This produced a statistically

significant result (p= 0.002). Additionally, estimated marginal means, estimates of effect size, and observed power demonstrated a large effect size and strong power (Table 8). There was a difference in maternal observed means of 13.19 ng/mL 25[OH]D at lactation, which may also have clinical implications.

Table 8. Observed and estimated means for maternal 25[OH]D at lactation.

Variable	Control Group	Experimental Group	P Value	Estimate of Effect Size	Observed Power
Observed Mean (ng/mL)	22.38 ± 2.82	35.57 ± 8.87	0.007	N/A	N/A
Estimated Mean (ng/mL)	21.9 ± 2.48	35.98 ± 2.3	0.002	0.632	0.961

Observed mean (± SD) and estimated mean (± SEM) for maternal 25[OH]D at lactation. Significant differences between experimental and control groups were determined at $p \leq 0.05$.

None of the 6 control group maternal participants had sufficient 25[OH]D status at lactation, whereas 4 of the 7 experimental group participants achieved sufficiency, demonstrating a 688% benefit increase of the intervention on achieving sufficiency. Additionally, mean maternal 25[OH]D level at lactation was highly correlated with mean maternal 25[OH]D level at delivery ($p < .0001$), but not at enrollment ($p = 0.370$) demonstrating the impact of maternal vitamin D supplementation during pregnancy.

Considering that maternal 25[OH]D serum level at both delivery and lactation is a function of the participant's enrollment 25[OH]D serum level, further analysis was

undertaken to separate the impact of the intervention from the impact of maternal enrollment 25[OH]D over the course of the entire intervention (Figure 8). In order to achieve this, a repeated measures ANCOVA was used to determine the combined impact of vitamin D supplementation on delivery and lactation 25[OH]D levels, while adjusting for enrollment maternal 25[OH]D level. By using this method, it is possible to assess the impact of the intervention over time and to estimate the means of the groups without the impact of enrollment input. The results showed that there were no within subjects effects (F= 1.032; P = 0.334), indicating no difference in the outcome over time. The between groups analysis produced estimated marginal means, which indicate what the means of the groups would have been had the covariate of enrollment not had an impact. The estimated marginal mean (± SEM) for the experimental group was 34.52 (± 2.35 ng/mL)

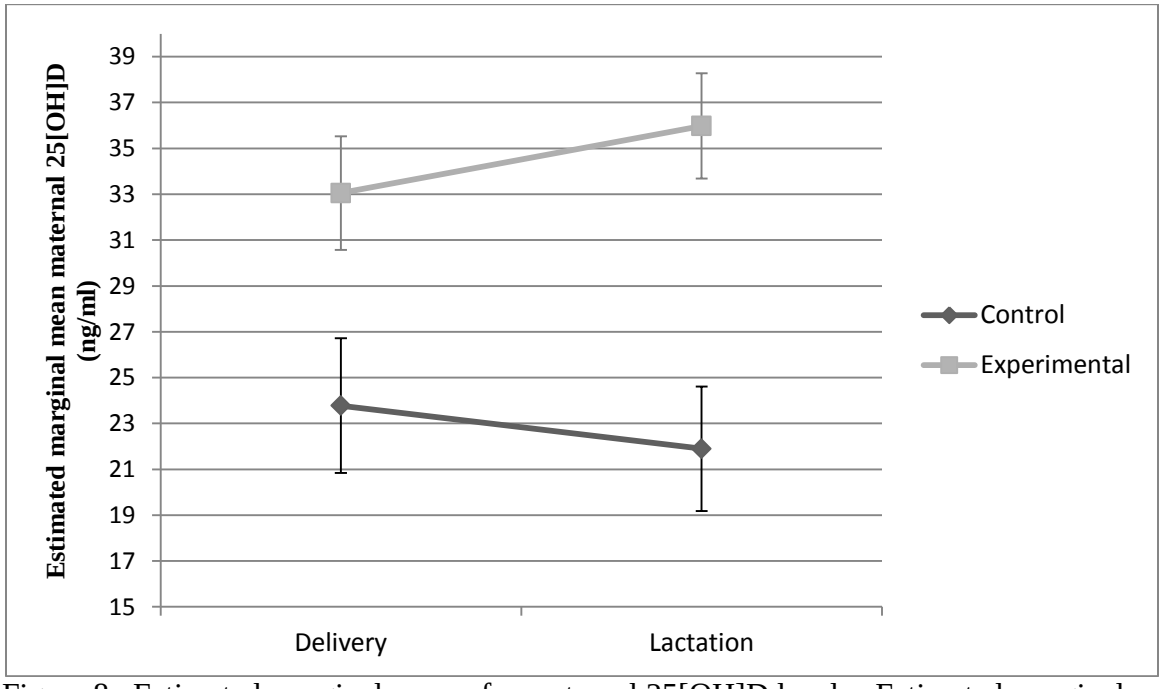


Figure 8. Estimated marginal means for maternal 25[OH]D levels. Estimated marginal means for maternal 25[OH]D serum levels at delivery and lactation for the control and experimental groups were significantly different (p=0.007). Significant differences between experimental and control groups were determined at $p \leq 0.05$.

and for the control group was 22.84 (± 2.54 ng/mL). This was statistically significant (p = 0.007), with estimated power of 0.857 and estimated effect size of 0.531. The difference between estimated means was 11.68 ng/mL, which is a greater than 50% increase between control and experimental means.

Impact of Maternal Vitamin D Supplementation on Infant 25[OH]D at Delivery

Hypothesis 3: Infants born to women who receive supplemental vitamin D starting in the early third trimester of pregnancy will have significantly higher 25[OH]D levels at birth compared to control infants.

A t-test of independent variables was used comparing the experimental and control group means for infant serum 25[OH]D at delivery. There was a significant difference in mean (± SD) serum 25[OH]D levels between infants in the experimental group and the control group at delivery (32.33 ± 6.15 vs. 23.67 ± 5.50 ng/mL, p=0.021). The difference in means was 8.66 ng/mL, which may have clinical implications beyond its statistical significance. Additionally, infant mean serum 25[OH]D level was highly correlated with maternal serum 25[OH]D levels at delivery (p = .001). In order to separate the impact of maternal enrollment 25[OH]D levels from the impact of the intervention, a univariate ANCOVA was used as described above. Estimated marginal means for infant 25[OH]D at delivery were significantly different (32.57 ± 2.17 vs. 23.38 ± 2.35 in the experimental and control groups respectively, p= 0.017) with a medium-large effect size (0.450) and fairly strong power (0.732) (Table 9).

One of the 6 control group infants reached defined level of sufficiency and 5 of the 7 infants in the experimental group achieved sufficient levels at delivery. This equates to a 328% benefit increase to the infants in the experimental group.

Table 9. Observed and estimated means for infant 25[OH]D at delivery.

Variable	Control Group	Experimental Group	P Value	Estimate of Effect Size	Observed Power
Observed Mean (ng/mL)	23.67 ± 5.50	32.33 ± 6.15	0.021	N/A	N/A
Estimated Mean (ng/mL)	23.38 ± 2.35	32.57 ± 2.17	0.017	0.450	0.732

Observed mean (± SD) and estimated mean (± SEM) for infant 25[OH]D at delivery. Significant differences between experimental and control groups were determined at $p \leq 0.05$.

Impact of Maternal Vitamin D Supplementation on Infant 25[OH]D at Lactation

Hypothesis 4: Infants who exclusively breastfeed from a mother receiving supplemental vitamin D will have significantly higher 25[OH]D levels than infants exclusively breastfeeding in the control group at 4-6 weeks postpartum.

In order to test this hypothesis, a t-test of independent variables was used comparing experimental and control group infant data. The experimental group infants had a mean (± SD) 25[OH]D level of 24.9 (±12.81) ng/mL and the control group infants 16.98 (±8.71) ng/mL ($p = 0.216$). There was a difference in means of 7.92 ng/mL, which may have clinical implications despite the lack of statistical significance (Figure 9).

Figure 9. Influence of maternal supplementation on infant 25[OH]D levels at delivery and lactation. Mean infant 25[OH]D levels in the experimental and control groups demonstrating a decrease in both groups with the experimental group maintaining a mean 25[OH]D level approximately 8 ng/mL greater than the control group infants. Significant differences between experimental and control groups were determined at $p \leq 0.05$.

Considering that infant 25[OH]D serum level at lactation is a function of the maternal enrollment 25[OH]D serum level, further analysis was undertaken to separate the impact of the intervention from the impact of maternal enrollment. A univariate ANCOVA revealed no significant difference in means ($p= 0.256$) with a small effect size (0.127) and very low power (0.193). Table 10 displays these findings along with the estimated marginal means for infant 25[OH]D at lactation.

In order to evaluate the impact of the intervention on infant 25[OH]D over the course of the entire intervention, a repeated measures ANCOVA was used to analyze differences in mean 25[OH]D levels between the control and experimental group infants

Table 10. Observed and estimated means for infant 25[OH]D at lactation.

Variable	Control Group	Experimental Group	P Value	Estimate of Effect Size	Observed Power
Observed Mean (ng/mL)	16.98 ±8.71	24.9 ± 12.81	0.216	N/A	N/A
Estimated Mean (ng/mL)	16.99 ± 4.79	24.89 ± 4.43	0.256	0.127	0.193

Observed mean (± SD) and estimated mean (± SEM) for infant 25[OH]D at lactation. Significant differences between experimental and control groups were determined at $p \leq 0.05$.

at delivery and lactation, while adjusting for enrollment maternal serum 25[OH]D level. By using this method, it is possible to assess the impact of the intervention over time while controlling for the impact of maternal enrollment input. The results showed that there were no within subjects effects (F= 0.166; P = 0.693), indicating no difference in the outcome over time. The between subjects effect indicated that infants in the experimental group achieved an estimated marginal mean of 28.73 ± 2.78 ng/mL and the control infants 20.19 ± 3.01 ng/mL (p= 0.065). The estimated power was 0.466 with an estimated effect size of 0.301. The overall impact of the intervention for infants at delivery and lactation approached significance. It is of greater clinical significance that there was a difference between estimated means of 8.54 ng/mL, with a medium effect size of 0.3 although adequacy of power was lost.

There was no statistically significant correlation between maternal and infant 25[OH]D level at lactation (p=0.163). None of the 6 control group infants were sufficient

and 2 of the 7 experimental group infants were sufficient at lactation, consistent with a 337% benefit increase to those infants breastfeeding from a mother receiving the intervention.

Impact of Vitamin D Supplementation on Maternal Cytokine Production

Secondary Hypothesis 1: Women with higher 25[OH]D levels will have decreased levels of pro-inflammatory cytokines.

Maternal means for pro-inflammatory cytokines TNF-α and IL-6 were compared in maternal plasma between the experimental and control groups using t-tests of independent variables. There were no significant differences between the experimental and control groups for these pro-inflammatory markers at enrollment, delivery, or lactation (Table 11). The mean minimum detectable dose for the IL-6 assay used was 0.039 pg/mL. All participants had values well above this mean minimum detectable dose. The mean minimum detectable dose for the TNF-α assay used was 1.6 pg/mL. Ten samples fell below this minimum detectable dose amount. All TNF-α and IL-6 samples were analyzed in triplicate.

In order to evaluate the impact of maternal vitamin D supplementation independently from the impact of maternal pro-inflammatory cytokine levels at enrollment, TNF-α and IL-6 were analyzed using the ANCOVA method adjusting for enrollment. When maternal pro-inflammatory cytokine TNF-α was analyzed using ANCOVA, adjusting for enrollment TNF-α level, there was no significant difference between the experimental and control groups in TNF-α at delivery or lactation.

Table 11. Maternal pro-inflammatory plasma cytokine levels.

Variable		Enrollment	Delivery	Lactation
TNF-α (pg/mL)	Control	4.45 ± 6.88	12.63 ± 12.11	6.35 ± 8.28
	Experimental	3.01 ± 3.10	12.36 ± 13.58	8.69 ± 8.78
IL-6 (pg/mL)	Control	6.99 ± 3.94	5.27 ± 1.81	11.10 ± 6.45
	Experimental	5.47 ± 1.85	4.08 ± 3.39	14.57 ± 6.60

Mean (± SD) maternal plasma TNF-α and IL-6 at enrollment, delivery, and lactation for the control and experimental groups were not significantly different.
Significant differences between experimental and control groups were determined at $p \leq 0.05$.

When maternal pro-inflammatory cytokine IL-6 was analyzed using ANCOVA, adjusting for enrollment IL-6 level, there was no significant difference between the experimental and control of IL-6 at delivery or lactation. This demonstrates that maternal vitamin D supplementation did not impact the production of these circulating pro-inflammatory cytokines.

Secondary Hypothesis 2: Women with higher 25[OH]D levels will have increased levels of an anti-inflammatory cytokine.

In order to test this hypothesis, t-tests of independent samples were used to compare the group means of the anti-inflammatory cytokine IL-10 in the experimental and control groups at enrollment, delivery, and lactation (Table 12). There were no statistical differences in IL-10 between groups, suggesting that maternal vitamin D supplementation did not influence circulating maternal plasma IL-10 levels. The mean detectable dose for the assay used was 0.09 pg/mL. Ten of the samples fell below this minimum detectable amount and an additional 4 samples had undetectable IL-10 levels.

Additionally, 24 samples were run in duplicate and 13 in single due to lack of funding to purchase enough assay trays for analysis in triplicate.

Table 12. Maternal anti-inflammatory plasma cytokine levels.

Variable		Enrollment	Delivery	Lactation
IL-10 (pg/mL)	Control	0.12 ± 0.12	0.33 ± 0.34	0.10 ± 0.15
	Experimental	0.13 ± 0.07	0.12 ± 0.08	0.11 ± 0.06

Mean (± SD) maternal plasma IL-10 levels in the experimental and control groups at enrollment, delivery, and lactation were not significantly different between groups. Significant differences between experimental and control groups were determined at $p \leq 0.05$

Because IL-10 levels at delivery and lactation are impacted by maternal enrollment circulating IL-10, the impact of maternal vitamin D supplementation on differences in IL-10 were analyzed using the univariate ANCOVA method adjusting for enrollment IL-10 plasma level. The impact of vitamin D supplementation on plasma IL-10 demonstrated a fairly robust effect size of 0.290 with a power of 0.446 and $p = 0.071$ (Table 13).

Table 13. Maternal vitamin D supplementation influence on maternal IL-10 plasma levels.

Group	Estimated Marginal Mean (pg/mL)	Effect Size	Power	P Value
Control Group	0.28 ± 0.06	0.290	0.446	0.071
Experimental Group	0.11 ± 0.06			

Estimated marginal means (± SEM) for maternal plasma IL-10 in the experimental and control groups adjusted for enrollment IL-10 plasma level. Significant differences between experimental and control groups were determined at $p \leq 0.05$.

The difference between group means demonstrates an effect that opposes the hypothesis, with the control group having a higher mean IL-10 value than the experimental group, therefore this hypothesis was rejected.

Summary of Key Findings

In summary, study results demonstrate that random sampling was achieved and the participants in the experimental and control groups did not differ in any enrollment measures. Analysis reveals a statistically significant impact of prenatal and postnatal vitamin D supplementation on mean maternal 25[OH]D level by delivery and continued through lactation when the impact of enrollment 25[OH]D is excluded. The intervention showed strong effect size and power. Analysis of infant data reveals statistically significant impact of the intervention on mean 25[OH]D at delivery, but by lactation this impact is lost and there is only a minimal correlation between maternal and infant 25[OH]D levels by 4-6 weeks postpartum. Adjusting for maternal enrollment 25[OH]D level brought the difference in mean infant 25[OH]D levels at delivery and lactation toward significance with a moderate effect size and low power. Minimal correlations were found between maternal 25[OH]D levels and inflammatory markers. There was no impact of vitamin D supplementation on pro-inflammatory (TNF-α and IL-6) or anti-inflammatory (IL-10) cytokine production. An in-depth discussion of the meaning and impact of these results can be found in Chapter 5.

CHAPTER V

DISCUSSION

The purpose of this study was to add novel information to improve understanding of the transfer of vitamin D from mother to infant during breastfeeding. Specifically, it was novel to start vitamin D supplementation in participants during pregnancy and continue that supplementation through 4-6 weeks of lactation, which had not been previously done. This study also added new information regarding the impact of vitamin D supplementation on maternal cytokine production during pregnancy, birth and lactation. This chapter presents an overview of the study, an overview of the methodology utilized, and an evaluation of the findings presented in Chapter 4 with respect to the current literature. This discussion is framed by the DOHaD hypothesis, which underpins the significance of the findings. This chapter also provides a discussion of the limitations of the study, recommendations for future research, and implications for nursing research, practice, education, and policy.

