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Could Low Vitamin D Status Explain the Increased Rates of Hypertensive Disorder in Pregnancy in the US Population and in Non-Hispanic Black Women? An Examination of NHanes 2001-2006

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TITLE PAGE

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HYPERTENSIVE DISORDERS IN PREGNANCY IN THE US POPULATION AND IN NON-
HISPANIC BLACK WOMEN? AN EXAMINATION OF NHANES 2001-2006

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A Thesis Submitted to the Graduate Faculty of Georgia State University in Partial
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APPROVAL PAGE

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2006

by Michelle Leander - Griffith

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DEDICATION

To my mother and father for their love, hard work and sacrifice. To Wyatt for his never ending support and encouragement. To my wonderful children, thanks for your patience throughout this process.

ABSTRACT

Michelle Leander-Griffith.

 Could Low Vitamin D Status Explain the Increased Rates of Hypertensive Disorders in Pregnancy in the US Population and in non-Hispanic Black Women? An Examination of NHANES 2001-2006
(Under the direction of Dr. Ike Okosun)

Background: The incidence of Hypertensive Disorders in Pregnancy (HDP) is increasing in the US and is linked to serious long and short-term health problems for both mother and fetus. Vitamin D has been shown to have direct influence on molecular pathways involved in pregnancy. However a link between vitamin D status and HDP in Pregnant women has not been established.

Objectives: The purpose of this study is to determine (1) the association between vitamin D deficiency and the occurrence of (HDP) and (2) whether non-Hispanic Black women (NHB) are at greater risk for HDP due to low vitamin D status.

Methods: Pregnant females in the National Health and Nutrition Examination Survey (NHANES) study from 2001 to 2006 were used in this study. Participant's response to interview questions and laboratory results were taken into account to determine HDP status. Logistic regression was used to determine the association between vitamin D status and HDP.

Results: Pregnant women with low vitamin D status (25(OH)D < 20ng/ml) were 1.123 (95%CI: 0.808-1.56) times more likely to have HDP compared to women who were vitamin D sufficient. This association was not significant. NHB women did not show a significant increased risk for HDP.

Conclusions: Low vitamin D status during pregnancy may lead to an increased risk for Hypertensive Disorders in Pregnancy. However more research on larger sample size is needed to determine the true extent of the association of vitamin D status with HDP in the general population and that of non-Hispanic Black women.

Key words; Maternal hypertension, preeclampsia, pregnancy

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Chapter I – Introduction

About 5-10% of all pregnancies in the United States are affected by hypertensive disorders in pregnancy (HDP), which contributes substantially to maternal mortality (Wagner et al., 2007). HDPs encompass a spectrum of conditions such as preeclampsia, eclampsia, and gestational as well as chronic hypertension (Wagner et al., 2007). The incidence of hypertensive disorders during deliveries has increased from 67.2 per 1000 deliveries in 1998 to 81.4 per 1000 deliveries in 2006, and is associated with an enhanced burden of severe obstetric morbidity (Kuklina et al., 2009). This trend is alarming as a growing body of evidence confirms that HPD impacts public health beyond the apparent immediate increased risk of maternal morbidity and mortality (Bilhartz and Bilhartz, 2010). In addition to latent maternal risk for heart disease, women with preeclampsia have neonates who are often pre-term and of low birth weight (Bilhartz and Bilhartz, 2010). According to the Hospitalization Utilization Project (HCUP) Nationwide Patient Survey, the economic burden of HDP in the US for 2003 was a staggering $2.3 billion (AHRQ.gov, 2012), with approximately 204, 868 pregnant women admitted to hospital.

Risk factors for HDP include chronic hypertension, obesity, multifetal gestation, history of preeclampsia, pre-gestational diabetes, extremes of gestational age, and pre-existing medical conditions (such as chronic hypertension, diabetes melitius, renal disease thrombophilias, black race, low SES, low vitamin D and some maternal infections) (Saibi et al., 2005; Mostello et al., 2002). Women with gestational hypertension are at a higher risk of progressing to severe hypertension, preeclampsia or eclampsia in the short term (Saudan et al., 1998; Barton et al., 2002), and to chronic

hypertension, ischemic heart disease and stroke in the long term (Bellamy et al., 2007).
Most of our understanding of hypertensive disorders in pregnancy, and the associated
severe obstetric complications, is informed by studies of preeclampsia and eclampsia
(Zang et al., 2003).

Epidemiology of HDP

According to a 2006 report of the World Health Organization, hypertension in
pregnancy is said to be responsible for 16% of all deaths in developed countries, 9% in
Africa and Asia and over 25% in Latin America and the Caribbean (Khan et al., 2006).
Most often, 10 to 20% of Gestational hypertension progresses to preeclampsia, which is
one of the most common causes of maternal and fetal morbidity and mortality (Hauth et
al., 2000; Saudan et al., 1998).

Preeclampsia, one of the most severe forms of HDP, affects about 5% to 7% of all
pregnancies, and places women at a greater risk for placental abruption, cerebral
hemorrhage, acute renal failure, disseminated intravascular coagulation, pulmonary
edema, circulatory collapse and eclampsia, which itself can lead to seizures, coma and
death, and are at increased risk for delayed growth, low birth weight and preterm birth
(American Academy of Family Physicians, 2006).

Non-Hispanic Black women are at an increased risk for HDPs, as confirmed by
longitudinal studies in NC and NY (Miranda et al., 2010; Tanaka et a., 2007; Samadi et
al. 1996). One potential cause of this disparity is vitamin D, which has direct influence on
molecular pathways involved in the pathogenesis of preeclampsia and the high
prevalence of vitamin D deficiency in Non-Hispanic Black women (Bondar et al., 2007;
Dror, 2011). Because of the strong biological plausibility of vitamin D's role in

preeclampsia and data suggesting that maternal vitamin D deficiency may increase the risk of preeclampsia and fetal growth restriction (Bodnar et al., 2010), further investigation is warranted in understanding the role of vitamin D in HDP.

Vitamin D

Vitamin D is a pleiotrophic secosteriod hormone that is primarily known for its role in bone metabolism and mineral homeostasis (Grundman et al., 2011). Until recently, Vitamin D deficiency was well known for its role in causing rickets, which leads to softening of bones and the occurrence of multiple fractures in children. Vitamin D has no biological activity on its own; however, we now know that a hormonal form with diverse biological activities is generated when vitamin D is converted to 1α, 25dihydroxyvitamin D ($1, \alpha\ 25(OH)_2D$ (Shin et al., 2010). Evidence also exist that vitamin D is able to regulate key target genes associated with implantation of the embryo, troboblast invasion and implantation tolerance (Evans et al., 2004).

In recent years, increased attention has been focused on vitamin D because of the report by Shin et al. (2010). This report found that, depending on country of residence and other factors, 20 -85% of pregnant women are deficient in vitamin D, a lack that has been linked to the non-classical actions of this hormone: preeclampsia, insulin resistance, and gestational diabetes mellitus (Shin et al., 2010).

Disparity of Preeclampsia

Previous research highlights an intractable disparity of maternal health outcomes between African American women and Caucasian women in the US (Bodnar et al., 2010). In particular, a 2003 study by Zang and colleagues found that, in addition to having a higher incidence of hypertensive disorders, African American women tend to be

at greater risk for the most severe HDP-related complications (Kozak and Lawerence, 1999). This occurs despite the fact that extensive research on HDP and other aspects of prenatal care has led to no substantial improvements in predicting or preventing the disorder (Saibi et al., 2005).