Background of the Study

Vitamin D deficiency among pregnant and lactating women is common and increasing in both prevalence and incidence (Bendall, de Costa, Woods, & Howat, 2012; Brannon, 2012; Collins-Fulea et al., 2012; Dror, King, Durand, & Allen, 2011b; Hollis et

al., 2011; Merewood et al., 2010; Wagner, Taylor, Johnson, & Hollis, 2012; Wagner, McNeil et al., 2013b). There is a growing body of evidence that suggests that vitamin D deficiency during pregnancy is associated with increased risk of pregnancy complications including gestational diabetes (Baker et al., 2012; Clifton-Bligh et al., 2008; Maghbooli et al., 2008; Senti et al., 2012; Soheilykhah et al., 2010) and preeclampsia (Baker et al., 2010; Haugen et al., 2009; Robinson et al., 2010). *In utero* exposure to deficient maternal nutriture is hypothesized to program the fetus for later development of childhood and adult disease (D. J. Barker, 1997; D. J. Barker, 2000; D. J. Barker, Eriksson, Forsen, & Osmond, 2002; Young, 2001). Several researchers postulate that *in utero* vitamin D deficiency may be a key aspect to programming of later disease (Kaludjerovic & Vieth, 2010; J. McGrath, 2001; Thandrayen & Pettifor, 2012; Weiss & Litonjua, 2011). Specifically, exposure to vitamin D deficiency during fetal development seems to increase risk for asthma, wheeze, respiratory infections, eczema, type 1 diabetes, and general poor innate immune response (Camargo et al., 2011; Cooper et al., 2011; Erkkola et al., 2011; Karatekin, Kaya, Salihoglu, Balci, & Nuhoglu, 2009; Madden et al., 2012; Marjamaki et al., 2010; Miyake et al., 2010; Walker et al., 2011).

Following birth, the vitamin D deficient infant is at risk for continued vitamin D deficiency when exclusively breastfed by a vitamin D deficient mother (Hollis & Wagner, 2004a; Saadi et al., 2009; Taylor, Wagner, & Hollis, 2006; Thiele et al., 2013; Wagner et al., 2008). Breast milk is perfectly suited to meet the nutritional needs of the growing infant, however, when the mother is vitamin D deficient her milk will also be vitamin D deficient (Basile et al., 2006; Hollis & Wagner, 2004b; Saadi et al., 2009; Thiele et al., 2013; Wagner et al., 2006). Programming of later disease does not end at

birth as the infant continues to demonstrate plasticity in developing mechanisms to adjust to its environment. As the first and only food the exclusively breastfed infant receives, breast milk serves to impact programming, potentially through epigenetic changes. Due to these multiple health impacts, maternal health and nutrition should be maximized to benefit both mother and infant. In two recent studies, researchers looked at the epigenetic impact of vitamin D (Pereira et al., 2012; Zhu et al., 2013). The authors reported that vitamin D has effects across the genome by regulating demethylation (Pereira et al., 2012). Additionally, severely vitamin D deficient African American adolescents experience differences in methylation in their leukocyte DNA, providing a potential explanation for the impact of vitamin D on the immune system (Zhu et al., 2013). As further epigenetic research is completed, it seems likely that more evidence will emerge about the impact of vitamin D on the epigenome and the resulting changes in gene expression.

Breast milk vitamin D deficiency is associated with hypocalcemic seizures, particularly amongst dark skinned infants living at higher latitudes, as well as rickets (Balasubramanian et al., 2006; Bodnar et al., 2007; Camadoo et al., 2007; Dawodu & Wagner, 2007; Greer, 2008; Salama & El-Sakka, 2010; Specker et al., 1985; Teaema & Al Ansari, 2010). Except for exploring the impact of early vitamin D exposure on multiple sclerosis and schizophrenia (Fernandes de Abreu, Landel, & Feron, 2011; Hanwell & Banwell, 2011; J. J. McGrath et al., 2010), the relationship between vitamin D deficiency during infancy and adult onset diseases such as cardiovascular, autoimmune, and endocrine diseases is wholly unevaluated at this point (Lucas, Ponsonby, Pasco, & Morley, 2008). In light of the impact on pregnancy related disease and early childhood

disease, it is critical that researchers determine the most efficient way to bring a majority of women and their breastfed infants up to a sufficient vitamin D serum level and then evaluate the impact on disease outcomes.

Purpose Statement and Research Hypotheses

Current research has focused on determining appropriate timing and dosage of maternal and infant vitamin D supplementation to maximize the potential for achieving sufficient serum vitamin D levels (Hollis & Wagner, 2004a; Hollis, 2008; Hollis & Wagner, 2011; Hollis et al., 2011; Wagner et al., 2010; Wagner & Hollis, 2011; Wagner et al., 2012; Wagner et al., 2013b). However, despite this focus, there are currently no published studies that evaluate the impact of maternal vitamin D supplementation initiated during pregnancy and continued through lactation on the vitamin D status of the breastfed infant. There is a lack of consensus and understanding of the maternal 25[OH]D level that corresponds to maternal vitamin D sufficiency or fetal vitamin D sufficiency. This lack of consensus and understanding continues in to lactation with divergent recommendations for maternal 25[OH]D level that will correspond with sufficiency in the mother and the exclusively breastfed infant. Both the pregnancy and lactation determinations are further compounded by the lack of understanding of what amount of vitamin D supplementation will elicit a given serum 25[OH]D level. It is widely recognized that there is a high prevalence of maternal vitamin D deficiency (defined either as a 25[OH]D level < 32ng/mL or < 20 ng/mL) due to factors of skin color, norms of dress, and lifestyles that include little time in the sun. Yet, researchers and clinicians are not yet able to recommend a specific vitamin D supplementation regimen or a specific 25[OH]D serum level to maximize health. It will take further

research, looking at disease outcomes and biomarkers of sufficiency in pregnant women, their infants, and their infants in to adulthood, to fully elucidate the impact of vitamin D supplementation and doses necessary to prevent disease.

The objective of this study was to identify the combined effect of maternal prenatal and postnatal vitamin D supplementation on vitamin D transfer to infants through breast milk, leading to adequate vitamin D status in infants. In order to meet this objective the following hypotheses were tested:

Central hypothesis: Maternal supplementation with vitamin D during pregnancy and lactation will significantly increase circulating vitamin D levels during lactation in mothers and their exclusively breastfed infants:

1. Women who receive supplemental vitamin D starting in the early third trimester of pregnancy will have significantly higher 25[OH]D serum levels compared to control participants by delivery;
2. Women who receive supplemental vitamin D starting in the early third trimester of pregnancy and continue with this dosing during the early postpartum will maintain significantly higher serum 25[OH]D levels than control participants during lactation;
3. Infants born to women who receive supplemental vitamin D starting in the early third trimester of pregnancy will have significantly higher 25[OH]D levels at birth compared to control infants;
4. Infants who exclusively breastfeed from a mother receiving supplemental vitamin D will have significantly higher 25[OH]D levels than infants exclusively breastfeeding in the control group at 4-6 weeks of age.

Due to immune and inflammatory modulating effects of vitamin D, the following secondary hypotheses were tested:

1. Women with higher vitamin D levels will have decreased levels of pro-inflammatory cytokines (TNF-α and IL-6);
2. Women with higher vitamin D levels will have increased levels of an anti-inflammatory cytokine (IL-10).

Review of the Methodology

To generate novel information about the impact of continuous prenatal to postpartum maternal vitamin D supplementation on the vitamin D status of breastfeeding infants, a randomized controlled trial design was used. This design allows for creation of two otherwise equal groups to receive two different interventions. The control group received a prenatal vitamin containing the typical 400 IU of vitamin D plus a placebo capsule, while the experimental group received the same prenatal vitamin plus a capsule containing 3400 IU of additional vitamin D, for a total of 3800 IU daily. Participants were recruited between 24-28 weeks gestation and provided with written and verbal informed consent if they desired to participate. They were provided with study pills in 30 day increments, to be taken daily through lactation. Each participant was contacted by the PI every 2 weeks for evaluation of compliance with study protocol and to arrange for the next meeting, which occurred approximately every 30 days. Data were gathered at enrollment, at delivery, and again at lactation, including maternal health characteristics, dietary intake, and infant supplementation with formula or vitamin D. Blood samples were collected from the maternal participants at enrollment, delivery, and lactation to assess 25[OH]D status and cytokine levels, as well as from the infant participants at

delivery and lactation to assess 25[OH]D status. Data were entered into SPSS Version 21 and analyzed for normality. Analysis included descriptive statistics including mean and variance measures (standard error of the mean/standard deviation), t-tests for differences between groups, and ANCOVA to determine impact of the intervention on continuous data while adjusting for enrollment values. Data were analyzed to determine relative benefit increase of achieving sufficient 25[OH]D levels for maternal and infant participants. A p value of ≤ 0.05 was set for determination of significance.

Evaluation of Study Findings

Overview of Current Research

This study is the first to use a randomized controlled trial method to investigate the impact of maternal vitamin D supplementation on the vitamin D status of maternal participants and their breastfed infants. There are several studies that used a longitudinal observation method to determine the typical course of vitamin D status for pregnant and lactating women and their infants (Bendall et al., 2012; Collins-Fulea et al., 2012; Dror et al., 2011b; Merewood et al., 2010; Merewood et al., 2012). There are a few additional studies that used a randomized controlled trial method to observe the impact of vitamin D supplementation on pregnant women (Hollis et al., 2011; Wagner et al., 2013b). There are 3 randomized controlled trials of lactating women using higher dose vitamin D supplementation to impact the 25[OH]D status of participants and their infants (Hollis & Wagner, 2004b; Saadi et al., 2009; Wagner et al., 2006). However, this is the first study that spans pregnancy and lactation with an intervention aimed at impacting maternal and infant 25[OH]D status. The findings from this approach contribute new knowledge regarding the timing and dosage of maternal vitamin D supplementation needed during

pregnancy to result in maternal and infant vitamin D sufficiency. Additionally, this study is the first to investigate the impact of vitamin D supplementation on maternal production of pro- and anti-inflammatory cytokines *in vivo*. There is a growing body of evidence that 25[OH]D level impacts immune response (Akbar & Zacharek, 2011; Di Rosa et al., 2012; Thota et al., 2013; Tiosano et al., 2013). There has been investigation in to the normal patterns of cytokine production during pregnancy, labor, and the postpartum (Denney et al., 2011; Palm et al., 2013). Other studies have used *in vitro* methods to elicit cell responses to bacterial endotoxin, using this as a model of chorioamnionitis or uterine infection during pregnancy (Thota et al., 2013). This is the first study to investigate differences in cytokine production *in vivo* between control and vitamin D supplemented groups of pregnant women. The findings offer additional information regarding the ability of vitamin D to mediate the inflammatory response in the pregnant woman.

Influence of Maternal Vitamin D Supplementation During Pregnancy

Of the 20 women screened for participation, 2 did not meet inclusion criteria, and 2 chose not to participate. The resulting 16 participants were enrolled into the study during the summer months of 2012. Random assignment was predetermined and after receiving written and verbal informed consent, the participants were randomized to either the control or experimental group. After randomization, one participant from each group chose to leave the study, and an additional participant in the control group was found to not meet exclusion criteria and was exited from the study (control N= 6, experimental N= 7). All participants self-identified as white ethnicity. The two groups were compared to assess for homogeneity and appropriate random sampling. There was no difference

between groups in terms of age, body mass index, gestation, or parity at enrollment. Blood samples from participants at enrollment were batch analyzed for 25[OH]D level. There was no difference in baseline values between the two groups. Of the 13 participants 5 (38%) had sufficient 25[OH]D values (> 32 ng/mL) at enrollment. This finding of low prevalence of sufficient vitamin D status is of particular interest for two reasons: all participants were of white ethnicity and all participants were recruited toward the end of the summer season. Although this study took place at a northern latitude, having light skin pigmentation allows for the greater production of vitamin D in a given amount of time exposed to the sun compared to having darker pigmentation. Additionally, the summer months are accompanied by access to sunshine and typical outdoor activities. Therefore, these 25[OH]D levels should reflect the participants' peak 25[OH]D level for the year. The low prevalence of vitamin D sufficiency in the sample indicates that a majority of white women in this region remain vitamin D deficient even when endogenous vitamin D production is possible. It can be inferred that women with darker skin pigmentation would have even lower rates of sufficiency, even during the summer months. The finding of 38% vitamin D sufficiency in the early third trimester correlates well with other studies. Bodnar et al. (2007) found 53% sufficiency amongst pregnant white women in the northeast, Collins-Fulea, Kilma, and Wegienka (2012) found 21% of white pregnant women to be sufficient, Dror et al. (2011) found a sufficiency rate of 46% in women at delivery, Merewood et al. (2010) found a sufficiency rate of 62%, and Li et al. (2011) found a rate of sufficiency of 35% amongst pregnant women in Canada (Bodnar et al., 2007; Collins-Fulea et al., 2012; Dror et al., 2011b; W. Li et al., 2011; Merewood et al., 2010). Rates of maternal 25[OH]D sufficiency in

pregnancy are much lower for African American, Arab American, and women who cover with a hijab or other cultural norms of dress that cover most of the skin (Bodnar et al., 2007; Collins-Fulea et al., 2012; Dawodu et al., 2013; Dror et al., 2011b).

Food frequency questionnaires were analyzed revealing a mean vitamin D dietary intake of 529 IU/day with no difference between groups. All participants self-reported 100% daily prenatal vitamin ingestion (usually containing 400 IU vitamin D), suggesting a total average dietary plus supplement intake of about 1000 IU. Interestingly, maternal dietary vitamin D intake did not correspond to maternal 25[OH]D level. The mean dietary intake in this study is higher than, but consistent with, findings of other studies (Dror et al., 2011b; Merewood et al., 2010), except for the recent large randomized controlled trial by Hollis et al. (2011). Hollis et al. (2011) found mean maternal dietary vitamin D intakes across pregnancy of less than 200 IU daily. Dror et al. (2011) and Merewood et al. (2010) found a strong correlation between maternal dietary vitamin D intake and maternal and neonatal 25[OH]D level at birth. It may be that the findings of this study are different because this study included an intervention whereas Dror et al. (2011) and Merewood et al. (2010) are solely observational. Of additional interest, if the participants in this study were ingesting an average of 1000 IU daily this was still inadequate to produce a sufficient 25[OH]D level in a majority of the participants. This signals that perhaps the foods fortified with vitamin D actually contain less vitamin D than stated or that participants overestimated the amount of certain foods they were ingesting. In fact, the participant with the highest reported dietary vitamin D intake (> 2 standard deviations above the mean) had the lowest 25[OH]D level by lactation as did her infant.

Analysis of the impact of the intervention on maternal 25[OH]D level at delivery reveals a significant difference between groups when controlling for enrollment 25[OH]D level. At delivery, the mean maternal 25[OH]D level in the experimental group was 32.48 ng/mL compared to the control group mean of 24.44 ng/mL. One of the 6 participants in the control group had sufficient 25[OH]D status and 3 of the 7 participants in the experimental group had sufficient 25[OH]D status, demonstrating a 157% benefit increase of the intervention on achieving sufficiency. In regards to the impact of the intervention by delivery, findings correspond well with the two other contemporary intervention studies completed during pregnancy (Hollis et al., 2011; Wagner et al., 2013a). Hollis et al. (2011) had a similar intervention group (initiating the intervention at 12-16 weeks gestation) receiving 4000 IU of supplemental vitamin D daily and found at delivery a mean 25[OH]D level of 44.4 ng/mL, which was significantly different than the control group mean of 31.56 ng/mL ($p < 0.0001$). These means are higher than those in this study, which is most likely due to the longer study period and the impact of lower latitude for location of the Hollis et al. (2011) study. Wagner et al. (2013) randomized pregnant women between 12-16 weeks gestation to receive either 2000 IU or 6000 IU of vitamin D daily, without a control group. Maternal 25[OH]D level increased from a baseline mean of 22.7 ng/mL to 37.9 ng/mL in the 6000 IU daily group. This was amongst a majority African American or Hispanic population. The findings from this study are similar to those of these other two randomized trials, thus confirming the findings of a significant difference in mean maternal 25[OH]D level by delivery. Both the Hollis et al. (2011) and Wagner et al. (2013) studies began at 12-16 weeks gestation, therefore offering a much longer duration of the intervention to have an effect, compared

to this study, which initiated the intervention at approximately 28 weeks gestation. Hollis et al. (2011) and Wagner et al. (2013) both demonstrated highly statistically significant results at delivery, which was confirmed by this study when controlling for maternal enrollment 25[OH]D levels.

Influence of Maternal Vitamin D Supplementation During Lactation on Maternal 25[OH]D

By lactation, the experimental group participants achieved a mean 25[OH]D level of 35.57 ng/mL and the control group mean of 22.38 ng/mL. None of the 6 control group participants had sufficient 25[OH]D status, whereas 4 of the 7 experimental group participants achieved sufficiency, demonstrating a 688% benefit increase of the intervention on achieving sufficiency. Over the course of the intervention, mean maternal 25[OH]D level in the control group steadily declined while the mean maternal 25[OH]D level in the experimental group steadily increased. Comparing findings to those of other researchers demonstrates some consistency. This study differs in that it spanned pregnancy and lactation, whereas the interventions reported by Hollis and Wagner (2004) and Wagner et al. (2006) were both initiated at one month postpartum. Hollis and Wagner (2004) spanned from 1 month postpartum to 4 months postpartum using supplementation of 4000 IU of vitamin D daily. In the intervention group, the mean maternal 25[OH]D level increased from 32.9 ng/mL to 44.5 ng/mL demonstrating a greater increase than was found in the study being described here. Wagner et al. (2006) completed a 6 months study (1 to 7 months postpartum) using 6400 IU of vitamin D supplementation daily. Participants had a mean 25[OH]D at baseline of 34 ng/mL, which increased to 58.8 ng/mL at the end of the study. Wagner et al. (2006) conducted a much

longer study with a higher dose of vitamin D supplementation and achieved higher mean 25[OH]D levels compared to the Hollis and Wagner (2004) or this study. However, all three studies demonstrate that long term use of vitamin D supplementation in lactation does not provoke adverse outcomes and will increase maternal 25[OH]D levels.

Influence of Maternal Vitamin D Supplementation on Infant 25[OH]D at Delivery

Analyzing the impact of the intervention on infant 25[OH]D level at delivery, the experimental group infants achieved a mean 25[OH]D level of 32.33 ng/mL compared to the control group infants mean 25[OH]D level of 23.67 ng/mL. In terms of ability to reach sufficient 25[OH]D levels at delivery, 1 of the 6 control group infants was sufficient (corresponded to the one mother sufficient at delivery), and 5 of the 7 infants in the experimental group were sufficient. Comparing the two groups for relative benefit increase demonstrates a 328% benefit increase to the infants in the experimental group. The mean infant 25[OH]D level at delivery in the experimental group infants in this study is very similar to the findings of Wagner et al. (2013) who demonstrated a infant mean 25[OH]D level of 27.0 ng/mL in the 4000 IU daily group at delivery. The mean infant 25[OH]D level at delivery in the experimental group infants in this study differs however, from the Hollis et al. (2011) findings of mean 25[OH]D amongst the experimental group infants of 10.6 ng/mL. Although studies by Hollis et al. (2011) and Wagner et al. (2013) were initiated in early pregnancy, the infants in the 4000 IU daily groups achieved a lower mean 25[OH]D level by delivery than in the study being described here. The lower mean 25[OH]D level of the infants at delivery in the experimental group may be due to methodologic differences in that Hollis et al. (2011) and Wagner et al. (2013) both used cord blood for neonatal assessment of 25[OH]D level and if cord blood was not available

they used blood samples taken from the infant up to 2 weeks after delivery. As evidenced by the results presented in Chapter 4, infant 25[OH]D level seems to drop rapidly after delivery, therefore a method that includes collecting infant blood up to 2 weeks after birth may be skewing the results in Wagner et al. (2013) and Hollis et al. (2011) reports. In almost all studies investigating neonatal vitamin D status at birth, the researchers used cord blood for assessment of neonatal 25[OH]D level (Bodnar et al., 2007; Bowyer et al., 2009; Dror et al., 2011b; Hollis et al., 2011; Novakovic et al., 2012; Viljakainen et al., 2010; Wagner et al., 2013a; Wang et al., 2010). It is commonly assumed that neonatal 25[OH]D level will be 60-80% of maternal as described in several review articles (Barrett & McElduff, 2010; Brannon, 2012; Kovacs, 2008). This assumption is upheld by Hollis et al. (2011) with neonatal mean 25[OH]D level only reaching 40% of maternal at delivery, and by Wagner et al. (2013) with neonatal mean 25[OH]D level reaching 71% of maternal, both in the 4000 IU daily group. However, several other studies would refute this assumed difference between neonatal and maternal 25[OH]D levels at delivery because they in fact found mean neonatal levels to be higher than mean maternal (Bowyer et al., 2009; Novakovic et al., 2012; Viljakainen et al., 2010; Wang et al., 2010). For the results of the study being discussed here, for participants receiving 3800 IU vitamin D daily the mean 25[OH]D level at delivery was 32.48 ng/mL and 32.33 ng/mL for their infants, demonstrating a nominal difference. This study used infant heel stick blood collection methods, not cord blood, for analysis. This technique was described in only one other study, Merewood et al. (2010). Merewood et al. (2010) describe that some vitamin D deficient participants had infants who were not vitamin D deficient and some vitamin D replete participants had vitamin D

deficient infants. Overall, they found that neonatal 25[OH]D levels were about 70% of maternal at delivery. The difference between the findings of the study being described here and those of Merewood et al. (2010) may be due to this being an intervention study, which may change the dynamic of maternal-fetal vitamin D transfer.

Influence of Maternal Vitamin D Supplementation on Infant 25[OH]D at Lactation

Looking at infant mean 25[OH]D levels by lactation, the experimental group infants had a mean of 24.9 ng/mL and the control group 16.98 ng/mL. The combined impact of maternal vitamin D supplementation on infant 25[OH]D at delivery and lactation approached statistical significance when controlling for maternal enrollment 25[OH]D level. There was a reasonably strong effect size at lactation, but power was lost due to small sample size. Nonetheless, there was about a 8 ng/mL observed difference between groups in mean 25[OH]D level in the infants at lactation. The difference of 8 ng/mL is an almost 50% increase in mean 25[OH]D value for the experimental group infants compared to control group infants. At lactation, none of the 6 control group infants were sufficient and 2 of the 7 experimental group infants were sufficient, consistent with a 337% benefit increase to those infants breastfeeding from a mother receiving the intervention. Both the control and experimental group infants demonstrated a drop in mean 25[OH]D level from delivery to lactation of about 8ng/mL with the experimental group infants starting and ending about 8 ng/mL above their control group counterparts. Looking again at studies of similar design and method, Hollis and Wagner (2004) demonstrate a rise in infant 25[OH]D during lactation among infants breastfeeding from a mother being supplemented with 4000 IU daily from 13.4 ng/mL to 30.8 ng/mL. Wagner et al. (2006), after 6 months of maternal supplementation with 6400

IU vitamin D daily, demonstrated a rise in infant 25[OH]D from 14 ng/mL to 46 ng/mL. Both of these studies demonstrate a greater increase in mean infant 25[OH]D than was seen in the results of the study being described here because of longer study period, higher dose of vitamin D supplementation, and perhaps greater maternal compliance with study protocol. In addition, Hollis and Wagner (2004) and Wagner et al. (2006) both had larger sample sizes by their final data point.

Influence of Maternal Vitamin D Supplementation on Maternal Cytokines

Both pro-inflammatory (TNF-α and IL-6) and an anti-inflammatory cytokine (IL-10) levels were analyzed at enrollment, delivery, and lactation for maternal participants. There were no observable differences in mean cytokine levels between the experimental and control groups at enrollment. A lack of difference between group mean cytokine levels would be expected at enrollment, but after initiation of the intervention it was expected that TNF-α and IL-6 would be lower in the experimental group and IL-10 would be lower in the control group. The hypothesized differences in group means were not observed and there were no statistically significant differences in group means for any of the cytokine results. There are limited other studies to compare these findings with, but Denney et al. (2011) looked at TNF-α, IL-6 and IL-10 across the course of uncomplicated pregnancies. Denney et al. (2011) report that TNF-α and IL-6 significantly decreased over the course of pregnancy, which is in opposition to the findings of this study. Denney et al. (2011) report no change in IL-10 over the course of pregnancy, which is consistent with the findings of this study. Palm et al. (2013) found a significant increase in IL-6 over the course of pregnancy, which remained higher during the postpartum period. Although the study being discussed here did not find an increase

in IL-6 from enrollment to delivery, there was an increase in IL-6 at lactation. Palm et al. (2013) report no change in TNF-α over pregnancy and postpartum, which is consistent with this study. Barrera et al. (2011) investigated the effects of calcitriol (1-25-dihydroxy vitamin D) on IL-10 in cultured human trophoblast cells. Barrera et al. (2011) report that calcitriol inhibited IL-10 production. The findings of the study being discussed here do not support this *in vitro* finding as IL-10 was not reduced in pregnant or lactating women receiving vitamin D supplementation. The findings for IL-10 are somewhat limited in this study as most of the plasma samples were analyzed in duplicate and some in single, as opposed to the recommended triplicate, because of lack of available space on the tray used for analysis.

Maternal and Infant Health Outcomes

In regards to health outcomes at delivery, there were no significant differences between groups in terms of gestation at delivery, maternal body mass index at delivery, mode of delivery, infant birth weight, infant birth length, or infant head circumference. The lack of differences between groups regarding health outcomes is consistent with both Hollis et al. (2011) and Wagner et al. (2013) who found no statistically significant difference in health outcomes between the experimental and control groups in the intervention studies in pregnant women. Wagner et al. (2013) note that there was a decline in rates of infection, preterm labor, and preterm birth with increases in maternal 25[OH]D, but this was not statistically significant. Merewood et al. (2009) have previously demonstrated an increased risk of cesarean section with declining 25[OH]D levels, but the study being discussed here was not powered for this finding and did not appreciate any difference in mode of delivery (Merewood, Mehta, Chen, Bauchner, &

Holick, 2009). There may be risks to health with vitamin D deficiency that are not able to be evaluated within the confines of the study being discussed here.

Limitations

All research studies are limited in one way or another by factors out of the control of the researcher. The first limitation of this study is in regards to participant loss to follow-up. There are difficulties inherent in using pregnant and lactating women as participants in that they have many demands on their time and attention, namely their older children and family obligations. Participants may have been hesitant to return for their final study visit knowing that their infant was going to experience a heel stick or that they were going to experience a blood draw, even though this was fully explained at enrollment. They may have had additional hesitations due to the harsh winter weather being experienced around the time that many of the participants were expected for their final visit. Although enrollment numbers were powered appropriately, more women were lost to follow-up than estimated and this has the potential to have affected the findings.

This study was also limited by lack of racial diversity. All participants self identified as white and because of the large impact that skin color plays in endogenous vitamin D production, it would be beneficial to evaluate a population with diversity of skin color. Additionally, there is a fairly robust population of non-white women living in the northern plains who probably have higher prevalence of vitamin D deficiency and therefore need particular attention from the medical and research communities.

Conduct of the study was interrupted by the PI moving after establishment of a study site with IRB approvals in place. This necessitated relationship building with a

new site and new research partners in order to complete the study. This took several months and interrupted the study process. However, despite the disruption at the time, this also allowed for further exploration of methodology and creation of new partnerships for future research.

Implications for Nursing

This study offers an exploration of the impact of continuous prenatal to lactation maternal vitamin D supplementation on the vitamin D status of pregnant and lactating women and their infants. Conducting a randomized controlled trial comes with multiple layers of complexity that are extremely beneficial to learn how to manage while in a research-focused doctoral degree program. As a relatively small pilot project, this study adds novel, significant findings to the science of perinatal health while simultaneously offering the PI invaluable training for future projects. This study has implications for nursing research, practice, education, and policy with potential to advance the science.

Implications for Nursing Research

Nurses should seek out opportunities for participating in clinical research that use the DOHaD hypothesis as its foundation. Nurses can contribute to this field using the lens of holism that is a hallmark of nursing. By taking a holistic view, nurses are able to consider all aspects of the human experience when designing and implementing clinical research trials. The nursing perspective would benefit the science as well as participants and patients. This study demonstrates the importance of the researcher being directly interactive with participants in order to improve retention and intervention fidelity. Nurses are well suited to creating a trusting relationship with participants that would benefit clinical research.

This study has helped to further understanding, but there are several strategies for future investigation that could be made to add further knowledge to the field. It would be recommended to do a larger study with a more diverse population. This study has demonstrated a very robust effect size of maternal supplementation on breastfed infant 25[OH]D level. Using these findings to power a future study of similar method demonstrates the need to enroll approximately 30 women total to result in statistically significant impact in the infants, including consideration for withdrawal from the study and loss to follow-up. In order to study our most vulnerable populations, it would be recommended that at least 15 of the recommended total 30 participants be women of color. This would allow for loss to follow-up as well as investigation of the protocol's impact on non-white women and their infants.

In addition to repeating this study in a larger, more diverse population, it would be recommended to implement the protocol prior to pregnancy. As was learned in the discovery of the impact of folic acid supplementation on the decrease in neural tube defects leading to recommendation of universal folic acid supplementation in all women of childbearing age, implementing vitamin D supplementation prior to pregnancy may impact the early stages of placental and embryonic development. This could in turn result in decreased pregnancy and infant morbidity or improved long-term health outcomes. Completing a study that would begin in adolescence and span through pregnancy and lactation would be supported by DOHaD hypotheses and potentially impact the health of multiple generations. As part of the study being discussed here, participants consented to be contacted in the future, allowing for evaluation of impact of

the intervention on infant outcomes including atopic disease and asthma and wheeze, or any childhood diseases evidenced by current literature.

Implications for Nursing Practice

Based on the findings of this study in conjunction with those of other researchers (Hollis & Wagner, 2004b; Hollis et al., 2011; Saadi et al., 2009; Wagner et al., 2006; Wagner et al., 2013a), it is time for clinical practice to be altered to reflect current understanding. It is clear that a majority of pregnant women and breastfed infants are not vitamin D sufficient. Vitamin D deficient pregnant women go on to be vitamin D deficient lactating women, which results in vitamin D deficient breastfed infants. When we advise lactating women that their exclusively breastfed infant needs supplementation with any outside substance, we run the risk of undermining her belief in breastfeeding as the ideal nutrition for her infant. By supplementing pregnant and lactating women with vitamin D, we are able to impact the vitamin D status of the women as well as their infants and in turn improve health across at least two generations. It should now be recommended that all women take enough supplemental vitamin D to raise their serum 25[OH]D levels above 32 ng/mL in order to provide for vitamin D adequacy prior to pregnancy starting, and throughout the woman's life. Further investigation is needed to determine if there is a dose of vitamin D that can be broadly recommended that would replete a majority of women with vitamin D. While this investigation continues, it would be recommended that women of all ages have their serum 25[OH]D level analyzed and take supplementation necessary to titrate this value above 32 ng/mL. This recommendation is inconsistent with the current IOM recommendation, which is based on bone health only. Further evidence is needed to bolster the recommendation of serum

25[OH]D to exceed 32 ng/mL, which is based on multiple other health outcomes beyond bone health.

Implications for Nursing Education

Vitamin D deficiency amongst pregnant and lactating mothers and their infants is highly prevalent throughout the United States (Collins-Fulea et al., 2012; Dror et al., 2011b; Hollis & Wagner, 2004b; Merewood et al., 2010; Wagner et al., 2006). Lifestyle factors such as avoiding sun exposure and using sunscreen have increased the prevalence of vitamin D deficiency, but other patient specific factors such as skin color or latitude at, which the person lives greatly impact endogenous vitamin D production.

Nurses should be educated about the modifiable and non-modifiable risk factors for vitamin D deficiency among pregnant and lactating women. In addition, nurses should understand the health implications of vitamin D deficiency for women and their infants, both short and long term. When educating nurses about fundamental theories of nursing practice, the DOHaD hypothesis should be included in that conversation in order to bring awareness of this most important theory to nursing practice. Nurses are educated to take a broad and holistic view of the patient and educating them about the possible multi-generational impacts of fetal exposures will bring depth of understanding to nursing education.

Implications for Nursing Policy

Although the IOM defines 25[OH]D sufficiency as > 20 ng/mL, there is evidence that bone health is affected, along with rates of cancer and psychological illness, at levels < 32 ng/mL. Additionally, the IOM recommendation of 600 IU daily of vitamin D would not be adequate to produce vitamin D sufficiency in a majority of women, even defined

as > 20 ng/mL. The Endocrine Society has taken a broader view, as their mandate allows, and recommends up to 2000 IU vitamin D daily for 25[OH]D deficient adults including pregnant women. Understanding of vitamin D sufficiency in pregnancy is rapidly changing. Researchers are finding that production of the biologically active $1,25[OH]_2D$ during pregnancy is maximized when 25[OH]D levels reach 40 ng/mL (Hollis et al., 2011; Wagner et al., 2013a). This knew understanding led Wagner et al. (2013a) to define vitamin D sufficiency in pregnancy as a 25[OH]D level > 40 ng/mL rather than the ≥ 32ng/mL that is typically cited as sufficient. As further research is completed in participants across the lifespan, including pregnant and lactating women and their infants, researchers will gain a better understanding of the biological markers associated with sufficiency. As a consequence of better understanding of a biologically based definition of sufficiency for different stages of life, policy should reflect the new understanding and recommendations from all agencies should provide recommendations that are congruent with improved health outcomes. If recommendations had improved consistency, policy could reflect this consistency and health would be improved amongst the population.

Concluding Remarks

This study demonstrated that adequate maternal vitamin D supplementation that spans pregnancy and lactation results in increased 25[OH]D levels in the mother and her breastfed infant. Although not always statistically significant, the observed differences between the experimental and control groups demonstrate clinically significant differences. Researchers need to continue this line of inquiry so that evidence based recommendations can be given to pregnant and lactating women about appropriate dosing

of vitamin D supplementation in order to negate giving infants anything other than breast milk. Current recommendations of 600 IU daily fail to bring a majority of women to sufficiency, which has the potential to impact their health and the health of future generations. Vitamin D has the potential to impact fetal and infant programming of health outcomes, including mechanisms as fundamental as the action of the innate immune system. Supported by understanding of the DOHaD hypothesis, clinicians have the potential to impact the health of multiple generations with interventions initiated before or during pregnancy, and future research is needed to support the interventions enacted in the clinical setting.

APPENDICES

Appendix A
Institutional Review Board Approvals

UNIVERSITY OF UND NORTH DAKOTA

INSTITUTIONAL REVIEW BOARD
c/o RESEARCH DEVELOPMENT AND COMPLIANCE
DIVISION OF RESEARCH
TWAMLEY HALL ROOM 106
264 CENTENNIAL DRIVE STOP 7134
GRAND FORKS ND 58202-7134
(701) 777-4279
FAX (701) 777-6708
www.und.edu/dept/rdc/regucomm/IRB

December 23, 2010

Doria Keesling
2110 Wylie Ave.
Missoula, MT 59802

Dear Ms. Keesling:

We are pleased to inform you that your project titled, "Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants" (IRB-201012-148) has been reviewed and approved by the University of North Dakota Institutional Review Board (IRB). The expiration date of this approval is December 22, 2011. Your project cannot continue beyond this date without an approved Research Project Review and Progress Report.