Link between vitamin D and preeclampsia

The link between vitamin D and preeclampsia has been observed as early as 2007 by Bodnar et al., who claimed that, a 50nmol/l decrease in $25(OH)D_3$ concentration doubles the risk of preeclampsia I pregnant women. The link between vitamin D and preeclampsia was documented again by Robinson and colleagues (2011), who found that vitamin D may influence fetal growth through placental mechanisms. Furthermore, a study examining the correlation between mid-gestation vitamin D deficiency and the risk of severe preeclampsia, reported that women who developed preeclampsia had lower mid-gestation concentrations of maternal 25OHD compared to controls (Baker et al, 2010).

GAPS in the Literature

Most research findings from the past five years implicate vitamin D in preeclampsia; however studies linking vitamin D to other hypertensive disorders in pregnancy, is lacking. Even studies that link vitamin D and preeclampsia lack sufficient power and nation-wide representation and cannot negate the effects of a single location. *Significance* – Determining whether vitamin D deficiency is, indeed, related to HDP, will inform actions needed to remedy such a deficiency. This in turn will be instrumental in reducing an important public health problem. Our findings will add to the body of scientific evidence on the role that vitamin D deficiency plays in the occurrence of HDP.

Results of this study may provide insight as to whether or not vitamin D supplementation in pregnant women could prevent adverse outcomes for the mother as well as the fetus. Since HDPs include preeclampsia, which is a well-known and studied disorder, literature on preeclampsia will be used in this study to inform many aspects of HDP.

Purpose of Study

This cross-sectional study aims to determine (1) the association between vitamin D deficiency and the occurrence of Hypertensive Disorders in pregnancy and (2) whether African American women are at greater risk for preeclampsia due to their low levels of vitamin D. These associations are important since in addition to being a major contributor to maternal and fetal morbidity and mortality, HPD also causes short and long term health effects as previously mentioned.

Chapter II – Literature Review

HDPs affect about 10% of all pregnancies in the US and contribute significantly to maternal mortality (Wagner et al., 2007). These disorders encompass a spectrum of conditions such as preeclampsia, eclampsia, and gestational as well as chronic hypertension (Wagner et al., 2007). This definition is supported by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy and includes eclampsia/preeclampsia superimposed on chronic hypertension, gestational hypertension and chronic hypertension (Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000). Mild hypertension and gestational hypertension are the most common of the HDPs (Kuklina et al., 2009). Preeclampsia is thought to be responsible for 18% of all maternal deaths in the US (Preeclampsia Foundation, 2006) and in 2002 was responsible for 56 maternal deaths per 100,000 live births (National Vital Statistics, 2004) and 71 neonatal deaths per 100,000 live births (American College of Obstetricians and Gynecologists, 2005).

A 2008 study using the National Hospital Discharge Survey (NHDS) found significant increases of preeclampsia and gestational hypertension, by 25 and 84%, respectively, in the period between 1987 and 2004 (Wallis et al., 2008). Additional support of this trend has found that hypertensive disorders during delivery have increased significantly, from 67.2 per 1000 deliveries in 1998 to 81.4 per 1000 deliveries in 2006, thus enhancing the burden of severe obstetrical morbidity that is associated with the disorder (Kuklina et al., 2009). Other findings noted by Wallis and coworkers, reported that the rates of preeclampsia and gestational hypertension increased significantly by 25

and 184%, respectively, from 1987 to 2004 using the National Hospital Discharge Survey (Wallis et al., 2008): they also point out that a change in clinical guidelines for gestational hypertension in the 1990's may have led to an exaggeration in the rates of the disorder.

Main Clinical Disorders of HDP

HDPs are comprised of the following clinical disorders: namely chronic hypertension, gestational hypertension, pre-eclampsia and chronic hypertension with superimposed preeclampsia. Precise definition of these conditions remains a major challenge in studying them (Hutcheon et al., 2011).

Preeclampsia consists of new-onset hypertension and proteinurea after 20 weeks of gestation, and is a multi-systemic pregnancy-specific disorder (Bodnar et al., 2007). The etiology of preeclampsia is unknown, but we know that it is unique to human pregnancy (Saibi et al., 2005). The hypertension portion of the disorder is characterized by systolic blood pressure of at least 140mm Hg, diastolic pressure of 90mm Hg, measured on two occasions, at least 4-6 hours apart, following the 20th week of gestation in women who are otherwise normotensive (Saibi et al., 2005). Proteinuria, on the other hand, is characterized by the excretion of more than 300mg of protein in the urine every 24 hours (Saibi et al., 2005). At a physiological level, preeclampsia is characterized by an abnormal vascular response to the implantation of the placenta, which is linked to increased systemic vascular resistance, increased platelet aggregation, activation of the coagulation system, and endothelial dysfunction (Report of the National High Blood Pressure Education, 2000).

Chronic Hypertension is defined as pre-existing hypertension, which is detected before pregnancy, or prior to 20 weeks of gestation (Report of the National High Blood Pressure Education, 2000); it is also defined as hypertension that is first diagnosed after 20 weeks gestation and continues past 12 weeks postpartum (Hutcheon et al., 2011). In the US, this condition affects 1 to 4% of women aged 18 – 29 years old, and 5 to 15% of women 30 to 39 years old from 1999 to 2004) (Cutler et l., 2008). In 2004, an estimated 1.7% of pregnancies in the US were complicated by preexisting hypertension. Women with chronic hypertension have a threefold increased risk of perinatal mortality, preterm delivery, maternal death, and small-for-gestational-age-infants, compared to normotensive women (Rey and Couturier, 1994; McCowan et al., 1996).

Gestational Hypertension develops after 20 weeks of gestation, and returns to normal within 12 weeks post partum period (Hutcheon et al., 2011); it is seen in 2 to 3% of all pregnancies in the US (Klemmenson et al., 2005; Wallis et al., 2008), with rates increasing from 10.7 to 30.6 per 1000 deliveries between 1987 and 2004 (1 Klemmenson et al., 2005). Gestational hypertension presents an increased risk of preeclampsia: 17% of women who have this condition go on to develop preeclampsia during pregnancy (Sudan et al., 1998). However, even though women with gestational hypertension are at increased risk for obstetric complications, it is considerably less hazardous to the mother compared to other HDPs (Wallis et al., 2008).

Chronic Hypertension with superimposed pre-eclampsia involves the occurrence of new onset proteinuria, thrombocytopenia, or any other features of the pre-eclamptic syndrome in women with chronic hypertension during pregnancy (Report of the National High Blood Pressure Education, 2000).

Eclampsia is characterized by seizures in the presence of preeclampsia, with no alternative explanation (Bilhartz and Bilhartz, 2010), and could lead to coma and death (American Academy of Family Physicians, 2006). Its incidence in the US was about 8.2 per 10,000 between 1996 and 2004 (Wallis et al., 2008). However this condition has declined in recent years (Hutcheon et al., 2011).

Hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome is a severe form of preeclampsia, and manifests as elevated liver enzymes and low blood platelet count (Report of the National High Blood Pressure Education, 2000).