As principal investigator for a study involving human participants, you assume certain responsibilities to the University of North Dakota and the UND IRB. Specifically, an unanticipated problem or adverse event occurring in the course of the research project must be reported within 5 days to the IRB Chairperson or the IRB office by submitting an Unanticipated Problem/Adverse Event Form. Any changes to or departures from the Protocol or Consent Forms must receive IRB approval prior to being implemented (except where necessary to eliminate apparent immediate hazards to the subjects or others.)

All Full Board and Expedited proposals must be reviewed at least once a year. Approximately ten months from your initial review date, you will receive a letter stating that approval of your project is about to expire. If a complete Research Project Review and Progress Report is not received as scheduled, your project will be terminated, and you must stop all research procedures, recruitment, enrollment, interventions, data collection, and data analysis. The IRB will not accept future research projects from you until research is current. In order to avoid a discontinuation of IRB approval and possible suspension of your research, the Research Project Review and Progress Report must be returned to the IRB office at least six weeks before the expiration date listed above. If your research, including data analysis, is completed before the expiration date, you must submit a Research Project Termination form to the IRB office so your file can be closed. The required forms are available on the IRB website.

If you have any questions or concerns, please feel free to call me at (701) 777-4079 or e-mail michellebowles@mail.und.edu.

Sincerely,

Michelle L. Bowles, M.P.A.
IRB Coordinator

MLB/jle

Enclosures

Altru
HEALTH SYSTEM

April 23, 2012

Doria K. Thiele
College of Nursing
400 Oxford St., Stop 9025
Norther Plains Center for Behavioral Research Room 340D
Grand Forks, ND 58202-9025

RE: Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants. (ST-103).

Dear Ms. Thiele,

We are pleased to inform you that your project has been reviewed and approved by Altru Health System Institutional Review Board (IRB). The expiration date of this approval is April 19, 2013. Your project cannot continue beyond this date without a continuing review approval from the Altru IRB. Also, it is very important that you keep your current address accurate with the IRB office, as if we don't hear about your study's status; we will automatically terminate your study.

Research investigators are responsible for obtaining informed consent and for ensuring that no human subject will be involved in the research prior to obtaining the consent. Only copies of the most recent approved consent form may be used.

As principal investigator for a study involving human participants, you assume certain responsibilities to Altru Health System and the Altru IRB. Specifically, any adverse events or protocol changes that occur must be reported to the IRB immediately. It is your obligation to inform the IRB in writing if you would like to change aspects of your approved project, prior to implementing such changes.

All Full Board and Expedited proposals must be reviewed at least once a year. Approximately ten months from your initial review date, you will receive a letter stating that approval of your project is about to expire. If a complete "Continuing Review Form" is not received as scheduled, your project will be terminated and you must stop all research procedures, recruitment, enrollment, interventions, data collection and data analysis. If your research, including data analysis, is completed before the expiration date, you must submit a Research Project Termination/Completion Report form to the IRB office so your file can be closed. The required forms are available on the IRB websites (on altrunet and on altru.org).

If you have any questions or concerns, please feel free to call me at (701) 780-6161 or e-mail me at mreese@altru.org.

Sincerely,

Marie-Laure Reese
IRB Coordinator

Enclosures

Altru HEALTH SYSTEM

Institutional Review Board (IRB)

Research Project Action Report

Pub to Doria 4/23/12

Revised 5/10/11

Date: April 20, 2012 IRB # ST-103

Principal Investigator: Doria K. Thiele

Department: Nursing Phone # 406-210-4890

Address to which notice of approval should be sent: 400 Oxford St, Stop 9025, Northern Plains Center for Behavioral Research Room 340D, Grand Forks, ND 58202

Research Coordinator: Phone #

Project Title: Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants.

The above referenced project protocol and informed consent was reviewed by the Altru Health System Institutional Review Board on 4/20/12 and the following action was taken:

FULL BOARD APPROVAL w/Minor Modifications:

☐ Project has been approved on _____ with **Minor Modifications required**. This study can not be started until revisions have been made and submitted, and final IRB approval has been granted.

FULL BOARD APPROVAL:

☒ Project has been approved on 4/20/12 Next scheduled review is on 4/19/13

APPROVAL GRANTED BY ONE REVIEWER:

☐ Final project has been approved on _____ Next scheduled review is on _____

☐ Project approved. **EXPEDITED REVIEW NO.** _____ This approval is VALID UNTIL _____

☐ Project approved. **EXEMPT CATEGORY NO.** _____ This approval is VALID UNTIL _____
As long as approved procedures are followed. No periodic review scheduled unless so stated in Remarks area

☐ Project approval **Denied** or Project approval **Tabled** (see REMARKS SECTION for further information)

☐ Amendment approved _____

☐ Administrative change approved _____

☐ Protocol revision approved _____

☒ Revised consent form approved **Consent Form.**

☒ Other **New Study.**

REMARKS:

Signature of Chairperson or Designated IRB Member
Altru Health System Institutional Review Board

4/20/12
Date

ALTRU HEALTH SYSTEM

APPROVAL TO CONDUCT RESEARCH STUDY
AT ALTRU HEALTH SYSTEM

Name: Doria K. Thiele

Date: 3/6/2012

Address: 400 Oxford St Stop 9025 Grand Forks ND 58202-9025

Telephone Number(s): 406-210-4890 (w)

Department/College College of Nursing UND

PROJECT: Maternal Vitamin D Supplementation To Correct Deficiency In Mothers And Breastfed Infants

Your request to conduct the above named study at an Altru Health System facility involving employees or patients as participants, and/or requiring facility resources has been reviewed. The following action has been taken:

✓ Permission to conduct the study is granted

_____ Permission to conduct the study will be granted upon completion of the following:

_____

_____

_____ Permission to conduct the study is denied for the following reason(s):

_____

_____

_____

RECOMMENDATIONS/REMARKS:

Signature | Administrative Director Medical Specialty Care (Title) | 3-8-12 (Date)

L:\Organization Approve -Student\Approval from Kerry.doc

REPORT OF ACTION: PROTOCOL CHANGE
University of North Dakota Institutional Review Board

Date: 3/15/2012 **Project Number:** IRB-201012-148

Principal Investigator: Keesling Thiele, Doria

Department: Nursing

Project Title: Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants

The above referenced project was reviewed by a Designated Member for the University's Institutional Review Board on March 21, 2012 and the following action was taken:

☒ Protocol Change approved. **Expedited Review** Category No. 2, 7
Next scheduled review must be before: November 16, 2012

☒ **Copies of the attached consent form with the IRB approval stamp dated March 21, 2012 must be used in obtaining consent for this study.**

☐ Protocol Change approved. **Exempt Review** Category No.
This approval is valid until _____ as long as approved procedures are followed.
No periodic review scheduled unless so stated in the Remarks Section.

☐ **Copies of the attached consent form with the IRB approval stamp dated must be used in obtaining consent for this study.**

☐ Minor modifications required. The required corrections/additions must be submitted to RDC for review and approval. **This study may NOT be started UNTIL final IRB approval has been received.**
(See Remarks Section for further information.)

☐ Protocol Change approval **deferred. This study may not be started until final IRB approval has been received.**
(See Remarks Section for further information.)

☐ Protocol Change **disapproved. This study may not be started until final IRB approval has been received.**

REMARKS: **Any unanticipated problem or adverse occurrence in the course of the research project must be reported within 5 days to the IRB Chairperson or RDC by submitting an Unanticipated Problem/Adverse Event Form.**

Any changes to the Protocol or Consent Forms must receive IRB approval prior to being implemented (except where necessary to eliminate apparent immediate hazards to the subjects or others).

PLEASE NOTE: **Requested revisions for student proposals MUST include adviser's signature. All revisions MUST be highlighted.**

☒ Education Requirements Completed. (Project cannot be started until IRB education requirements are met.)

cc: Dr. Cindy Anderson

Signature of Designated IRB Member
UND's Institutional Review Board

3-21-12
Date

If the proposed project (clinical medical) is to be part of a research activity funded by a Federal Agency, a special assurance statement or a completed 310 Form may be required. Contact RDC to obtain the required documents.

REPORT OF ACTION: PROTOCOL CHANGE

University of North Dakota Institutional Review Board

Date: 9/18/2012 **Project Number:** IRB-201012-148

Principal Investigator: Keesling Thiele, Doria

Department: Nursing

Project Title: Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants

The above referenced project was reviewed by a Designated Member for the University's Institutional Review Board on September 23, 2012 and the following action was taken:

☒ Protocol Change approved. **Expedited Review** Category No. 2 & 7
Next scheduled review must be before: November 16, 2012
☒ **Copies of the attached consent form with the IRB approval stamp dated September 23, 2012 must be used in obtaining consent for this study.**

☐ Protocol Change approved. **Exempt Review** Category No.
This approval is valid until _____ as long as approved procedures are followed.
No periodic review scheduled unless so stated in the Remarks Section.
☐ **Copies of the attached consent form with the IRB approval stamp dated must be used in obtaining consent for this study.**

☐ Minor modifications required. The required corrections/additions must be submitted to RDC for review and approval. **This study may NOT be started UNTIL final IRB approval has been received.**
(See Remarks Section for further information.)

☐ Protocol Change approval **deferred. This study may not be started until final IRB approval has been received.**
(See Remarks Section for further information.)

☐ Protocol Change **disapproved. This study may not be started until final IRB approval has been received.**

REMARKS: **Any unanticipated problem or adverse occurrence in the course of the research project must be reported within 5 days to the IRB Chairperson or RDC by submitting an Unanticipated Problem/Adverse Event Form.**

Any changes to the Protocol or Consent Forms must receive IRB approval prior to being implemented (except where necessary to eliminate apparent immediate hazards to the subjects or others).

PLEASE NOTE: **Requested revisions for student proposals MUST include adviser's signature. All revisions MUST be highlighted and submitted to the IRB within 90 days of the above review date.**

☒ Education Requirements Completed. (Project cannot be started until IRB education requirements are met.)

cc: Dr. Cindy Anderson

Signature of Designated IRB Member — Date
UND's Institutional Review Board

9-23-12

If the proposed project (clinical medical) is to be part of a research activity funded by a Federal Agency, a special assurance statement or a completed 310 Form may be required. Contact RDC to obtain the required documents.

(Revised 10/2006)

Altru HEALTH SYSTEM

Institutional Review Board (IRB)

Research Project Action Report

Revised 5/10/11

Date: September 18, 2012 IRB # ST-103

Principal Investigator: Doria K. Thiele

Department: College of Nursing Phone # 406-210-4890

Address to which notice of approval should be sent: 400 Oxford St. Stop 9025; Grand Forks, ND 58202-9025

Research Coordinator: _____ Phone # _____

Project Title: Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants.

The above referenced project protocol and informed consent was reviewed by the Altru Health System Institutional Review Board on _____ and the following action was taken:

FULL BOARD APPROVAL w/Minor Modifications:

☐ Project has been approved on _____ with **Minor Modifications required**. This study can not be started until revisions have been made and submitted, and final IRB approval has been granted.

FULL BOARD APPROVAL:

☐ Project has been approved on _____ Next scheduled review is on _____

APPROVAL GRANTED BY ONE REVIEWER:

☐ Final project has been approved on _____ Next scheduled review is on _____

☐ Project approved. **EXPEDITED REVIEW NO.** _____ This approval is VALID UNTIL _____

☐ Project approved. **EXEMPT CATEGORY NO.** _____ This approval is VALID UNTIL _____
As long as approved procedures are followed. No periodic review scheduled unless so stated in Remarks area

☐ Project approval **Denied** or Project approval **Tabled** (see REMARKS SECTION for further information)

☐ Amendment approved _____

☐ Administrative change approved _____

☐ Protocol revision approved _____

☒ Revised consent form approved **Revised Consent Form, version 2 dated 9/12/12.**

☐ Other _____

REMARKS: clarification that separate research blood draws may occur + increased compensation for this inconvenience

Signature of Chairperson or Designated IRB Member
Altru Health System Institutional Review Board

9/20/12
Date

Version Date: 12/20/2005

Institutional Review Board (IRB)/Independent Ethics Committee (IEC) Authorization Agreement

Name of Institution or Organization Providing IRB Review (Institution/Organization A):
University of North Dakota IRB #1

IRB Registration #: IRB00001040 Federalwide Assurance (FWA) #, if any: FWA00000376

Name of Institution Relying on the Designated IRB (Institution B):
OHSU

FWA #: FWA 00000161

The Officials signing below agree that the OHSU may rely on the designated IRB for review and continuing oversight of its human subjects research described below: (*check one*)

(___) This agreement applies to all human subjects research covered by Institution B's FWA.

(_X_) This agreement is limited to the following specific protocol(s):

Name of Research Project: Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants
Name of Principal Investigator: Doria K. Thiele
Sponsor or Funding Agency: AWHONN
Award Number, if any: UND0016676

(___) Other (*describe*):_____

The review performed by the designated IRB will meet the human subject protection requirements of Institution B's OHRP-approved FWA. The IRB at Institution/Organization A will follow written procedures for reporting its findings and actions to appropriate officials at Institution B. Relevant minutes of IRB meetings will be made available to Institution B upon request. Institution B remains responsible for ensuring compliance with the IRB's determinations and with the Terms of its OHRP-approved FWA. This document must be kept on file by both parties and provided to OHRP upon request.

Signature of Signatory Official (Institution/Organization A):

Date: June 4, 2012

Print Full Name: Barry I. Milavetz, Ph.D. Institutional Title: Associate Vice President for Research

NOTE: The IRB of Institution A must be designated on the OHRP-approved FWA for Institution B.

Signature of Signatory Official (Institution B):

Date: 7/9/12

Print Full Name: Kara Drolet, PhD

Institutional Title: Associate Director

Appendix B
Support Letter

Altru
MAIN CLINIC

To: Doria K. Thiele, CNM, IBCLC, PhD Candidate
Principal Investigator

From: Michael R. Brown, MD
Chief, Obstetrics and Gynecology

Re: Support for Research Study

Date: January 22, 2012

This letter confirms support for your proposed study, "Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants". I have reviewed your proposal and have determined that this study is feasible and important.

There are minimal risks for the pregnant women enrolled in your study. Specimen and data collection will occur at our office at Altru Obstetrics and Gynecology Clinic. There will be no use of Altru Health System resources during this study.

Your protocol demonstrates minimal staff burden. Your involvement with our clinic will be limited to support from the nursing staff related to identifying participants in the late second trimester and alerting our research staff of participant admission for labor and delivery. Further, medical records support will be needed in data retrieval from the prenatal and postnatal record though this will be limited. Qualified members of your research team with experience as maternal/child professional nurses will retrieve data from medical records.

I look forward to the successful initiation of this study and to your findings as they have great potential to improve the health of women and their children.

Sincerely,

Michael R. Brown, MD

Appendix C
Advertisement

Looking for Pregnant Women for Vitamin D Study

Participants must be 18 & older, have a history of breastfeeding a previous baby for 4 or more weeks, and be able to start the study between 24 and 28 weeks of pregnancy. Participants should be planning to breast feed for at least one month.

This study looks at how vitamin D passes from mother to baby during pregnancy and then through breast milk.

Free high quality prenatal vitamin
Earn up to $40 at completion of the study

Doria Thiele, CNM, IBCLC, PhD Candidate
406-210-4890

Appendix D

Consent to Participate and Authorization for Access to Personal Health Information

COLLEGE OF NURSING
NURSING BUILDING
430 OXFORD STREET STOP 9025
GRAND FORKS ND 58202-9025
(701) 777-4174
FAX (701) 777-4096

Research Study: Maternal vitamin D supplementation to correct deficiency in mothers and breastfed infants.

Version 2: 9.12.2012

Principal Investigator: Doria Thiele, CNM, APRN, IBCLC
406-210-4890
400 Oxford Street Stop 9025 Northern Plains Center for Behavioral Research Room 340D Grand Forks, ND 58202-9025

Co-Investigator: Cindy Anderson, PhD, WHNP-BC, FAAN
701-777-4354
400 Oxford Street Stop 9025 Northern Plains Center for Behavioral Research Room 340D Grand Forks, ND 58202-9025

Consent to Participate

You are invited by Doria Thiele, CNM, APRN, IBCLC at the University of North Dakota to participate in a study to identify the ability of mothers to transfer vitamin D to their babies through breast milk. Vitamin D is something that we make in our skin when we get enough sun light or if we take it in supplements or certain foods. Adequate vitamin D has been linked with preventing diabetes, high blood pressure, breast and colon cancer, and several other diseases. Researchers are still not sure if women can pass enough vitamin D to their babies through breast milk, but recent studies show that this is possible if women themselves get enough vitamin D.

STUDY OVERVIEW

You will either be in a group that receives a prenatal vitamin plus a placebo pill without vitamin D, or the group that receives a prenatal vitamin plus another pill with vitamin D. You would take your supplements daily during pregnancy and through 4-6 weeks postpartum. Neither you nor the principal investigator, Doria Thiele, will be aware of which group you are in. Your participation in this study would require approximately 40 minutes of your time. This would include filling out a questionnaire about how frequently you eat certain foods and keeping track of anything you give your baby other than breast milk. You will meet with Doria Thiele or other member of the research team during your pregnancy to enroll in the study and obtain all the necessary paperwork. You would have your blood drawn at the beginning of your third trimester, within 72 hours of delivery, and lastly at your 4-6 week postpartum visit to test your vitamin D level and possible other related factors. We would also do a heel stick blood test on your baby within 72 hours of birth and at 4-6 weeks postpartum, to test the vitamin D level. In addition to testing for vitamin D levels in you and your baby we will also look at possible changes that occur "above" the genes inside your cells. These changes that occur might help us

identify risks for diseases that are associated with vitamin D deficiency. When we see you during your pregnancy visit, we will collect information related to the study from your medical records. This will be repeated at the 4-6 week visit. All information is kept confidential and your name will be removed from any data we collect. It is our aim to minimize any inconvenience or discomfort to you or your baby.
Your participation in this study is completely voluntary. You may refuse to participate or withdraw your participation at any time without penalty or loss of benefits. If you decide not to participate, you will continue to receive the standard of care throughout your pregnancy and postnatal visits.