Severity of Disease – Many of the proposed classifications for the severity of preeclampsia take into account the following: severity of hypertension (i.e. if systolic blood pressure >160mmHg, diastolic >=110mmHg at least on two occasions, 6 hrs apart) or both, time of delivery (whether delivery < 34 weeks) and aftermath (e.g. the occurrence fetal death, preterm birth or eclampsia (Ananth et al., 1997; Hernandez et al., 2009; McDonald et al., 2009). Some women also develop severe gestational hypertension that is not associated with nor accompanied by proteinuria. It is note worthy that compared with mild preeclampsia, gestational hypertension is associated with higher maternal and perinatal morbidities.

Epidemiology of HDP

As mentioned above, hypertensive disorders complicate about 5-10% of all pregnancies (Williams Obstetrics, 2010), and are the leading cause of maternal mortality in developed countries (Khan et al., 2006). Globally HDPs are responsible for 25, 000

maternal deaths in Africa, 22,000 deaths in Asia, 3,800 deaths in Latin America and the Caribbean, and 150 deaths in developed countries (Hutcheon et al., 2011).

Since preeclampsia is one of the most severe conditions of HDP, literature examining its epidemiology will be examined in this study.

A 2009 National Vital Statistics report, with 19 states reporting, revealed that preeclampsia rates were 43.6 per 1000 births in non-Hispanic whites, 49.1 per 1000 live births in non-Hispanic Blacks, and 38.0 per 1000 live births. These rates are highest for pregnant women under 20 years old, and in those 40 to 54 years old, with Black women in the latter group showing 1.5-fold increase compared to all races.

Preeclampsia affects between 2% and 7% of healthy nulliparous women. In 75 % of these cases, the disease is mostly mild, with late onset or intrapartum, conferring only a negligible increased risk for adverse pregnancy outcome (Saibi et al., 2003; Vatten and Skjaerven, 2004).

Preeclampsia

Preeclampsia is a common complication in pregnancy, affecting up to 10 percent of pregnant women, and often resulting in fetal growth restriction, premature delivery and low birth weight of affected neonates. Pathogenesis for this heterogeneous disorder varies depending on the risk factor profile. Risk factors include chronic hypertension, obesity, multifetal gestation, history of preeclampsia, pregestational diabetes, extremes of gestational age, and pre-existing medical conditions (such as chronic hypertension, diabetes mellitus, renal disease thrombophilias, black race, low SES, vitamin D and some maternal infections. (Saibi et al., 2005; Mostello et al., 2002).

Preexisting Medical Conditions include chronic hypertension, obesity, multifetal gestation, history of preeclampsia, pregestational diabetes, kidney disease, autoimmune disease and thrombophilias (Saibi et al., 2005; Mostello et al., 2002; Hutcheon et al., 2011).

Primitarity and Sperm Exposure: It is hypothesized that risk for preeclampsia is increased in women who have little exposure to their partner's sperm, as supported by the fact that nulliparous women are three times more likely to experience preeclampsia compared to multiparous women, and that multiparous women display a reduced risk of preeclampsia (Hutcheon et al., 2011).

Smoking has a protective effect with regard to preeclampsia, as shown by many studies; smoking reducing the incidence of preeclampsia in a dose dependent manner, by up to about 50% (England and Zhang, 2007).

Miscellaneous factors: A history of preeclampsia is a predictor preeclampsia in a subsequent pregnancy (Hutcheon et al, 2011); obesity, advanced gestational age, multiple pregnancies, infections, and residing at a high altitude are all factors that increase the risk of preeclampsia (Hutcheon et al., 2011). Interestingly enough, preeclampsia rates are higher when sunlight –dependent 25(OHD production is reduced in the winter months (Shin et al, 2010).

Maternal Effects – Women with severe eclampsia and preeclampsia have a 10- to 30-fold higher risk for complications such as acute renal failure, and pulmonary edema, and have a 70% increased risk of placental abruption (Hutchen et al., 2011). Preeclampsia is also the leading cause for admission to the intensive care unit during the puerperal period

(Loverro et al., 2001). Long term studies show that women with preeclampsia have a 3- to 4- fold increased risk of developing chronic hypertension, and an almost 2-fold increased risk of ischemic heart disease, stroke, and venous thromboembolism (Bellamy et al., 2007). Some women also develop severe gestational hypertension, which is not associated with, nor accompanied by proteinurea. Compared with mild preeclampsia, severe gestational hypertension is associated with higher maternal and perinatal morbidities. (Sibai et al., 2005).

Effects on the child – Preeclamptic women have a 4-fold increase in early delivery, largely due to iatrogenic effects, and neonates with a 2-fold increase in mortality, low apgar scores, febrile seizures, encephalopathy and elevated rate of admission to neonatal intensive care unit (Basso et al., 2006). The long-term effects of preeclampsia on the fetus include higher levels of diastolic and systolic blood pressures in childhood, and adolescence. In addition they are at increased risk for metabolic disorders, epilepsy and other complications (Wu et al., 2008; Wu et al., 2009).

Pathogenesis of Preeclampsia and the Implication of Vitamin D

The underlying cause of preeclampsia is not known, but one hypothesis states that preeclampsia is caused by the presence of placenta, or the maternal response to implantation of the placenta (Sibai et al., 2005). Historically, two schools of thought exist on the cause of preeclampsia: (i) vascular biologists believe that in the placenta, ischemia–reperfusion precedes oxidative stress which leads to vascular disease; and (ii) immunologists believe that preeclampsia results from a maternal-paternal maladaptation,

where the maternal alloimmune reaction is initiated by the rejection of the fetal allograft (Saibi et al., 2005).

There is evidence that suggest vitamin D's role in the pathogenesis of preeclampsia. As mentioned before, it is thought that the primary trigger of preeclampsia relates to impaired placentation, after which systemic maternal responses follow that produce the clinical signs and symptoms of the 2–stage disorder (Walker, 2000). The reduced tolerance to the developing fetus following maternal immune maladaption is thought to result in preeclampsia (Robertson et al., 2003).

Stage 1 of the disorder is characterized by reduced placental perfusion which follows abnormal placental implantation (Bodnar et al., 2007). Materials produced by the poorly perfused placenta is said to produce materials that initiate the subsequent sequelae of adverse health effects(Bodnar et al., 2007). Pathophysiological changes such as these, are suggested to be secondary to abnormal endothelial function which accompanies generalized increase in the inflammatory activation that constitutes normal pregnancy (Redman et al., 1999). One –25-dihydroxyvitamin D_3, the active form of vitamin D has been shown to control the transcription and function of genes connected to placental invasion, normal implantation and angiogenesis (Evans et al., 2004; Daftary and Taylor, 2006). In addition it is suggested that abnormal implantation is mediated by an inappropriate immune response between mother and baby (Bodnar et al., 2007). Immunomodulatory properties of 1,25-dihydroxyvitamin D_3 may be play a key role in this regard (Hewison, 1992)**. It follows that vitamin D deficiency may predispose a woman to increased inflammatory response** (Hewison, 1992). Vascular structure, and

function as well as vascular compliance, elasticity and intima media thickness are all better off in women optimally supplemented with vitamin D (Braam et al., 2004).

It is suggested that the domination by the T helper type 2 (TH2) cytokine response is one of the critical steps required to maintain normal pregnancy (Hypponen, 2005). Induction of Th2 cells is favorable to the survival to the survival of the fetus, while Th1-type reactions in the placenta is associated with spontaneous preterm delivery (El-Shazly et al., 2004). It has been suggested that $1,25(OH)_2D$ has an important role in promoting a shift to a Th2- dominated pattern in immune response (Zehnder at al., 2002). In addition, in vitro studies show that $1,25(OH)_2D$ increases the secretion of Th2 cytokines and inhibits the secretion of Th1 cytokines (Cantorna et al., 2000; Piccinni et al., 2000). However the exact mechanisms of these actions are not fully understood (Hyppönen, 2005). Figure 2 shows a summary of hypothesis for the pathogenesis of preeclampsia.
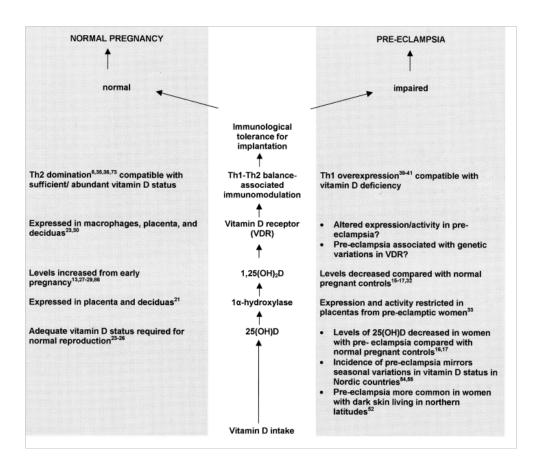
Figure 1. Comparison between normal and preeclamptic pregnancies. Hypothesis on the association between vitamin D and Preeclampsia (Hyppönen, 2005).

Many studies attempting to find the etiology of the disparity pregnancy outcomes have examined the contribution of differences of socioeconomic status, access to health care, other environmental and social causes (Swamy et al., 2010). A host factor examined by Swamy and colleagues is the genetic variability in the VDR gene. Research suggests that race specific allelic frequencies in the vitamin D receptor gene is potentially involved in health disparities (Swamy et al., 2010) . This study found that low infant birth weight was strongly associated with VDR genetic variability among NHB women but not NHW women. Swamy and colleagues suggest that the association between maternal VDR

genetic polymorphisms and birth weight is an example of genetic influences on fetal growth as well as a potentially important explanation for why there is a significant difference in birth weight between NHB and BHW women. Swamy and colleagues also speculate that VDR polymorphisms not only contribute to low birth weight, but may signify causal pathways for the effects of vitamin D on other pregnancy outcomes (Swamy et al., 2010).

It is possible that the chronic hypertension component of HDP may be explained by similar mechanisms that are found in the association of low vitamin D and high blood pressure in the population. One such mechanism is vitamin D's modulation of the RAS gene.RAS is known to regulate electrolyte and plasma volume homeostasis which affects blood pressure (Motiwala and Wang, 2012). Hypertension and overall cardiovascular risk results when RAS is inappropriately activated. Strong experimental evidence frome murine studies support vitamin D's role as a proximal inhibitor of RAS (Motiwala and Wang, 2012).

Previous research on Vitamin D and Preeclampsia

As discussed above, considerable research has identified as association between vitamin D deficiency and the increased risk of preeclampsia (Shin et al., 2010), and has shown that cohorts supplemented with Vitamin D showed a decreased risk of preeclampsia when compared to non-supplemented controls. Even though the exact mechanism whereby vitamin D leads to preeclampsia is unclear (Shin et al., 2010), it is hypothesized that low vitamin D levels destroy the normal Th1-to-TH2 cytokine balance, indicating that higher TH1 cytokine expression adversely affects embryo implantation (Hypponen, 2005). A 2007 study by Bondar and colleagues, concluded that vitamin D is

an independent risk factor for preeclampsia: more specifically, a 50nmol/L drop in 25(OH)D concentration doubled the risk of preeclampsia after adjusting for confounders. Consequences of low vitamin D levels during pregnancy on fetal and child health include low birth weight (Scholl and Chen, 2009), craniotabes (Yorifuji et al., 2006), softening of the skull bone that (an early sign of vitamin D deficiency). In addition, two recent retrospective studies have found a new association between maternal vitamin D deficiency and rickets-associated heart failure (Sullivan et al., 2008) and with acute lower respiratory heart failure. Health problems later in childhood, including improper bone development at 9 years of age (Shin et al., 2010), asthma (Litonjua and Weiss, 2007; Kinney et al., 2009), schizophrenia (Kinney et al., 2009), and type 1 diabetes (Shin et al., 2010) are also correlated with vitamin D deficiency during pregnancy.

Over the past 30 years, a vast amount of research has focused on the pathogenesis, pathophysiology, management, and treatment of preeclampsia (Raymond and Peterson, 2011). This body of research has also sought to find biomarkers that can be used to predict preeclampsia to find its association with other factors like smoking, stroke and cardiovascular disease (Raymond and Peterson, 2011). Previous research on the effects of vitamin D and preeclampsia have shown a positive association (Bodnar et al., 2010). Notably, the frequency of preeclampsia remains unchanged despite advances in prenatal care, and extensive research regarding this problem over the past decade has not led to substantial improvement in prediction or prevention of this disorder (Saibi et al., 2005). The reason for this lack of progress is thought to be our inadequate understanding of the

pathological mechanisms that result in preeclampsia (Saibi et al., 2005). One potential candidate for the cause of preeclampsia and HDP is vitamin D.

Evidence for vitamin D's implication in preeclampsia comes from various sources. Observed seasonal patterns in preeclampsia; higher incidence in winter and lower incidence in summer, suggests a role of sunlight hence vitamin D in this disorder (Bodnar et al., 2007; ToPoel et al., 2011). Other observational evidence comes from studies where women with preeclampsia have lower levels of circulating 25(OH)D than pregnant normotensive women (Bodnar et al., Halhali et al., 2000; 2007; Robinson et al., 2010)

Recent studies document an association between vitamin D deficiency and preeclampsia. In 2011, Robinson et al. examined the association of vitamin D levels and small-for–gestational-age (SGA) in 56 patients with early-onset severe preeclampsia (EOSPE), and found that 25(OH)D levels were lower in patients with SGA infants. The authors concluded that vitamin D may have an effect on fetal growth through placental mechanisms. In 2010 the same authors had found a similar result when they examined total 25OHD levels at the time of diagnosis of EOSPE. Furthermore a nested case control study of a possible association of midgestation vitamin deficiency and the risk of severe preeclampsia in 51 cases out of an overall cohort of 3992 pregnant women documented that women who developed preeclampsia had lower midgestation maternal 25OHD concentrations relative to control women (Baker et al., 2010). This trend of vitamin D and preeclampsia was, in fact, reported as early as 2007, when Bodnar and colleagues showed that, after adjustment for confounders, a 50nmol/l decrease in 25OHD concentration doubled the risk of preeclampsia. On the other hand, a large cohort study of

23,423 nulliparous women in Norway found that women taking 10-15microg/d of vitamin D supplement experienced a 27% decrease in preeclampsia compared to women who did not take the supplements; however no association with preeclampsia was found when the additional vitamin D intake was from food alone (Haugen et al., 2009).