STUDY SCHEDULE, PROCEDURES, RISKS, AND DISCOMFORT

You will be invited to participate by Doria Thiele, CNM, APRN, IBCLC. If you agree to participate the following will occur:

- Sign this consent form and have your questions answered
- Sign a form allowing access to your medical information related to your pregnancy, delivery, and postpartum course. You will receive a copy of both forms for your records
- At our first visit, a blood sample will be collected to establish your vitamin D levels and analyze the chemicals "above" your DNA as well as possible other related factors which may include but not limited to calcium and parathyroid hormone. You will also be asked to complete a Food Frequency Questionnaire that takes about 20 minutes.
- You will be provided with prenatal vitamins that contain 400 International Units (IU) of vitamin D. You will also receive a pill without vitamin D (placebo) or a pill containing a dose of vitamin D in the amount of 3400 IU. You will take your prenatal vitamin and extra capsule every day through your 4-6 week postpartum visit. Neither you nor the investigator will know which extra capsule you are getting.
- You will receive your vitamins in 30 day supply packs. A member of the research team will meet with you monthly at your prenatal visits to provide you with further vitamins and answer any questions. You will be asked to bring all used vitamin packs/bottles to your prenatal visits. Doria Thiele, Principal Investigator, will also call you each month to check that you do not have any unanswered concerns or questions.
- Once you deliver your baby, you will have a second blood test. A blood test will also be done on your baby by pricking his/her heel. These blood samples will be used to test vitamin D levels and the chemicals "above" the genes as well as possible other related factors which may include but not limited to calcium and parathyroid hormone. Both of these blood tests will be completed by hospital or research staff and every attempt will be made to complete them during other regularly scheduled blood draws, however, it may be necessary for either you or your baby to have an extra blood draw.
- During the early weeks postpartum, you will fill in a form each day recording if your baby received any infant formula, vitamin supplements, or foods other than breast milk.
- A member of the research team will meet with you again during your 4-6 week postpartum visit and request that you complete the Food Frequency Questionnaire again. A final blood draw for you and your baby will be completed at this time as well.

- The visits for the study will be coordinated with the hospital or your care provider so any extra visits will be minimized.
- Doria Thiele, Principal Investigator, may contact you in the future (up to 15 years from the end of the study) in order to evaluate health outcomes for you and your infant.

Procedures, Risks, and Discomfort

I. Height

If a recent height measurement is not included in your chart an instrument will measure your height.

Risks and Discomfort: There are no known risks for this procedure.

II. Food Frequency Questionnaire

You will be given a questionnaire that lists food items that are a usual part of the diet. You will be asked to mark the number of times you have eaten a particular food item each month, week and day. Foods are listed in categories that include dairy products, fruit and fruit juices, vegetables, snacks, sweets and beverages, eggs, meats fish, main dishes and breads and cereals. The purpose of marking these foods is to estimate usual nutritional intake.

Risks and Discomfort: There are no risks associated with this activity.

III. Blood Sampling

Blood totaling about 3 tablespoons will be taken from you for testing needed in this study. This will be accomplished using standard venipuncture at three visits. Blood totaling about 2 to 4 drops will be taken from your infant for analysis in this study. This will be accomplished using standard heel poke at two visits.

Risks and Discomfort: There may be discomfort for you when the needle enters the skin, lasting a few seconds. The discomfort due to the needle in the vein should be minimal, lasting less than one minute during collection of the blood. Your infant may experience discomfort with the heel poke, lasting a few seconds. The discomfort should be minimal and you may hold and comfort your infant however you wish during this procedure. Genetic information will not be used in establishing medical diagnoses.

IV. Infant Intake Tool

You will be asked to complete an infant intake tool for every day after your baby is born. This tool asks about formula intake for the day, brand of formula, and if the infant was given any other vitamin or supplement. This should take less than one minute per day and will be collected at your 4-6 week visit.

Risks and Discomforts: There are no risks associated with this activity.

V. Record Retrieval Tool and Postpartum Record Retrieval Tool

Medical record data related to your pregnancy and delivery will be collected when you join the study and 4-6 weeks after birth. This information will be kept locked and confidential at all times and your name will not appear on the collected information. The data collected will become part of the study results, but only in group form, never with any information that could tie you to the study. When the study is concluded, this information will be shredded for your protection.

Risks and Discomforts: There are no discomforts associated with this activity. Any risk of a privacy violation is mitigated by strict confidentiality procedures.

BENEFITS
You and/or your child may or may not receive a direct benefit as a result of participating in this study. However, the results of this study will provide helpful information about how vitamin D is passed to babies from breast milk.

COMPENSTATION
You will receive a gift card from Target Stores in the amount of $20 when we meet with you at delivery and one in the amount of $30 when we meet with you at 4-6 weeks postpartum.

COSTS
You will not be responsible for the costs of the prenatal vitamins, any supplements received, or any blood analysis for this study.

NEW FINDINGS
You will be notified of any new information that may affect your willingness to continue your participation in this study.

ADVERSE REACTIONS
If you have an adverse reaction (get hurt or sick) as a direct result of taking part in this study, immediate and appropriate medical treatment will be made available. However, you or your insurance carrier will be responsible for all medical costs associated with any adverse reaction or injury while taking part in this study. No compensation is available from study sponsors, except as permitted by law.

WITHDRAWAL
You may choose to discontinue your participation in the study at anytime without penalty. If you decide to withdraw from the study, we ask that you notify the principal investigator.

CONFIDENTIALITY
All information is kept confidential. You will be assigned an identification number that will be used to code your research data for computer entry. Paper copies of your personal information and medical data will be kept in a locked file, with access limited to approved staff members, auditors, such as the University of North Dakota Institutional Review Board and USDA auditors, Altru Health System's Institutional Review Board and other state or federal agencies as provided by federal regulations. Your signed consent form and data will be kept in separate locked files for at least 3 years. If, and when they are disposed of, your name and any identifying information will be shredded. Any results from your participation in this project may be published in a scientific journal or presented at professional conferences, but only in a form not identifiable with you.

STATEMENT OF PRIVACY RIGHTS
Please see the Authorization for Access to Personal Health Information form which must be signed to participate in the study.

This research study has been reviewed by the Altru Health System Institutional Review Board (IRB) and by the University of North Dakota IRB for the purpose of protecting your safety and rights. Both the Institutional Review Boards were instituted under Federal and State law to review studies such as this one in order to protect research participants from: unnecessary risks, risks that outweigh benefits, and procedures that are scientifically unnecessary.

QUESTIONS

You are free to ask questions at any time during the study. Contact the principal investigator, Doria Thiele, CNM, APRN, IBCLC at 406-210-4890 for any information or if problems arise during the study. She can be reached by mail at the College of Nursing, University of North Dakota, 400 Oxford Street Stop 9025, Grand Forks, North Dakota, 58202-9025. You may also contact Doria Thiele's academic advisor, Dr. Cindy Anderson, at any time during the study. She can be reached at 701-777-4354 or by mail at the above address. If you have any other questions or concerns, please call the Office of Research Development and Compliance at the University of North Dakota at 701-777-4279. Additionally, if you have any questions regarding your rights as a research subject, you may contact the Altru Health System IRB at 701-780-1750.

CONSENT

Your signature below indicates that you have read this form, have had the study explained, and have had any questions answered to your satisfaction, that you now understand what will be expected of you, that you agree to take part in this study, and that you authorize the use of your personal health information and agree to take part in this study.

A copy of this signed Informed Consent Statement will be given to you

I understand that my medical records and study records are confidential. However, representatives of the study sponsor, the U.S. Food and Drug Administration (FDA), or the Institutional Review Board (IRB) may need to inspect my medical and/or study records. By signing this consent, I am allowing this inspection.

_____ _____

Your Signature Date

_____

Your Name Printed

_____ _____

Study Team Member Date

_____

Study Team Member

UNIVERSITY OF NORTH DAKOTA

COLLEGE OF NURSING
NURSING BUILDING
430 OXFORD STREET STOP 9025
GRAND FORKS ND 58202-9025
(701) 777-4174
FAX (701) 777-4096

Research Study: Maternal vitamin D supplementation to correct deficiency in mothers and breastfed infants.

Version 2: 9.12.2012

Principal Investigator: Doria Thiele, CNM, APRN, IBCLC
406-210-4890
400 Oxford Street Stop 9025 Northern Plains Center for Behavioral Research Room 340D Grand Forks, ND 58202-9025

Co-Investigator: Cindy Anderson, PhD, WHNP-BC, FAAN
701-777-4354
400 Oxford Street Stop 9025 Northern Plains Center for Behavioral Research Room 340D Grand Forks, ND 58202-9025

Consent to Participate

You are invited by Doria Thiele, CNM, APRN, IBCLC at the University of North Dakota to participate in a study to identify the ability of mothers to transfer vitamin D to their babies through breast milk. Vitamin D is something that we make in our skin when we get enough sun light or if we take it in supplements or certain foods. Adequate vitamin D has been linked with preventing diabetes, high blood pressure, breast and colon cancer, and several other diseases. Researchers are still not sure if women can pass enough vitamin D to their babies through breast milk, but recent studies show that this is possible if women themselves get enough vitamin D.

STUDY OVERVIEW

You will either be in a group that receives a prenatal vitamin plus a placebo pill without vitamin D, or the group that receives a prenatal vitamin plus another pill with vitamin D. You would take your supplements daily during pregnancy and through 4-6 weeks postpartum. Neither you nor the principal investigator, Doria Thiele, will be aware of which group you are in. Your participation in this study would require approximately 40 minutes of your time. This would include filling out a questionnaire about how frequently you eat certain foods and keeping track of anything you give your baby other than breast milk. You will meet with Doria Thiele or other member of the research team during your pregnancy to enroll in the study and obtain all the necessary paperwork. You would have your blood drawn at the beginning of your third trimester, within 72 hours of delivery, and lastly at your 4-6 week postpartum visit to test your vitamin D level and possible other related factors. We would also do a heel stick blood test on your baby within 72 hours of birth and at 4-6 weeks postpartum, to test the vitamin D level. In addition to testing for vitamin D levels in you and your baby we will also look at possible changes that occur "above" the genes inside your cells. These changes that occur might help us

identify risks for diseases that are associated with vitamin D deficiency. When we see you during your pregnancy visit, we will collect information related to the study from your medical records. This will be repeated at the 4-6 week visit. All information is kept confidential and your name will be removed from any data we collect. It is our aim to minimize any inconvenience or discomfort to you or your baby.
Your participation in this study is completely voluntary. You may refuse to participate or withdraw your participation at any time without penalty or loss of benefits. If you decide not to participate, you will continue to receive the standard of care throughout your pregnancy and postnatal visits.

STUDY SCHEDULE, PROCEDURES, RISKS, AND DISCOMFORT
You will be invited to participate by Doria Thiele, CNM, APRN, IBCLC. If you agree to participate the following will occur:

- Sign this consent form and have your questions answered
- Sign a form allowing access to your medical information related to your pregnancy, delivery, and postpartum course. You will receive a copy of both forms for your records
- At our first visit, a blood sample will be collected to establish your vitamin D levels and analyze the chemicals "above" your DNA as well as possible other related factors which may include but not limited to calcium and parathyroid hormone. You will also be asked to complete a Food Frequency Questionnaire that takes about 20 minutes.
- You will be provided with prenatal vitamins that contain 400 International Units (IU) of vitamin D. You will also receive a pill without vitamin D (placebo) or a pill containing a dose of vitamin D in the amount of 3400 IU. You will take your prenatal vitamin and extra capsule every day through your 4-6 week postpartum visit. Neither you nor the investigator will know which extra capsule you are getting.
- You will receive your vitamins in 30 day supply packs. A member of the research team will meet with you monthly at your prenatal visits to provide you with further vitamins and answer any questions. You will be asked to bring all used vitamin packs/bottles to your prenatal visits. Doria Thiele, Principal Investigator, will also call you each month to check that you do not have any unanswered concerns or questions.
- Once you deliver your baby, you will have a second blood test. A blood test will also be done on your baby by pricking his/her heel. These blood samples will be used to test vitamin D levels and the chemicals "above" the genes as well as possible other related factors which may include but not limited to calcium and parathyroid hormone. Both of these blood tests will be completed by hospital or research staff and every attempt will be made to complete them during other regularly scheduled blood draws, however, it may be necessary for either you or your baby to have an extra blood draw.
- During the early weeks postpartum, you will fill in a form each day recording if your baby received any infant formula, vitamin supplements, or foods other than breast milk.
- A member of the research team will meet with you again during your 4-6 week postpartum visit and request that you complete the Food Frequency Questionnaire again. A final blood draw for you and your baby will be completed at this time as well.

- The visits for the study will be coordinated with the hospital or your care provider so any extra visits will be minimized.
- Doria Thiele, Principal Investigator, may contact you in the future (up to 15 years from the end of the study) in order to evaluate health outcomes for you and your infant.

Procedures, Risks, and Discomfort

I. Height

If a recent height measurement is not included in your chart an instrument will measure your height.

Risks and Discomfort: There are no known risks for this procedure.

II. Food Frequency Questionnaire

You will be given a questionnaire that lists food items that are a usual part of the diet. You will be asked to mark the number of times you have eaten a particular food item each month, week and day. Foods are listed in categories that include dairy products, fruit and fruit juices, vegetables, snacks, sweets and beverages, eggs, meats fish, main dishes and breads and cereals. The purpose of marking these foods is to estimate usual nutritional intake.

Risks and Discomfort: There are no risks associated with this activity.

III. Blood Sampling

Blood totaling about 3 tablespoons will be taken from you for testing needed in this study. This will be accomplished using standard venipuncture at three visits. Blood totaling about 2 to 4 drops will be taken from your infant for analysis in this study. This will be accomplished using standard heel poke at two visits.

Risks and Discomfort: There may be discomfort for you when the needle enters the skin, lasting a few seconds. The discomfort due to the needle in the vein should be minimal, lasting less than one minute during collection of the blood. Your infant may experience discomfort with the heel poke, lasting a few seconds. The discomfort should be minimal and you may hold and comfort your infant however you wish during this procedure. Genetic information will not be used in establishing medical diagnoses.

IV. Infant Intake Tool

You will be asked to complete an infant intake tool for every day after your baby is born. This tool asks about formula intake for the day, brand of formula, and if the infant was given any other vitamin or supplement. This should take less than one minute per day and will be collected at your 4-6 week visit.

Risks and Discomforts: There are no risks associated with this activity.

V. Record Retrieval Tool and Postpartum Record Retrieval Tool

Medical record data related to your pregnancy and delivery will be collected when you join the study and 4-6 weeks after birth. This information will be kept locked and confidential at all times and your name will not appear on the collected information. The data collected will become part of the study results, but only in group form, never with any information that could tie you to the study. When the study is concluded, this information will be shredded for your protection.

Risks and Discomforts: There are no discomforts associated with this activity. Any risk of a privacy violation is mitigated by strict confidentiality procedures.

BENEFITS
You and/or your child may or may not receive a direct benefit as a result of participating in this study. However, the results of this study will provide helpful information about how vitamin D is passed to babies from breast milk.

COMPENSTATION
You will receive a gift card from Target Stores in the amount of $20 when we meet with you at delivery and one in the amount of $30 when we meet with you at 4-6 weeks postpartum.

COSTS
You will not be responsible for the costs of the prenatal vitamins, any supplements received, or any blood analysis for this study.

NEW FINDINGS
You will be notified of any new information that may affect your willingness to continue your participation in this study.

ADVERSE REACTIONS
If you have an adverse reaction (get hurt or sick) as a direct result of taking part in this study, immediate and appropriate medical treatment will be made available. However, you or your insurance carrier will be responsible for all medical costs associated with any adverse reaction or injury while taking part in this study. No compensation is available from study sponsors, except as permitted by law.

WITHDRAWAL
You may choose to discontinue your participation in the study at anytime without penalty. If you decide to withdraw from the study, we ask that you notify the principal investigator.

CONFIDENTIALITY
All information is kept confidential. You will be assigned an identification number that will be used to code your research data for computer entry. Paper copies of your personal information and medical data will be kept in a locked file, with access limited to approved staff members, auditors, such as the University of North Dakota Institutional Review Board and USDA auditors, Altru Health System's Institutional Review Board and other state or federal agencies as provided by federal regulations. Your signed consent form and data will be kept in separate locked files for at least 3 years. If, and when they are disposed of, your name and any identifying information will be shredded. Any results from your participation in this project may be published in a scientific journal or presented at professional conferences, but only in a form not identifiable with you.

STATEMENT OF PRIVACY RIGHTS
Please see the Authorization for Access to Personal Health Information form which must be signed to participate in the study.

This research study has been reviewed by the Altru Health System Institutional Review Board (IRB) and by the University of North Dakota IRB for the purpose of protecting your safety and rights. Both the Institutional Review Boards were instituted under Federal and State law to review studies such as this one in order to protect research participants from: unnecessary risks, risks that outweigh benefits, and procedures that are scientifically unnecessary.