Not all research examining vitamin D and preeclampsia are consistent. A study in British Columbia Canada 49°N, investigated whether the vitamin D (serum 25-hydroxyvitamin 25(OH)D status is associated with a risk of preeclampsia or with adverse pregnancy outcomes in a group of 221 women with biochemical risk factors for preeclampsia. Study authors concluded that there is no difference in the rates of preeclampsia, gestational hypertension, or adverse pregnancy outcome as a result of 25OHD concentration, even though 78% of the women in the study were vitamin D insufficient (25OHD <75nmol/) and 53% were vitamin D deficient (25OHD <50nmol/l). This result is not surprising, because vitamin D deficiency and insufficiency are prevalent in the group at that location (Shand et al., 2010). Another study that showed no association calculated the free 25OHD levels by measuring the level of 25OHD and vitamin D binding protein in a group of 170 women in their first trimester of pregnancy: when 25OHD levels were compared in control women (n=131) to those in women who subsequently developed preeclampsia (n=39), first trimester levels of vitamin D binding protein and free 25OHD were similar, despite an association between higher first trimester blood pressures and ensuing preeclampsia (Powe et al., 2010).

Racial Disparity of Major Pregnancy Outcomes

Intractable disparities persist between black and white women in the US with respect to their rates of major pregnancy outcomes. (Disparity as defined by the IOM refers to differences that are above and beyond those that can be explained by differences in health status between two or more groups.) Earlier studies reveal profound disparities in the rates of major pregnancy outcomes like preterm birth, gestational diabetes and HDP among non-Hispanic black and non-Hispanic white women in the United States. In the United States, data from 2005 suggests that black women are 2.3 times more likely to experience intrauterine death of their fetus, or postnatal death of their infants than are white women. These disparities in turn, contribute to the disparate rates of infant and maternal mortality, and hence to the impacted health status of the communities of these women (Bryant et al, 2011). Examination of the major causes of infant death also reveals marked disparities. With respect to preterm births (defined as delivery at less than 37 weeks gestation), black women are 1.5 to 2.5 times more likely to experience infant death than are white women. Black women are 2.4-folf more likely to have preterm births (at less than 32 weeks gestation), and 2.1-fold more likely to have a low birth weight infant (Martin et al, 2006). In addition, black women are more likely than their white counterparts to develop preeclampsia accompanied by more severe complications, even after adjusting for women with chronic hypertension (4-7 in Bodnar and Simhan).

In an effort to highlight the etiology of maternal health disparities, many studies have shown how differences in socioeconomic status contribute to the disparity in pregnancy outcomes (Finch, 2003; Lynch et al., 2004). Studies examining how genetic

variability contribute to the disparity of maternal outcomes in NHB and NHW women

suggest that differences in the race-specific allelic frequencies in the vitamin D receptor

(VDR) gene, is potentially involved in these health disparities (Cooper and Umbach,

1996).

Vitamin D and the Disparity in Adverse Maternal Outcomes

Previous studies have illuminated profound disparities in the rates of major

pregnancy outcomes in non-Hispanic black and non-Hispanic white women in the United

States (Tanaka et al., 2007; Tucker et al., 2007; Bryant et al., 2010). These disparities in

turn, contribute to the (disparate) rates of infant and maternal mortality, hence the

contributing to the poor health status of the communities of these women (Bryant et al,

2011).

In the United States, data from 2005 suggests that black women were 2.3 times more

likely to experience intrauterine fetal death or death of their infant than white women.

An examination of the major causes of infant death also revealed considerable disparities.

With respect to preterm births, defined as delivery at less than 37 weeks gestation, black

women were 1.5 to 2.5 times more likely to experience this than white women. Black

women were 2.4 times likely to have very preterm births (less than 32 weeks gestation

and 2.1 times more likely to have a low birth weight infant. (Bodnar and Simhan, 2010).

Most of these conditions are the result of conditions such as HDP. In addition, black

women were more likely than white women to develop preeclampsia along with more

severe complications than their white counterparts even after adjusting for women with

chronic hypertension (Bodnar and Simhan, 2010).

Previous research on the disparity of adverse maternal health outcomes has examined many causes. They include the impact of stress, racism and related factors in pregnancy (Giscombe et al., 2005; Alio et al., 2010) as well as the role of socioeconomic factors and access to adequate healthcare (Dominguez, 2011). Studies examining the biological factors have looked at the variation of the VDR receptor in African American women (Swamy et al., 2011).

What is Vitamin D

Vitamin D was first discovered as a preventative treatment for rickets, a disease which results in softening bones, fractures and deformity in children (Shin et al., 2010); vitamin D's first known roles in bone and kidney are known as its classical actions (Shin et al., 2010), but since then, vitamin D is known to be involved in many non-classical processes (Shin et al, 2010). Vitamin D is a secosteriod hormone which provides and maintains sufficient calcium and phosphorous stores in the body to facilitate optimal metabolic functions. For humans, the primary source of vitamin D is synthesis in the skin from sunlight (Nesby-O'Dell et al., 2002). This is achieved when solar UVB radiation (wavelength, 290 to 315nm), penetrates the skin to convert 7-hydrocholesterol to previtamin D_3, which is then rapidly converted to vitamin D_3 in the presence of warmth from sunlight (Holick et al., 2007). Cutaneous absorption of UVB radiation is inhibited by levels of sunlight exposure (season, latitude and time of day), the use of sun block and dark skin pigmentation (Nesby-O'Dell et al., 2002). Limited dietary sources of vitamin D include fatty fish, fish oils, fortified foods and vitamin supplements (Nesby-O'Dell et al., 2002). Vitamin D_3 obtained from the skin and diet is transported to the liver where it is metabolized to 25-hyroxy vitamin D; the form used to determine an

individual's vitamin D status (Holick, 2007). From there it is transported to the kidney and other cells in the body. In the kidney, vitamin D is metabolized to its active form 1,25-dihydroxyvitamin D (Holick, 2007). Vitamin D deficiency is linked to a number of diseases including some common cancers, auto immune diseases, infectious diseases and cardiovascular diseases (Holick, 2007). Vitamin D exerts its biological activity by binding to a high-affinity receptor (VDR) which in turn acts like a ligand-activated gene transcription factor. Differences in allelic frequencies of VDR polymorphisms are race specific and have been confirmed by multiple studies (Cooper and Umbach, 1996; Valdivielso and Fernandez 2006; Swamy et al., 2010)

Vitamin D: UV The Original Source - How to Use It; Edward Gorham, PhD
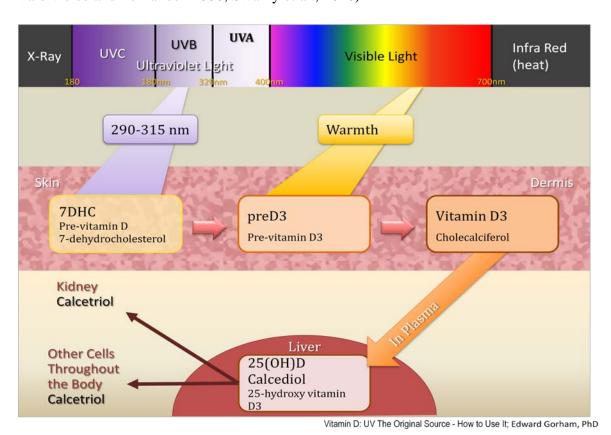
Figure 21. Shows how vitamin D is synthesized in the skin.