QUESTIONS

You are free to ask questions at any time during the study. Contact the principal investigator, Doria Thiele, CNM, APRN, IBCLC at 406-210-4890 for any information or if problems arise during the study. She can be reached by mail at the College of Nursing, University of North Dakota, 400 Oxford Street Stop 9025, Grand Forks, North Dakota, 58202-9025. You may also contact Doria Thiele's academic advisor, Dr. Cindy Anderson, at any time during the study. She can be reached at 701-777-4354 or by mail at the above address. If you have any other questions or concerns, please call the Office of Research Development and Compliance at the University of North Dakota at 701-777-4279. Additionally, if you have any questions regarding your rights as a research subject, you may contact the Altru Health System IRB at 701-780-1750.

CONSENT

Your signature below indicates that you have read this form, have had the study explained, and have had any questions answered to your satisfaction, that you now understand what will be expected of you, that you agree to take part in this study, and that you authorize the use of your personal health information and agree to take part in this study.

A copy of this signed Informed Consent Statement will be given to you

I understand that my medical records and study records are confidential. However, representatives of the study sponsor, the U.S. Food and Drug Administration (FDA), or the Institutional Review Board (IRB) may need to inspect my medical and/or study records. By signing this consent, I am allowing this inspection.

_____ _____

Your Signature Date

_____

Your Name Printed

_____ _____

Study Team Member Date

_____

Study Team Member

Maternal Vitamin D Supplementation to Correct Deficiency in Mothers and Breastfed Infants

Authorization for Access to Personal Health Information

A federal government rule has been issued to protect the privacy rights of patients. The rule is designed to protect the confidentiality of your personal health information. We are required by these new regulations to obtain your authorization to share personal health information that may reveal your identity.

What Information will be Used or Disclosed
For this research study, the health information to be used or disclosed includes information contained in your existing medical records and new information created or collected during this study. Your records may include information about your physical examinations, medical history, blood samples, and any other data collected or reviewed during the course of the study as described in the consent form. Specifically, we will be collecting vitamin D levels, as well as possible other related analysis, from obtained blood samples, information about the course of your pregnancy and delivery, and information about how you feed your infant in the first 4-6 weeks after birth.

Purpose for Use or Disclosure
The purpose for use or disclosure of information gathered will be to develop a better understanding of how vitamin D levels in the mother effect how much vitamin D her baby receives through breast milk. This study will help measure the safety and effectiveness of vitamin D supplementation for women and their breastfed babies.

Who May Use or Disclose Information
The persons and organizations that may use or disclose your individually identifiable health information may include: approved staff members, auditors, such as the Altru or University of North Dakota Institutional Review Board and USDA auditors, and other state or federal agencies as provided by federal regulations.

Who May Receive Information
The persons and entities that may receive your personal health information may include: Doria Thiele, CNM, APRN, Principal Investigator, Dr. Cindy Anderson, PhD, WHNP-BC, FAAN, Dr. David Roth, Statistical Consultant, and personnel at the Grand Forks Human Nutrition Research Center. The data sent by the principal investigator to the sponsor usually does not include your name, address, or social security number. However, the sponsor might review or copy all of your records to assure the quality of the study or for other uses allowed by law.

Every effort will be made to maintain confidentiality of information accessed. However, absolute confidentiality cannot be guaranteed. Once your personal health information is released it may be re-disclosed, at which point your health information will no longer be protected by federal privacy regulations.

Duration of Authorization
This authorization is effective until the end of this research study.

Right to Refuse, Withdraw or Cancel Authorization
You may refuse to sign this authorization. If you refuse to sign this authorization, you will not be able to take part in this study. However, you will not be penalized or lose any benefits to which you are otherwise entitled. You will continue to receive treatment for your condition.

You have the right to cancel this authorization or withdraw from this study at any time with no penalty. If you choose to do so, you must notify the principal investigator in writing at Doria Thiele, CNM, APRN, IBCLC at College of Nursing, University of North Dakota, 400 Oxford

Street Stop 9025, Grand Forks, North Dakota, 58202-9025. Data collected prior to cancellation of this authorization may be used in order to preserve the scientific integrity of the study.

Patient Access to Records
You have the right to access your medical records at any time. However, you will not be able to access study specific information until the study is completed, at which time your right of access will be restored.

Privacy Authorization
I have read this Privacy Authorization and have had my questions answered to my satisfaction at this time. I understand that by signing this consent, I authorize the release of my medical records and health information related to this study. I authorize the use, disclosure, review, duplication, storage and data transfer of my medical records and study information. I understand this information may be obtained by the persons and organizations stated above. I will receive a copy of this signed authorization.

Signature of Participant	Date	Name of Participant (Printed)

Or

Signature of Legal Representative (Printed)	Date	Name of Legal Representative

Relationship to Participant

Appendix E
Food Frequency Questionnaire

Food Frequency Questionnaire **ID Number:**_____ **Date:**_____

Instructions:

- Mark each line/food item only once (whatever is the most accurate – daily, weekly, or monthly).
- If there are any foods/beverages you eat on a regular basis that are not listed, please write them down in the space provided after each table.

Example:	each month		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Milk *(drank twice a day during past 3 months)*							X		
Chocolate milk *(did not drink at all during past 3 months)*	X								

During the last 3 months, how often did you eat or drink a serving of the foods or beverages listed here?

DAIRY PRODUCTS	**each month**		**each week**			**each day**			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Milk, 8 fluid ounces									
Chocolate milk (hot or cold), 8 fluid ounces									
Instant nonfat dry milk, ⅓ cup (powder)									
Evaporated milk (nonfat), ½ cup									
Cheese (plain or as part of a dish or sandwich), 1 ounce									
Yogurt, 1 cup									
Ice cream, 1 cup									
Butter, 1 teaspoon									
Margarine, 1 teaspoon									

What kind of milk do you usually drink?

☐ Skim ☐ 1% ☐ 2% ☐ Whole ☐ Soymilk ☐ Other:_____

Are there any other dairy products you ate/drank in the last 3 months not listed above? ☐ No ☐ Yes

If yes, please name:_____ If yes, how often:_____

FRUIT & FRUIT JUICES	**each month**		**each week**			**each day**			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Orange juice, 8 fluid ounces									
Orange juice (with Calcium & Vitamin D), 8 fluid ounces									
Other 100% fruit juice, 8 fluid ounces									
Lemonade, 8 fluid ounces									
Fruit drinks (Kool-Aid, Hi-C, Gatorade), 8 fluid ounces									
Apple or applesauce, 1 medium									
Banana, 1 medium									
Orange or grapefruit, 1 medium									
Watermelon, 1 medium wedge									
Strawberries, 1 cup whole									
Raisins or prunes, ½ cup									

Are there any other fruits/juices you ate/drank in the last 3 months not listed above? ☐ No ☐ Yes

If yes, please name:_____ If yes, how often:_____

Time: 1 2 3

Study 608 Food Frequency Questionnaire

VEGETABLES	each month		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Tomatoes or tomato juice, 1 medium									
Green beans, 1 cup									
Carrots, 1 cup									
Corn, 1 cup									
Broccoli or cauliflower, 1 cup									
Dried beans or peas, 1 cup prepared									
Winter (orange) squash, 1 cup									
Sweet potatoes or yams, 1 medium									
Spinach or other greens, 1 cup									
Potatoes (baked, boiled, or mashed), 1 cup									
French fries or hash browns, 1 medium order									
Vegetable soup, 1 cup									
Lettuce salad, 1 ½ cups									
Salad dressing or mayonnaise, 2 tablespoons									

Are there any other vegetables you ate in the last 3 months not listed above? ☐ No ☐ Yes

If yes, please name:_____ If yes, how often:_____

SNACKS, SWEETS, AND BEVERAGES	each month		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Chips (potato, corn, etc.), 1 ounce (about 15 chips)									
Peanuts, 1 ounce									
Mixed nuts, 1 ounce									
Sunflower seeds, 1 ounce									
Brownies or cookies, 1 piece (2-inch square)									
Pumpkin or sweet potato pie, 1 piece (⅛ of 9-inch pie)									
Other pie, 1 piece (⅛ of 9-inch pie)									
Pudding, ½ cup									
Jell-O gelatin, ½ cup									
Chocolate candy, 1 bar (about 1 ½ ounces)									
Other candy, 5 pieces									
Regular soda pop, 12 fluid ounces									
Sugar-free soda pop, 12 fluid ounces									
Coffee or tea, 8 fluid ounces									
Beer or wine cooler (other alcoholic drinks), 12 fluid ounces									

Are there any other snacks/sweets/beverages you ate/drank in the last 3 months not listed above? ☐ No ☐ Yes

If yes, please name:_____ If yes, how often:_____

Study 608 Food Frequency Questionnaire

EGGS, MEATS, FISH, MAIN DISHES, ETC.	each month		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Eggs, 2 large									
Bacon or sausage, 1 piece									
Peanut butter, 2 tablespoons									
Hamburger (prepared any way), 3 ounces prepared									
Chicken or turkey, 3 ounces prepared									
Pork chops, roast pork or ribs, 3 ounces prepared									
Steak or roast (beef, bison, venison), 3 ounces prepared									
Salmon, 3 ounces prepared									
Oil-packed canned tuna, 3 ounces prepared									
Water-packed canned tuna, 3 ounces prepared									
Fried fish or fish sticks, 3 ounces prepared									
Baked/broiled fish (halibut, walleye, cod), 3 ounces prepared									
Pickled herring, 2 pieces									
Sardines, 2 pieces									
Spaghetti or other pasta with sauce, 1 cup									
Macaroni and cheese, 1 cup									
Pizza, 2 small slices									
Stew, 1 cup									

Are there any other main dish items you ate in the last 3 months not listed above? ☐ No ☐ Yes

If yes, please name:_____ If yes, how often:_____

BREADS AND CEREALS	each month		each week			each day			
Number of times	0	1-3	1	2-4	5-6	1	2-3	4-5	6+
Oatmeal or other hot cereal, 1 cup prepared									
Cold cereal, 1 cup (1 ounce)									
White bread or rolls, 1 slice									
Whole wheat bread or rolls, 1 slice									
Flour tortilla, 1 medium									
Cornbread or corn tortilla, 1 medium									
Rice (white or brown), 1 cup									
Wild rice, 1 cup									
Popcorn, 2 cups popped									

What kind of cold cereal do you usually eat?

☐ Cheerios ☐ Frosted Flakes ☐ Raisin Bran ☐ Total ☐ Other:_____

Are there any other breads/cereals you ate in the last 3 months not listed above? ☐ No ☐ Yes

If yes, please name:_____ If yes, how often:_____

Study 608 Food Frequency Questionnaire

- Are there any other foods with added vitamin D that you ate/drank in the last 3 months? ☐ No ☐ Yes
- If yes, please name: _____ If yes, how often: _____

Please answer the following questions regarding your dietary history and lifestyle over the past THREE MONTHS:

1. Do you take vitamins or minerals? ____ No ____ Yes

2. If yes, check all nutrients and/or combinations (select individual vitamins/minerals if taken as single item per tablet/capsule).

____ Antacids w/ Calcium	Brand: _____	How many: ____ per Day / Week / Month (circle one)
____ Prenatal Vitamins	Brand: _____	How many: ____ per Day / Week / Month (circle one)
____ Multiple Vitamins	Brand: _____	How many: ____ per Day / Week / Month (circle one)
____ Multiple Vitamins + Iron	Brand: _____	How many: ____ per Day / Week / Month (circle one)
____ Multiple Vitamins/Minerals	Brand: _____	How many: ____ per Day / Week / Month (circle one)
____ B-Complex	Brand: _____	How many: ____ per Day / Week / Month (circle one)
____ Calcium + Vitamin D	Brand: _____	How many: ____ per Day / Week / Month (circle one)
____ Vitamin A	Amount per tablet/capsule: _____	How many: ____ per Day / Week / Month (circle one)
____ Vitamin C	Amount per tablet/capsule: _____	How many: ____ per Day / Week / Month (circle one)
____ Vitamin D	Amount per tablet/capsule: _____	How many: ____ per Day / Week / Month (circle one)
____ Vitamin E	Amount per tablet/capsule: _____	How many: ____ per Day / Week / Month (circle one)
____ Folic Acid	Amount per tablet/capsule: _____	How many: ____ per Day / Week / Month (circle one)
____ Calcium	Amount per tablet/capsule: _____	How many: ____ per Day / Week / Month (circle one)
____ Iron	Amount per tablet/capsule: _____	How many: ____ per Day / Week / Month (circle one)
____ Zinc	Amount per tablet/capsule: _____	How many: ____ per Day / Week / Month (circle one)
____ Other (please list) _____		How many: ____ per Day / Week / Month (circle one)
____ Don't Know		

3. Do you take herbal/other nutrition supplements, like Echinacea, Fish oil, Fiber, etc.? ____ No ____ Yes

4. If yes, please list name, amount per item, and number taken per day, week, or month for each: _____

5. Do you follow a special diet? ____ No ____ Yes

6. If yes, please check all that apply: ____ Diabetic ____ Low sodium ____ Low fat ____ Low cholesterol ____ High protein ____ Weight loss ____ Weight gain ____ Other diet (please name) _____
   ____ Vegetarian (Please circle the foods that you **DO NOT** eat.) Dairy Eggs Pork Fish Seafood/Shellfish Poultry Red Meat Other foods (please list) _____

7. Do you have any food allergies? (Please choose all that apply.) ____ No known food allergies ____ Eggs ____ Milk ____ Dairy foods ____ Nuts ____ Wheat ____ Other (please name) _____

8. How many days a week do you usually eat a: Morning meal _____ Midday meal _____ Evening meal _____

9. When do you snack? (Please choose all that apply.) ____ Morning ____ Afternoon ____ Evening ____ Do not snack

10. Where do you eat most of your meals? (Please choose all that apply.) ____ Home ____ Work ____ Restaurant/Cafeteria ____ Other (please list) _____

11. In your household, who does most of the food preparation? ____ Self ____ Spouse/partner ____ Restaurant/Cafeteria ____ Other (please name) _____

12. Within the past **THREE MONTHS** have you lost or gained weight? ____ No ____ Yes, lost ____ Yes, gained

13. If yes, how much did your weight change? _____ Pounds

Appendix F
Infant Intake Tool

ID#:_____

Age Weeks	Age Days	Vitamin D Supplement	Formula Intake Brand Name	Formula Intake Number of Ounces Eaten
Example	1	400 IU	None	None
Example 2	4	None	Similac Advance	1 oz after nursing X 8 nursings = 8 oz for the day
0	1			
	2			
	3			
	4			
	5			
	6			
	7			
1	8			
	9			
	10			
	11			
	12			
	13			
	14			
2	15			
	16			
	17			
	18			
	19			
	20			
	21			
3	22			
	23			
	24			
	25			
	26			
	27			
	28			
4	29			
	30			
	31			
	32			
	33			
	34			
	35			
5	36			
	37			
	38			
	39			
	40			
	41			
	42			
6	43			
	44			

Appendix G
Record Retrieval Form

ID Number:

Reviewed by; _____ On: _____/_____/_____

Instructions

Complete this medical chart abstraction form for each participant enrolled in the study.

A. Detailed Past Obstetrical History

LMP: ___/___/___ **EDC**:___/___/___ **Date of First Prenatal Visit:** ___/___/___ **Maternal Age at Time of Delivery:** _____

Gravida Status

G: ____	P: ____	T: ____	A: ____	L: ____

Pregnancy Outcome	Type of Delivery |__|__|	Birth Weight	Maternal Description	Maternal Code	Fetal/Infant Description	Fetal/Infant Code	If LB & Child NOT Living
1. **Date:** __ __/__ __/__ __ Time: _____ *mm/dd / yy* **GA:** |__|__| / |__|__| *wks days* **GA code:** |__|__| **Gender:** |__|__|		1) __ __/ __ __ *lbs / oz* ***OR*** 2) _____ *grams*	1)_____ 2)_____ 3)_____	1) |__|__| 2) |__|__| 3) |__|__|	1)_____ 2)_____ 3)_____	1) |__|__| 2) |__|__| 3) |__|__|	**Date of Death:** __ __/__ __/__ __ *mm /dd / yy* **Reason for death:** _____ **Code:** |__|__|

Outcome	GA (Gestational Age) code	Gender	Maternal Complications	Fetal/Infant Complications	Child NOT Living (Reason for Death)
LB=Live birth SB=Stillbirth SA=Spontaneous Abortion TA=Therapeutic Abortion EP=Ectopic Pregnancy MP=Molar Pregnancy	Enter time in weeks/days if documented. If number of weeks is not documented, enter: FT=full term(≥ 37+ weeks) NT=near term (32-36 wks) PT=early preterm (20-31 wks) ET=early termination (<20 wks)	01=male 02=female **Type of Delivery** 01=vaginal 02=op vaginal (forceps or vacuum) 03=C-section	00=None noted 01=Preeclampsia 02- Gestational Hypertention 03=Placenta previa 04=Placental abruption 05=Pre-gestational diabetes – Type I 06=Pre-gestational diabetes – Type II 07=Gestational diabetes 08=PPH, requiring blood transfusion 09=PTL requiring treatment 10=PPROM 99=Other	00=None noted 01-Intra-Uterine Growth Restriction 02- Small for Gestational Age 03- Large for Gestational Age 04-Respiratory Distress Syndrome 05-Meconium Aspiration 06=Other defect 07=Other genetic disease 08-Observe for sepsis 09-Hypoglycemia 99=Other	01=Cardiovascular 02=Congenital Defect 03=Respiratory 04-Prematurity 05-Sepsis 06-Unknown 99=Other

Coding Key:
-4 = Temporarily Missing (not currently available) -7 = Don't Know
-5 = Multiple Responses (needs review) -8 = Refused to Answer
-6 = Permanently Missing (not documented in chart) -9 = Does Not Apply

B. History of Medical Conditions

	Medical Condition	Present (circle one for each)	Absent (circle one for each)	Receiving Medication (circle one for each)	Not Receiving Medication (circle one for each)
1.	Asthma	1	2	1	2
2.	Seizure disorder	1	2	1	2
3.	Chronic hypertension	1	2	1	2
4.	Diabetes mellitus (type 1 & 2)	1	2	1	2
5.	Hyperthyroidism	1	2	1	2
6.	Hypothyroidism	1	2	1	2
7.	Valvular heart disease	1	2	1	2
8.	Other structural heart disease	1	2	1	2
9.	Coronary artery disease/ congestive heart failure	1	2	1	2
10.	Nephropathy/Nephrotic syndrome/ Glomerulonephritis	1	2	1	2
11.	Renal insufficiency/ renal failure	1	2	1	2
12.	Sickle cell anemia	1	2	1	2
13.	Thrombocytopenia	1	2	1	2
14.	Lupus erythematosus	1	2	1	2
15.	Antiphospholipid antibody syndrome	1	2	1	2
16.	Rheumatoid arthritis	1	2	1	2
17.	Ulcerative colitis/Crohn's disease	1	2	1	2
19.	Malignancy Specify _____ (please specify)	1	2	1	2
20.	Hepatitis B	1	2	1	2
21.	Hepatitis C	1	2	1	2
22.	Psychiatric Disorder Specify _____ (please specify)	1	2	1	2
23.	Other Specify _____	1	2	1	2

Medical Condition	(circle one for each) Present	Absent	Receiving Medication	Not Receiving Medication
24. Other Specify _____ (please specify)	1	2	1	2
25. Other Specify _____ (please specify)	1	2	1	2
26. Other Specify _____ (please specify)	1	2	1	2

C. Prescription Medication, Vitamins and Vaccines

	Medication, Vitamin, Vaccine Name	Code*	other	Trimester 01=Taken 02=Not taken 1st	2nd	3rd	Postpartum
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							

Coding Key:
-4 = Temporarily Missing (not currently available)
-5 = Multiple Responses (needs review)
-6 = Permanently Missing (not documented in chart)
-7 = Don't Know
-8 = Refused to Answer
-9 = Does Not Apply

Medications (100- 300 series)					Vitamins (500 series)	Vaccines (600 series)
Analgesics 101=Narcotic 102=NSAID 103=Aspirin 104=Acetaminophen	110=Antibiotics 120=Anticoagulants 130=Antidepressants 140=Anticonvulsants 150=Antihistamines	Antihypertensives 161= Aldomet 162= Labetolol 163= Ca-Channel Blockers 164= Beta-Blockers 165= Ace-Inhibitor 169 = Other antihypertensive	170=Antiemetics 180=Antipsychotics 190=Antivirals 200=Birth control pills 210=Chemotherapeutics 220=Diuretics 230=GI agents 240=Progesterone 260=Rhogam 270= Sleep Aide 280=Steroids	Thyroid Agents 251=Antithyroids (overactive) 252=Thyroid Replacement (under active) 399=Other Medication	510=Multi – vitamin 520=Iron 530=Folate 540=Calcium + D 599=Other vitamin	610=Influenza 620=Hepatitis B 630=Rubella 640=Varicella-zoster immune globulin (VZIG) 699=Other vaccine

D. Prenatal Care Visits

Instructions

Complete the Prenatal Care Flow sheet on all women enrolled in the study using medical records. Complete one row for each prenatal visit.

Visit	Date mm/ dd / yy	Weight lbs	Highest Blood Pressure mm Hg	Fundal Height cm	Fetal Heart Rate 01=present 02=absent	Fetal Movement 00=normal 01=decreased 02=absent	Urine Dipstick Proteinuria 01=Negative 02=Trace 03=+1 04=+2 05=+3 06=+4	Urine Dipstick Glucosuria 01=Negative 02=Trace 03= ≥ +1	Other Condition Specify
1.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
2.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
3.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
4.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						

5.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
6.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
7.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
8.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
9.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						

Visit	Date mm/ dd / yy	Weight lbs	Highest Blood Pressure mm Hg	Fundal Height cm	Fetal Heart Rate 01=present 02=absent	Fetal Movement 00=normal 01=decreased 02=absent	Urine Dipstick Proteinuria 01=Negative 02=Trace 03=+1 04=+2 05=+3 06=+4	Urine Dipstick Glucosuria 01=Negative 02=Trace 03= ≥ +1	Other Condition Specify
10.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
11.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
12.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
13.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
14.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
15.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
16.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
17.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						
18.	__ __/ __ __/ __ __		1. Sys __ __ __ 2. Dia __ __ __						

Coding Key:
-4 = Temporarily Missing (not currently available) -7 = Don't Know
-5 = Multiple Responses (needs review) -8 = Refused to Answer
-6 = Permanently Missing (not documented in chart) -9 = Does Not Apply

E. General Prenatal Labs

1. **Mother's Blood Type:** *(circle one)*

   A.....1
   B.....2
   O.....3
   AB.....4

2. **RH Factor:** *(circle one)*

   Positive.....1
   Negative.....2

b. Date of most recent RhIG given:
(mm/dd/yyyy)

___ ___ / ___ ___ / ___ ___

3. **HCT/HgB**:

	Date mm/ dd / yy	Result HgB g/dl	HCT %
a.	__ __/ __ __/ __ __	__ __ . __	__ __ . __
b.	__ __/ __ __/ __ __	__ __ . __	__ __ . __
c.	__ __/ __ __/ __ __	__ __ . __	__ __ . __

4. **GBS:**

	Date mm/ dd / yy	Result 01= Positive 02= Negative
a.	__ __/ __ __/ __ __	___ ___
b.	__ __/ __ __/ __ __	___ ___

5. **Urinalysis:**

	Date mm/ dd / yy	Result(s) 01=Negative 02=Protein 03=Ketones 04=Bacteria	If Other, specifyy	00 = Absent, 1+, 2+, 3+ or 4+, trace (5)
a.	__ __/ __ __/ __ __			___ ___
b.	__ __/ __ __/ __ __			___ ___
c.	__ __/ __ __/ __ __			___ ___
d.	__ __/ __ __/ __ __			___ ___

6. **Urine culture:**

	Date mm/ dd / yy	Result 01=Positive 02=Negative	If Positive, specify organism 01=GBS 02=E. coli 03=Other	If Other, specify
a.	__ __/ __ __/ __ __	___ ___	___ ___	Other: _____
b.	__ __/ __ __/ __ __	___ ___	___ ___	Other: _____
c.	__ __/ __ __/ __ __	___ ___	___ ___	Other: _____
d.	__ __/ __ __/ __ __	___ ___	___ ___	Other: _____

Coding Key:
-4 = Temporarily Missing (not currently available)
-5 = Multiple Responses (needs review)
-6 = Permanently Missing (not documented in chart)
-7 = Don't Know
-8 = Refused to Answer
-9 = Does Not Apply

7. Hepatitis B Surface Antigen:

	Date mm/ dd / yy	Result 01= Positive 02= Negative	If other, hep B serology: (use codes below)
a.	__ __/ __ __/ __ __	__ __	__ __
b.	__ __/ __ __/ __ __	__ __	__ __

Other hep B serology codes:

01=HbeAg ((Hepatitis E antigen)
02=Anti HBc IgM (IgM core antibody)
03=Anti-HBs (Surface antibody)
04=Anti–Hbe (e antibody)
05=HBV DNA

8. PPD: *(circle one)*

Positive..... 1
Negative..... 2

9. Chlamydia:

	Date mm/ dd / yy	Result 01= Positive 02= Negative
a.	__ __/ __ __/ __ __	__ __
b.	__ __/ __ __/ __ __	__ __
c.	__ __/ __ __/ __ __	__ __

10. Gonorrhea:

	Date mm/ dd / yy	Result 01= Positive 02= Negative
a.	___/___/___	___ ___
b.	___/___/___	___ ___
c.	___/___/___	___ ___

11. Diabetes screen:

	Date mm/ dd / yy	Blood Sugar Result Fasting	1 Hour	Random
		_____.___	_____.___	_____.___
		☐ mg/dl	☐ mg/dl	☐ mg/dl
a.	___/___/___	☐ mmol/L	☐ mmol/L	☐ mmol/L
		_____.___	_____.___	_____.___
		☐ mg/dL	☐ mg/dl	☐ mg/dl
b.	___/___/___	☐ mmol/L	☐ mmol/L	☐ mmol/L

12. GTT:

	Date mm/ dd / yy	Blood Sugar Result Fasting	1 hr	2 hr	3 hr
		___ ___ ___ . ___	___ ___ ___ . ___	___ ___ ___ . ___	___ ___ ___ . ___
		☐ mg/dl	☐ mg/dl	☐ mg/dl	☐ mg/dl
a.	___/___/___	☐ mmol/L	☐ mmol/L	☐ mmol/L	☐ mmol/L
		___ ___ ___ . ___	___ ___ ___ . ___	___ ___ ___ . ___	___ ___ ___ . ___
		☐ mg/dl	☐ mg/dl	☐ mg/dl	☐ mg/dl
b.	___/___/___	☐ mmol/L	☐ mmol/L	☐ mmol/L	☐ mmol/L

F. Specialized Testing

Instructions

Complete the Specialized Prenatal Labs on all women enrolled prospectively into the study who have results of specialized testing in their charts. All questions refer to the mother. If the number of tests done of a type exceeds the number of rows provided, enter the results from the first prenatal lab tests on the first row. Use the remaining rows to enter the most recent results chronologically.

1. Specialized testing:

(circle one)

No 0

Yes 1

1. Hemoglobin (HgB) A1C:

	Date mm/ dd / yy	Result %
a.	__ __/ __ __/ __ __	__ __ . __
b.	__ __/ __ __/ __ __	__ __ . __
c.	__ __/ __ __/ __ __	__ __ . __
d.	__ __/ __ __/ __ __	__ __ . __

3. Twenty-four hour urine protein:

	Date mm/ dd / yy	Result mg/24 hr
a.	__ __/ __ __/ __ __	__ __ __ __ __
b.	__ __/ __ __/ __ __	__ __ __ __ __
c.	__ __/ __ __/ __ __	__ __ __ __ __

2.

3.

4. Pre-Eclampsia Labs:

4.

Test	Date Done mm/dd/yy	Result	Units
LDH	/ /		IU
	/ /		IU
	/ /		IU
	/ /		IU
AST (SGOT)	/ /		IU
	/ /		IU
	/ /		IU
	/ /		IU
ALT (SGPT)	/ /		IU
	/ /		IU
	/ /		IU
	/ /		IU

Uric Acid	/ /		mg/dL
	/ /		mg/dL
	/ /		mg/dL
	/ /		mg/dL
BUN	/ /		mg/dL
	/ /		mg/dL
	/ /		mg/dL
	/ /		mg/dL
Creatinine	/ /		mg/dL
	/ /		mg/dL
	/ /		mg/dL
	/ /		mg/dL
Hemoglobin	/ /		g/dL
	/ /		g/dL
	/ /		g/dL
	/ /		g/dL

Platelets	/ /		K/uL
	/ /		K/uL
	/ /		K/uL
	/ /		K/uL
Urine Prot/ Creat Ratio	**Date** mm/dd/yy	**Ratio Result**	
	/ /		urine total protein ___ mg/dL urine creatinine, random ___ mg/dL
	/ /		urine total protein ___ mg/dL urine creatinine, random ___ mg/dL
	/ /		urine total protein ___ mg/dL urine creatinine, random ___ mg/dL
	/ /		urine total protein ___ mg/dL urine creatinine, random ___ mg/dL
24- hour urine protein	/ /	**Result**	mg/24 hr
	/ /		mg/24 hr
	/ /		mg/24 hr
	/ /		mg/24 hr

5. Other Lab Tests:

	Specify Test	Date Done mm / dd / yy	Result 01=Normal 02= Abnormal
a.		__ __ / __ __ / __ __	
b.		__ __ / __ __ / __ __	
c.		__ __ / __ __ / __ __	
d.		__ __ / __ __ / __ __	

6. Fetal Surveillance: Non-Stress Tests (NSTs):

	Date mm/ dd / yy	Result 01= Reactive 02= Non-reactive, reassuring 03= Nonreactive, nonreassuring: requires further testing or delivery
a.	__ __ / __ __ / __ __	__ __
b.	__ __ / __ __ / __ __	__ __
c.	__ __ / __ __ / __ __	__ __
d.	__ __ / __ __ / __ __	__ __
e.	__ __ / __ __ / __ __	__ __
f.	__ __ / __ __ / __ __	__ __
g.	__ __ / __ __ / __ __	__ __
h.	__ __ / __ __ / __ __	__ __
i.	__ __ / __ __ / __ __	__ __
j.	__ __ / __ __ / __ __	__ __

G. In-Patient Stay

Date	Highest Blood Pressures	On $MgSO_4$? (circle one)		
__ __/__ __/__ __	1. Sys __ __ __ 2. Dia __ __ __	yes no	**If yes:** Start date: Start time:	End date: End time
__ __/__ __/__ __	1. Sys __ __ __ 2. Dia __ __ __	yes no	**If yes:** Start date: Start time:	End date: End time
__ __/__ __/__ __	1. Sys __ __ __ 2. Dia __ __ __	yes no	**If yes:** Start date: Start time:	End date: End time
__ __/__ __/__ __	1. Sys __ __ __ 2. Dia __ __ __	yes no	**If yes:** Start date: Start time:	End date: End time

Appendix H
Postpartum Record Retrieval Form

ID Number:

Reviewed by: _____ On: _____/_____/_____

Instructions

Complete this medical chart abstraction form for each participant enrolled in the study.

A. Detailed Past Obstetrical History

LMP: ___/___/___ **EDC**:___/___/___ **Date of First Prenatal Visit:** ___/___/___ **Maternal Age at Time of Delivery:** _____

Gravida Status				
G: ____	P: ____	T: ____	A: ____	L: ____

Pregnancy Outcome	Type of Delivery	Birth Weight				If LB & Child NOT Living
	____		Maternal Description	Code	Fetal/Infant Description	Code	
1. **Date:** __ __/__ __/__ __ *mm/dd/yy* **Time:** _____ **GA:** ____/____ *wks days* **GA code:** ____ **Gender:** ____	1) __ __/__ __ *lbs/oz* ***OR*** 2) _____ *grams*		1)_____ 2)_____ 3)_____	1) ____ 2) ____ 3) ____	1)_____ 2)_____ 3)_____	1) ____ 2) ____ 3) ____	**Date of Death:** __ __/__ __/__ __ *mm/dd/yy* **Reason for death:** _____ **Code:** ____

Outcome	GA (Gestational Age) code	Gender	Maternal Complications	Fetal/Infant Complications	Child NOT Living (Reason for Death)
LB=Live birth SB=Stillbirth SA=Spontaneous Abortion TA=Therapeutic Abortion EP=Ectopic Pregnancy MP=Molar Pregnancy	Enter time in weeks/days if documented. If number of weeks is not documented, enter: FT=full term(≥ 37+ weeks) NT=near term (32-36 wks) PT=early preterm (20-31 wks) ET=early termination (<20 wks)	01=male 02=female **Type of Delivery** 01=vaginal 02=op vaginal (forceps or vacuum) 03=C-section	00=None noted 01=Preeclampsia 02- Gestational Hypertention 03=Placenta previa 04=Placental abruption 05=Pre-gestational diabetes – Type I 06=Pre-gestational diabetes – Type II 07=Gestational diabetes 08=PPH, requiring blood transfusion 09=PTL requiring treatment 10=PPROM 99=Other	00=None noted 01-Intra-Uterine Growth Restriction 02- Small for Gestational Age 03- Large for Gestational Age 04-Respiratory Distress Syndrome 05-Meconium Aspiration 06=Other defect 07=Other genetic disease 08-Observe for sepsis 09-Hypoglycemia 99=Other	01=Cardiovascular 02=Congenital Defect 03=Respiratory 04-Prematurity 05-Sepsis 06-Unknown 99=Other

Coding Key:
-4 = Temporarily Missing (not currently available) -7 = Don't Know
-5 = Multiple Responses (needs review) -8 = Refused to Answer
-6 = Permanently Missing (not documented in chart) -9 = Does Not Apply

ID Number:

B. Prescription Medication, Vitamins and Vaccines – Received Since Delivery

Medication, Vitamin, Vaccine

	Name	Code*	other
1.	_____	__ __	__ ____ ____ __
2.	_____	__ __	__ ____ ____ __
3.	_____	__ __	__ ____ ____ __
4.	_____	__ __	__ ____ ____ __
5.	_____	__ __	__ ____ ____ __
6.	_____	__ __	__ ____ ____ __
7.	_____	__ __	__ ____ ____ __
8.	_____	__ __	__ ____ ____ __
9.	_____	__ __	__ ____ ____ __
10.	_____	__ __	__ ____ ____ __