UVB radiation (wavelength, 290 to 315nm) penetrates the skin to convert 7-hydrocholesterol to previtamin D_3, which is then rapidly converted to vitamin D3 in the presence of warmth from the sunlight (Holick et al., 2007; Gorham UCSD, 2010).

Individuals could be vitamin D deficient for many reasons; they include older age, higher BMI, dark skin pigmentation (melanin absorbs UVB photons), some medications, liver disease, chronic kidney disease and living above 35˚ in winter months. Fig. 2 shows the absorption of UVA and UVB rays by the skin. Fig 2a shows UVA and UVB radiation penetrating skin in the absence of melanin. However in the presence of melanin, UVB radiation does not penetrate the skin well since melanin in a natural sunscreen and absorbs UVB radiation.
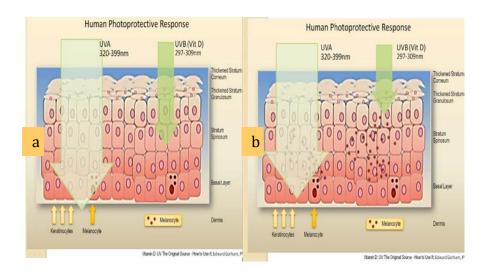
Figure 3. The absorption of UVA and UVB rays by the skin. Fig 2a shows melanin penetrating skin in the absence of melanin. However in the presence of melanin, UVB radiation does not penetrate well since it is absorbed by melanin (Gorham UCSD, 2010).

Vitamin D exists in two forms: vitamins D_2 or ergocholeciferol, and vitamin D_3 cholecalciferol. Vitamin D_2 is found in plants (Lewis et al., 2010) and is one third less potent than vitamin D_3, which is present in many fewer foods such as oily fish (salmon, mackerel, herring) and, to some extent, egg yolks (Lewis et al., 2010). In fact, wild

caught salmon has an average of 500 – 1000 IU vitamin D_3 versus 100 – 250 IU vitamin D_2 per 100g (Chen et al., 2007). In the US, some food products like juice, breads, yogurts and cheeses are fortified with vitamin D. Many individuals also take multivitamins supplements that contain vitamin D, or supplements that contain only vitamin D (Holick et al., 2008.). Because it is not if not obtained from the diet, vitamin D is synthesized from a steroid precursor (Shin et al., 2010) and thus it is considered to not be a true vitamin (Holick et al., 2007).

Classical and Non classical actions of Vitamin D

Classical actions of vitamin D take place in the kidney, liver and intestine, where it is involved in the regulation of calcium and phosphate absorption, and thus affects bone synthesis and metabolism (Shin et al). Vitamin D obtained from sunlight or ingestion is transported to the liver, where it is hydroxylated by various mitochondrial and microsomal P450 enzymes to form 25 hydroxycalciferol (25 (OH)D) (Lewis et al ., 2010). It is then processed in the kidney where and converted to its bioactive form calcitrol and is subsequently involved in regulating calcium deposits in the bone, thereby playing a major role in bone health (Alpert and Shaikh, 2007). However recent studies show that more than 30 human tissues express the vitamin D receptor (VDR) and are therefore able to respond to 1,25 $(OH)_2D$ (Shin et al); these data implicate vitamin D and its receptor in a number of additional roles including immune function, cell proliferation and differentiation, and hormone secretion (Shin et al). In particular, 1,25(OH)2D is believed to reduce the activity of the adaptive immune system while enhancing the activity of the innate immune system (Shin et al., 2010), and it also has anti-proliferative and pro-differentiation functions in a variety of cell types (Shin et al., 2010). During

pregnancy, vitamin D deficiency is linked to the non-classical actions of this hormone, leading to preeclampsia, insulin resistance and gestational diabetes mellitus (Shin et al., 2010).

Vitamin D in Pregnancy

As mentioned above, vitamin D status is reflected in circulating 25(OH) concentrations, with the normal range being between 32ng/ml to 80ng/ml; values below 32ng/ml are designated as being deficient (Shin et al., 2010). According to a NCHS news brief (March, 2011), The Institute of Medicine (IOM) issued a guideline that vitamin D levels sufficient levels of 25(OH)D were above 20mg/ml (CDC.gov Data Brief 59, 2011). It is now widely accepted that adequate intake of vitamin D intake is essential for maternal health, and is key for the prevention of adverse maternal outcomes (Shin et al., 2010). Given that well over 20 -85% of pregnant women are vitamin D deficient, depending on the country of residence and other factors (Shin et al., 2010), it is critical that we understand the mechanisms whereby vitamin D deficiency leads to these adverse outcomes.

Little is known about the mechanisms underlying the non-classical actions of vitamin D. The theoretical pathogenesis is that vitamin D levels is thought to impair the normal balance of the Th1 cytokine expression thus damaging the immunological functioning tolerance of the embryo to implantation (Bodnar, 2007 OR Baker, 2010). The CYP27B1 and CYP24A1 are genes that code enzymes that regulate levels of $1,25(OH)_2D$, which is responsible for binding to the VDR to induce genomic and non genomic responses (Shin et al., 2010).

Research shows that all components for vitamin D signaling pathway are expressed in the human placenta (Shin et al., 2010) and, moreover, that human decidual and placental tissues are able to synthesize $1,25(OH)_2D$ as well as $24,25(OH)_2D$. (Weisman and Harell, 1979). Experiments with trophoblasts, the precursors to cells of the human placenta, document that when these cells are exposed to E. coli in the presence of vitamin D, they exhibit lower rates of infection and of cell death (Shin et at., 2010), making the case for vitamin D supplementation to reduce infection during pregnancy. This theory was further supported by study by Bodnar et al. (2009), who showed that the unadjusted mean serum concentration of 25(OH)D was lower in patients with bacterial vaginosis (BV) than in women without BV, and that the in prevalence of BV decreased as 25(OH)D increased to 80nmol/L.

Prevalence of vitamin D insufficiency

Bondar et al., (2006) investigated the prevalence of vitamin D insufficiency in black and white pregnant women in the Pittsburg Pennsylvania area by race and season, and found that 29.2% and 54.1% of black women had vitamin deficiency and insufficiency, respectively, compared to 5% and 42.1% of white women, respectively. Moreover, after adjusting for confounders, a small mean increase in maternal 25(OH) concentrations in black and white women was also noted from winter to summer. The authors concluded that black and white pregnant women had a high risk of vitamin D deficiency when they reside in the northern US even while 90% of the study population was compliant with vitamin supplementation (Bodnar et al., 2006). Scragg and colleagues, after an examination of the third NHANES survey, found that adjusted mean serum 25(OH)D concentrations were lowest in NHB when compared to Mexican

Americans and NHW (Scragg et al., 2007).Just recently, a study by Ganji and colleagues found that overall, the mean serum 25(OH)D decreased by 9% in all participants of the NHANES study from 1988-1994 to 2001 -2006 and the prevalence of 25(OH)D <30nmol/L increased from 5 to 10 percent in all participants but in blacks it increased from 22 to 88% (Gangi et al., 2011).

Gaps in Research

In the past years research has come to light implicating vitamin D in preeclampsia. However there are few experimental studies that examine the association of vitamin and HDP. In addition studies examining vitamin D and preeclampsia lack sufficient power and national representativeness to negate the effects of a single location. In this study I propose to use a nationally representative sample in the National Health and Nutrition Survey to determine the association of low vitamin D on pregnant women with hypertensive pregnancy related disorders.