Medications (100- 300 series)					**Vitamins (500 series)**	**Vaccines (600 series)**
Analgesics 101=Narcotic 102=NSAID 103=Aspirin 104=Acetaminophen	110=Antibiotics 120=Anticoagulants 130=Antidepressants 140=Anticonvulsants 150=Antihistamines	Antihypertensives 161= Aldomet 162= Labetolol 163= Ca-Channel Blockers 164= Beta-Blockers 165= Ace-Inhibitor 169 = Other antihypertensive	170=Antiemetics 180=Antipsychotics 190=Antivirals 200=Birth control pills 210=Chemotherapeutics 220=Diuretics 230=GI agents 240=Progesterone 260=Rhogam 270= Sleep Aide 280=Steroids	Thyroid Agents 251=Antithyroids (overactive) 252=Thyroid Replacement (under active) 399=Other Medication	510=Multi – vitamin 520=Iron 530=Folate 540=Calcium + D 599=Other vitamin	610=Influenza 620=Hepatitis B 630=Rubella 640=Varicella-zoster immune globulin (VZIG) 699=Other vaccine

ID Number:

C. Maternal Surgeries/Hospitalizations Since Delivery

	Type of Surgery	Days Hospitalized	Able to Breastfeed?	Long Term Implications
1				
2				
3				
4				

D. Maternal Diagnoses Since Delivery

	Medical Diagnosis	Medications Prescribed?	Able to Breastfeed?	Expected Duration
1				
2				
3				
4				
5				
6				

Medications (100- 300 series)					**Vitamins (500 series)**	**Vaccines (600 series)**
Analgesics 101=Narcotic 102=NSAID 103=Aspirin 104=Acetaminophen	110=Antibiotics 120=Anticoagulants 130=Antidepressants 140=Anticonvulsants 150=Antihistamines	Antihypertensives 161= Aldomet 162= Labetolol 163= Ca-Channel Blockers 164= Beta-Blockers 165= Ace-Inhibitor 169 = Other antihypertensive	170=Antiemetics 180=Antipsychotics 190=Antivirals 200=Birth control pills 210=Chemotherapeutics 220=Diuretics 230=GI agents 240=Progesterone 260=Rhogam 270= Sleep Aide 280=Steroids	Thyroid Agents 251=Antithyroids (overactive) 252=Thyroid Replacement (under active) 399=Other Medication	510=Multi – vitamin 520=Iron 530=Folate 540=Calcium + D 599=Other vitamin	610=Influenza 620=Hepatitis B 630=Rubella 640=Varicella-zoster immune globulin (VZIG) 699=Other vaccine

ID Number:

E. Infant Diagnoses Since Delivery

	Medical Diagnosis	Hospitalized?	Medications Prescribed?	Able to Breastfeed?	Expected Duration
1					
2					
3					
4					
5					
6					

F. Infant Feeding Practices

1. Infant has never received any formula
2. Infant received small amounts (≤ 3 oz) formula in the first week
3. Infant has received small amounts(≤ 3 oz) of formula several times
4. Infant has received small amounts (≤ 3 oz) of formula weekly
5. Infant receives small amounts (≤ 3 oz) of formula a few times per week
6. Infant receives full feedings (≥3 oz) of formula a few times per week
7. Infant receives full feedings (≥3 oz) of formula once a day
8. Infant receives full feedings (≥3 oz) of formula several times a day
9. Infant receives only formula feedings

Appendix I
Adverse Event Forms

UNIVERSITY OF UND NORTH DAKOTA

COLLEGE OF NURSING
NURSING BUILDING
430 OXFORD STREET STOP 9025
GRAND FORKS ND 58202-9025
(701) 777-4174
FAX (701) 777-4096

September 7, 2012

Dear Members of the UND Institutional Review Board,

On September 4th 2012 we were notified by Doria Thiele that one of the participants in her study was hospitalized overnight for a kidney stone. She also provided a recent article published by Dr. Hollis regarding the safety and efficacy of Vitamin D supplementation. The chair of Doria's committee, Dr. Cindy Anderson, was in contact with one of the participant's care providers and the nurse practitioner was unconcerned that this is associated with her participation in the study as well and feels it was only related to her increased risk with pregnancy.

Our immediate feedback to Doria was to ascertain, if possible, the results of any in-hospital lab studies, in particular the participant's 25(OH)D level. However, no labs were drawn as the patient's care providers are familiar with pregnancy-associated kidney stones and were unconcerned about her participation in Ms. Thiele's study.

The researchers were able to obtain the baseline vitamin D level for the participant. It was 74.5 nmol/L (greater than or equal to 80 nmol/L is considered "sufficient" by most contemporary vitamin D researchers and Ms. Thiele is using that as her cut-off for the study, with 225 nmol/L as the cut-off for maximum level). The participant's 25(OH)D level was certainly not too high, and we don't anticipate that it will be raised above the maximum cut-off by her on study treatment.

Our recommendation to Ms. Thiele was continue with the study as planned but additional bloodwork/investigation may be warranted if this patient or other patients experience similar events.

Sincerely,

On behalf of the members of Ms. Thiele's Data Safety Monitoring Board
Dr. Jody Ralph
Dr. Cindy Anderson
Dr. Liz Tyree
Dr. Edward Sauter
Dr. Leah Whigham

ADVERSE EVENT/UNANTICIPATED PROBLEM REPORT

RR 411-A 10/18/06

Adverse Event/Unanticipated Problems Involving Risk to Participants or Others

Protocol deviations, violations, adverse events, and/or unanticipated problems involving risks to participants or others may be reported to the IRB by anyone. It does not require the signature of the principal investigator. SUBMIT (1) COPY OF THIS FORM TO THE IRB WITHIN 5 CONSECUTIVE DAYS OF KNOWLEDGE OF EVENT/PROBLEM.

DATE SUBMITTED: ___9/11/2012 **IRB PROPOSAL NUMBER:** __IRB-201012-148_____
PROJECT TITLE: _____Maternal vitamin D supplementation to correct deficiency in mothers and breastfed infants_____
PRINCIPAL INVESTIGATOR: ____Doria K. Thiele_____

1. **DESCRIBE TYPE OF EVENT:**

- ☐ Event which in the opinion of the Principal Investigator (1) was unexpected, and (2) was related to the research procedures;
- ☐ Event that requires prompt reporting, according to the protocol, to the sponsor;
- ☐ Accidental or unintentional change to the IRB-approved protocol that involves risks or has the potential to recur;
- ☐ Deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant;
- ☐ Publication in the literature, safety monitoring report, interim result, or other finding that indicates an unexpected change to the risk/benefit ratio of the research;
- ☐ Breach in privacy/confidentiality/data security/loss of study data that may involve risk to that individual or others;
- ☒ Independent safety monitoring reports or data and safety monitoring board reports;
- ☐ Complaint of a participant that indicates an unanticipated risk or which cannot be resolved by the research staff;
- ☐ Incorrect labeling/dosing of study medication or test article

(**NOTE**: EVENTS THAT DO NOT FIT INTO THE ABOVE CATEGORIES DO NOT REQUIRE REPORTING TO THE IRB UNTIL THE RESEARCH PROJECT REVIEW AND PROGRESS REPORT IS FILED FOR CONTINUING REVIEW. HOWEVER, THE EVENT MAY REQUIRE REPORTING TO THE SPONSOR OR DATA MONITORING PLAN.)

2. **EVENT/PROBLEM DESCRIPTION:** Provide a description of the event/problem including the timing of study treatment, dosing, or intervention with start and stop dates of relevant research interventions. Include all relevant lab information pertinent to this adverse event or unanticipated problem:
One participant, now 33 weeks gestation, had an episode of a kidney stone that necessitated over night stay in the hospital with stent placement to remove the stone. The participant entered the study on 8/2. No lab work was completed at the hospital as they consider this a common occurrence in pregnancy. Her 25-Hydroxy Vitamin D upon entering the study was slightly insufficient at 74.5 nmol/L.

3. **IN THE OPINION OF THE PRINCIPAL INVESTIGATOR, WAS THIS EVENT:**

UNANTICIPATED?	☒YES ☐NO	(It was unforeseeable at the time of its occurrence)
SERIOUS?	☐YES ☒NO	(It adversely alters the research risk/benefit relationship)
RELATED?	☐YES ☒NO	(It is likely to have been caused by research procedures)

4. **PLEASE INDICATE TYPE OF REPORT:** ☐INITIAL (FIRST) REPORT OF EVENT/PROBLEM
☒FOLLOW-UP REPORT

IF A MEDWATCH REPORT (FDA FORM 3500) HAS BEEN SUBMITTED TO THE FDA, PLEASE ATTACH IT TO THIS REPORT AND SKIP QUESTIONS 5-9

5. **DATE OF EVENT/PROBLEM:** Informed on 9/4/12

6. **PARTICIPANT IDENTIFIER:** 612-0006 *(DO NOT INCLUDE NAME OR PERSONAL IDENTIFIERS)*

7. **PARTICIPANT AGE:** 43

8. **IDENTIFY DRUG/BIOLOGIC/DEVICE/TREATMENT/INTERVENTION** (if applicable): Vitamin D 3400 IU/day OR placebo. Prenatal vitamin daily.

IND # (if applicable)
IDE # (if applicable)

9. **LIST 3 – 4 KEYWORDS DESCRIBING THE EVENT/PROBLEM** (e.g., loss of confidentiality, nausea and vomiting): kidney stone in pregnancy

10. **HAS A DATA SAFETY MONITOR (DSM) REVIEWED THIS EVENT/PROBLEM** (CHOOSE ONE):

☒IF YES, CHOOSE ONE:
- ☒ A COPY OF THE DSM'S REVIEW OF THE EVENT/PROBLEM IS ATTACHED
- ☐ THE DSM HAS NOT REVIEWED THE EVENT/PROBLEM
- ☐ DSM REVIEW IS PENDING

☐STUDY DOES NOT HAVE A DSM

11. **THIS EVENT/PROBLEM IS** (CHOOSE ONE OF THE FOLLOWING):

- ☐ Currently described as a risk in the informed consent document and does not require submission of an amendment.
- ☒ Not listed as a risk in the informed consent document and submission of an amendment is not recommended at this time. Please explain: Pregnancy is a risk factor for kidney stones and it is not believed that this is related to the study or that other participants are at increased risk because of their participation.
- ☐ Not listed as a risk in the informed consent document and requires submission of an amendment.

12. **HAS THE PI BEEN NOTIFIED OF THIS EVENT/PROBLEM AND RECEIVED A COPY OF THIS REPORT?**

☒YES ☐NO The PI should be notified of all protocol deviations, protocol violations, adverse events, and/or unanticipated problems involving risks to participants or others. The PI is responsible for the accurate documentation, investigation, and follow-up of all protocol deviations, protocol violations, adverse events, and/or unanticipated problems involving risks to participants or others that are possibly related to study participation.

13. **NUMBER OF PARTICIPANTS ENROLLED IN THE STUDY:** 14

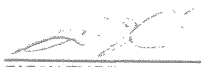
14. **IF A PARTICIPANT WAS INVOLVED, WILL HE/SHE CONTINUE WITH THE STUDY?** ☒YES ☐NO
IF NO, DATE STOPPED: _____

15. **WHAT ACTIONS HAVE BEEN TAKEN? WHAT WILL BE DONE TO MINIMIZE THE CHANCE OF REOCCURRENCE?**
The case has been reviewed by the DSMB. Both the DSMB and the Principal Investigator agree this was not study related, but should it re-occur we will consider other options for this participant.

16. **HAS THIS EVENT/PROBLEM BEEN REPORTED TO THE SPONSOR?** ☐YES ☒NO
If *"NO"*, please provide rationale for not reporting: Event not likely a direct result of the study intervention.

17. **SPONSOR'S RESPONSE (*IF APPLICABLE*):**

18. **ADDITIONAL COMMENTS:**



SIGNATURE

9/11/2012
DATE

ROLE IN STUDY

Altru HEALTH SYSTEM

Institutional Review Board (IRB)
Adverse Event Report

Revised 5/10/11
All serious adverse events, whether occurring at the local study site or at other study sites, are to be reported to the Altru Health System IRB using this form.

Principal Investigator: Doria K. Thiele IRB # ST-103

Project Name: Maternal vitamin D supplementation to correct deficiency in mothers and breastfed infants.

Date of Event: 9/4/2012 Date Known to You: 9/4/2012

Name of Study Drug, Device or Procedure: (not study name) Vitamin D 3800 IU/Daily, Or, Placebo (400 IU daily from prenatal vitamin)

Name of Adverse Event: kidney stones

Adverse Event #: 1

Initial Report (Yes or No): Yes Follow-up report #

Detailed description of adverse event and action taken (use additional pages or attach additional documentation, if necessary):

One of the study participants developed a kidney stone which necessitated an over-night stay in the hospital with ureter stent placement. Kidney stones are relatively common in pregnancy. She has been in the study for 1 month and is now 33 weeks gestation. Upon learning of this incident, her primary care clinic was contacted and they reported they felt it was not study related. They also reported that no labs were done at the hospital as this is considered to be solely pregnancy related. I then contacted the Data Safety Monitoring Board with the information and they are currently working on reviewing the data and drafting a report. The participant's baseline circulating vitamin D level (25[OH]D) was 74.5 nmol/L (80 nmol/L to 225 nmol/L is considered sufficient/normal), so she did not enter the study with an unusually high 25[OH]D.

Outcome of adverse event: (Check all that apply)

___	Death	___	Disability/incapacity		
___	Required Intervention	___	Life threatening	___	Congenital anomaly/birth defect
X	Hospitalization-initial or prolonged	___	Other (Explain):		

Did this event occur in the above listed study?	X Yes	___ No
Is this type of adverse event described in the consent form approved by this IRB?	___ Yes	X No
Did this event occur to a subject enrolled in the study at Altru Health System?	X Yes	___ No

If yes, was this event reported to: ___ *Study Sponsor* __X__ *Co-Investigators*

Relationship of the Event to any research treatment appears to be: (**Sponsor assessment**)

___ Unknown ___ Not related ___ Unlikely ___ Possibly ___ Probably ___ Related

Relationship of the Event to any research treatment appears to be: (**PI assessment** – site where event occurred)

___ Unknown X Not related ___ Unlikely ___ Possibly ___ Probably ___ Related

Signature of person reporting: ___ Date: ___

Printed name of person reporting: ___

Signature of Principal Investigator: [signature] Date: 9/7/2012

Printed name of Principal Investigator: DORIA THIELE

IRB USE ONLY

Will this event require a change in timeframe for continuing review? ___ Yes ___ No

Comments:

Will the event require changes in the consent form used at this site? ___ Yes ___ No

Comments:

This adverse event report has been reviewed and approved by:
Signature of IRB Chair/designee: _____ Date: _____

*Completed by:*_____

Altru HEALTH SYSTEM

Copy to Doria 9/20/12

Institutional Review Board (IRB)
Adverse Event Report

Revised 5/10/11
All serious adverse events, whether occurring at the local study site or at other study sites, are to be reported to the Altru Health System IRB using this form.

Principal Investigator: Doria K. Thiele IRB # ST-103

Project Name: Maternal vitamin D supplementation to correct deficiency in mothers and breastfed infants.

Date of Event: 9/4/2012 Date Known to You: 9/4/2012

Name of Study Drug, Device or Procedure: (not study name) Vitamin D 3800 IU/Daily, Or, Placebo (400 IU daily from prenatal vitamin)

Name of Adverse Event: kidney stones

Adverse Event #: 1

Initial Report (Yes or No): Yes Follow-up report #

Detailed description of adverse event and action taken (use additional pages or attach additional documentation, if necessary):

One of the study participants developed a kidney stone which necessitated an over-night stay in the hospital with ureter stent placement. Kidney stones are relatively common in pregnancy. She has been in the study for 1 month and is now 33 weeks gestation. Upon learning of this incident, her primary care clinic was contacted and they reported they felt it was not study related. They also reported that no labs were done at the hospital as this is considered to be solely pregnancy related. I then contacted the Data Safety Monitoring Board with the information and they are currently working on reviewing the data and drafting a report. The participant's baseline circulating vitamin D level (25[OH]D) was 74.5 nmol/L (80 nmol/L to 225 nmol/L is considered sufficient/normal), so she did not enter the study with an unusually high 25[OH]D.

Outcome of adverse event: (Check all that apply)

___	Death	___	Disability/incapacity		
___	Required Intervention	___	Life threatening	___	Congenital anomaly/birth defect
X	Hospitalization-initial or prolonged	___	Other (Explain):		

Did this event occur in the above listed study?	X Yes	___ No
Is this type of adverse event described in the consent form approved by this IRB?	___ Yes	X No
Did this event occur to a subject enrolled in the study at Altru Health System?	X Yes	___ No

If yes, was this event reported to: ___ *Study Sponsor* _X_ *Co-Investigators*

Relationship of the Event to any research treatment appears to be: (**Sponsor assessment**)

___ Unknown ___ Not related ___ Unlikely ___ Possibly ___ Probably ___ Related

Relationship of the Event to any research treatment appears to be: (**PI assessment** – site where event occurred)

___ Unknown X Not related ___ Unlikely ___ Possibly ___ Probably ___ Related

Signature of person reporting: Cindy Anderson Date: 9/7/12

Printed name of person reporting: CINDY ANDERSON, faculty advisor

Signature of Principal Investigator: [signature] Date: 9/7/2012

Printed name of Principal Investigator: DORIA THIELE

IRB USE ONLY

Will this event require a change in timeframe for continuing review? ___ Yes X No

Comments:

Will the event require changes in the consent form used at this site? ___ Yes X No

Comments:

This adverse event report has been reviewed and approved by:
Signature of IRB Chair/designee: _____ Date: 9/19/12

*Completed by:*_____

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