Conclusion

   There appears to be consistent evidence in the literature linking vitamin D deficiency and preeclampsia. In this study we will use research based on preeclampsia to inform various aspects of HDP since they are related. Non-Hispanic Black women have an increased risk for HDP as well as vitamin D deficiency. Herein we will attempt to investigate whether low vitamin D status is associated with increased risk if HDP. *Importance* – If indeed vitamin D deficiency is related to HDP and preeclampsia, this correlation can inform future studies, which hopefully will ultimately lead to a decrease in HDPs, hence a decrease in adverse maternal and fetal outcomes. In addition Vitamin D

supplementation in pregnant women could be further investigated in order to prevent adverse outcomes for the mother as well as the fetus.

This study will utilize the NHANES survey, to conduct a cross sectional analysis of HDP in pregnant women across the US from 2001 to 2006. The association of vitamin D status and HDP will be examined with respect to race.

Chapter III - Methods

Study Purpose

The purpose of this cross-sectional study was to determine (i) the association of vitamin D deficiency with the occurrence of preeclampsia and (ii) whether African American women are at greater risk for preeclampsia due to low vitamin D status.

Data Source

Data used in this study were obtained from the National Health and Nutrition Examination Survey (NHANES) of the National Center for Health Statistics (NCHS). The NHANES used a stratified, multistage, probability sample design to undertake nationally representative survey on the civilian non-institutionalized population in the US. As of 1999, NHANES has been conducted annually, with data released in 2-year cycles for public use. Data were derived from interviews, physical examinations, and laboratory. For the personal interviews, participants signed consent forms, and health questionnaires were administered by interviewers. Participant's answers to demographic, socioeconomic, dietary and health-related questions were recorded with use of Computer-Assisted Personal Interview Technology. A Mobile Examination Center (MEC) was employed to facilitate physical examinations and collection of blood and urine samples. Adolescents, persons aged ≥60 years, non-Hispanic blacks, low-income persons, and Hispanics/Mexicans were oversampled, to obtain reliable estimates for these groups. To obtain a stratified, multistage probability sample for 2001 to 2006, multiple sample stages included: (1) sampling units of single counties or small groups of low population

counties, (2) a block or group of blocks within those counties, (3) clusters of households within those blocks, and (4) and one or more persons within those households. Starting in 2003, the survey content was held as constant as possible for each two year period, to ensure consistency with the data release cycle. This allowed analysis of two or more "cycles" (e.g. 2001-2002, 2003-2004 and 2005-2006) in addition to evaluation of data from any single two-year cycle. In fact combining combination of two or more two-year cycles is strongly recommended to generate estimates with superior statistical reliability. Data sets (documents) were examined to verify that data items collected in all of the combined years were similar in wording and methods.

Study Participants

For our study, we used interview and examination data from the NHANES survey for 2001-2002, 2003-2004, and 2005-2006. The data for this study was collected between January 2001 and December 2002, and include interviews of 11, 039 individuals of all ages (10,477 were examined in MECs). The 2003-2004 NHANES survey comprises data for 12, 862 individuals of all ages (9,643 were examined in MECs), collected between January 2003 and December 2004. The 2005-2006 NHANES survey used data from 11,862 individuals of all ages (9,950 were examined in MECs): these data were collected between January 2005 and December 2006. For all years, several files contained the data and appropriate documentation for the survey interview and examination components. All NHANES protocols were reviewed by the NCHS review board prior to data collection. This study utilized updated serum 25(OH)D data released by the NCHS in November 2010.

Periodically NHANES data files are updated by NCHS which replaces previous data files. In November of 2010, the NCHS issued a data advisory for measurements of serum 25(OH)D because of changes (method bias and imprecision) in the performance of the serum 25 OHD assay over time. Users were informed that 25(OH)D data from NHANES 2003-2004 and 2005-2006 were adjusted for assay drifts. The advisory recommended that researchers use the newly available assay-adjusted data rather than the unadjusted data previously available (CDC.gov., 2011).

Measurement of Vitamin D: Venipuncture was used to collect blood samples in the MEC as per standard protocols. The Diasorin RIA kit assay (Stillwater, MN) was used to measure serum 25(OH)D concentrations ate h National Center of Environmental Health, CDC Atlanta GA.

Demographic Variables

The demographic variables used in this study were gender, age and race/ethnicity, with the exclusion of all males. One inclusion criterion for age was that the women be of child bearing age in the US - namely, 15 to 49 years old.

Age: The demographic age was self reported, and was recorded as a whole number in years at the time of screening. Age was then classified into the following three categories: 15 to 20 years, 21 – 35 years, and 36 – 44 years.

Gender: Gender was self reported; this study only included females.

Ethnicity: Ethnicity was self reported, and was obtained from responses to the survey question on race and Hispanic origin. Ethnicity was categorized into the following groups: Other racial groups, Non-Hispanic White and Non-Hispanic Black. This variable

was recoded as follows: "1" for Other Racial groups, "2" for Non-Hispanic White and "3" for Non-Hispanic Black.

Pregnancy Status: For all years, pregnancy tests were performed on all female subjects 12 to 59 years old and on menstruating females 8 to 11 years old with use of the Icon 25 hCG test kit (Beckman Coulter), which detects human chorionic gonadotrophin (hCG) in urine or serum.

Body mass Index: BMI was calculated from measurements of participants' weight and height. A BMI of <18.5 was designated as underweight, BMI of 18.5 to 25 – normal weight, BMI 25 to 30 – overweight and BMI >30 was considered obese. In later analysis the overweight and obese categories were combined for simplification.

Hypertensive Disorder in pregnancy: HDP was determined by a study participant satisfying any one of the following: (1) Pregnant and confirmed by urine pregnancy test. In this study those who were pregnant were coded as 1 and non pregnant coded as 2. (2) A positive answer to the question: 'ever been told you have High Blood Pressure' "1" for yes and "2" for no. (3) A positive answer to the question 'Are you now taking prescription for Hypertension "1" for yes and "2" for no. (4) Mean Systolic Blood Pressure (MSBP) > 140 mmHg, calculated by computing the average of the second and third systolic blood pressure reading or Mean Diastolic Blood Pressure > 90 mmHg calculated by computing the average of the second and third blood diastolic pressure reading. Therefore a subject meeting any one of these requirements was coded as having Hypertensive Disorder in pregnancy.

Vitamin D status: This was determined by the concentration of 25-OHD (ng/ml) (range 2 to 86 ng/ml) in the serum of participants, and was measured in ng/ml in the NHANES survey. 25-OHD concentration of <20ng/ml was considered as deficient, while concentrations of >20ng/ml and above, was considered as sufficient. Study participants missing this variable were excluded from this study. For additional analysis, vitamin D status was further categorized into three categories; deficient (<20ng/ml), insufficient (21-30ng/ml) and sufficient (>30ng/ml) for additional analysis.

Delimitations: The study included pregnant women only between the child bearing ages of 15 and 44 years old.

Confounding Variables

Confounding variables for preeclampsia in this study were high BMI, race, diabetic status, and age. Race was recoded as described above and diabetic status was determined as positive if the subject satisfied anyone of the following criteria: (i) Has a doctor ever said you had diabetes? "1" for yes and "2" for no. (ii) Do you take pills to lower blood sugar? (iii) Participant's glycohaemoglobin levels are > 6%.

Confounders for low vitamin D were used and included anticonvulsants, glucocorticoids, HAART (AIDS medications), antirejection medication. Subjects taking anticonvulsants or glucocorticoids were coded as having a vitamin D depressant. Any of the following drugs can suppress vitamin D levels: Oxcarbazepine, Carbamazepine, Phenobarbital or Hydrocortisone. Use of vitamin D as a supplement was presumed when the participant answered positively to the question 'Have you used a vitamin D supplement within the past 30 days?'. A 'yes' answer was scored as 1, while 0 indicated that no supplement was

used. Once the final data set containing only pregnant women was obtained, women who had missing values for vitamin D were excluded from the study.

Statistical Analysis

NHANES data from 2001 to 2006 were organized, truncated and analyzed with use of the Statistical Package for the Social Sciences (SPSS) version 18.0 and SAS version 9.2. The distribution of race, ethnicity, education level and household income for the total sample was obtained with use of descriptive analysis. The independent variable analyzed was vitamin D status, and the dependent variable was HDP; control variables were age in years, race/ethnicity, education level, diabetic status, and BMI.

Mean values of continuous values were compared across HDP states using the independent t-tests. The distribution of categorical variables was reported in percentage across HDP status. Chi squared tests were then used to determine which confounders were significantly different among women who experienced HDP verses those who did not. The distribution of categorical variables was across vitamin D status (deficient verses sufficient) as also analyzed using bivariate methods. Chi squared tests were then used to determine which confounders were significantly different among women with different vitamin D status. Univariate logistic regression was conducted for each covariate in the study Multivariate logistic regression was performed with step-wise selection for all possible covariates of HDP. The variables remaining were then described. Statistical significance was reported based on 95% confidence intervals and p-values of 0.05.

Chapter IV – Results

Initially the study population consisted of 1037 study participants. However once subjects who had missing values for vitamin D, the main independent variable, were removed from the study population, 803 subjects were eligible for the study. The basic characteristics of the study population of pregnant women ages 15 to 44 years, are described in table 1. The distribution of age, race/ethnicity, annual house hold income, education level, HDP status, vitamin D status, the presence of liver disease and vitamin D status were all examined. Regarding race, there were 359 (44.71%) NHW, 135 (16.81%) NHB and 309 (38.48%) subjects of other racial backgrounds. The mean age of the participants was 26.28 yrs (SD=5.80), and mean vitamin D concentration was 22.50ng/ml (SD 8.97). Pregnant women aged 21 to 35 years were the largest group at 54.8%, followed by the 36-44 year age group at 30.5%. NHW were the largest ethnic group at 44.71%, followed by other racial groups at 38.5% and 16.81% NHB. Women with an annual house hold income of $20,000 to $55,000 had the largest representation at 40.34%. Almost half of the population had high school and some college.

With respect to the independent variables, 340 (42%) had low vitamin D (25(OH)D<20ng/mL), vitamin D deficient), mean vitamin levels were 22.50ng/ml (SD=8.97). Thirteen subjects (1.62%) had liver disease, 56 (7%) were diabetic and 674 (84% used supplements (vitamin D supplementation assumed). The mean BMI was 24.39 (SD=6.79). For BMI sub categories of underweight, normal weight, overweight and obese; the overweight sub category had the highest number of subjects with 263 (37.73%). The proportion of HDP in the study population was 24%.

Table 2 describes the characteristics of the study participants across HDP states. Study participants with HDP were more likely to be non-Hispanic white, have an annual house hold income between \$20,000 and \$55,000, have some high school or college and of normal weight. The only variables that were significant were for BMI and diabetic status and supplement use (95% CI; *P*= 0.009, 0.0354 and 0.0225 respectively). Table 3 shows the result of bivariate analysis of the independent variable across vitamin D deficient verses vitamin D sufficient states. The only variable that was significant was use of supplements (P-0.0012).

Univariate logistic regression of the independent variables was conducted to determine association with the variable and HDP. These results are shown in table 4. Overall, low vitamin D status showed an increased risk of HDP (OR; 1.123) but this was not significant (95%CI; 0.808, 1.56). The only significant positive association with HDP at the 0.05 confidence level was BMI. For the vitamin D deficiency subcategories, i.e. vitamin D deficiency, insufficiency and sufficiency, the reference group was vitamin D sufficiency. Based on the reference group (vitamin D sufficiency) estimated OR for vitamin D deficiency and insufficiency was shown in table 5. Subjects who were vitamin D deficient were 0.853 times less likely to have HDP than sufficient subjects. Based on the table, confidence interval between 95% CI, vitamin D status was not significantly associated with HDP under the condition of $\alpha = 0.05$ For the diabetic status an OR of 0.545 was observed, but this was not significant (95% CI; 0.307, 0.966) under the condition of $\alpha = 0.05$.

Multivariate Logistic Regression

Stepwise multivariate logistic regression was used to select which covariates had a significant effect on HDP from all possible covariates. Only one variable, BMI (Body Mass Index) remained. The goodness of fitness of the selected model was confirmed by Hosmer-Lemeshow test: Chi-square test statistics is 3.3270 with d.f of 8, and p-value is 0.9122.

The estimated parameters for multivariate logistic regression and the estimated OR with 95% of confidence intervals are shown on the tables below. The results of the table 5 shows that increased BMI had significantly associated odds of HDP as indicated by odds ratio 1.040 (95%CI: 1.015, 1.066).

Table 1. Basic Characteristics of the study population from NHANES 2001 to 2006

Variable		Number (%)
<u>Demographic Variables</u>		
Female		803
Age Group	*Mean*	26.29 (SD=5.81)
	15-20 yrs	118 (14.69)
	21-35 yrs	440 (54.79)
	36-44	245 (30.51)
Race/Ethnicity	*Non-Hispanic White*	359 (44.71)
	Non-Hispanic Black	135 (16.81)
	Mixed/ Other	309 (38.48)
[a]**Annual Household Income**	*Less than $20,000*	181(23.94)
	$20,000.00 -$55,000	305 (40.34)
	$55,000+	270 (35.71)
[b]**Education Level**	*Less than High School*	179 (26.17)
	High School Some College	332 (48.54)
	College Grad	173 (25.29)
<u>Dependent Variable</u>		
HDP	*Yes*	197 (24.16)
	No	609 (75.84)
<u>Independent Variables</u>		
Vitamin D Level/Status (ng/ml)	*Mean*	22.50 (SD=8.97)
	Low	340 (42.34)
	Not Low	463 (57.66)
Liver Disease	*Yes*	13 (1.62)
	No	790 (98.38)
[c]**Diabetic Status**	*Yes*	56 (7.04)
	No	740 (92.96)
BMI	*Mean*	24.39 (SD=6.80)
	Underweight	237 (29.51)
	Normal Weight	263 (32.75)
	Overweight	170 (37.73)
	Obese	133 (16.56)
Use of Supplements	*Yes*	674 (83.94)
	No	129 (16.06)
Vitamin D categories	*Vitamin D Deficient*	304 (37.85)
	Vitamin D Insufficient	386 (48.07)
	Vitamin D Sufficient	113 (14.07)

aMissing 47, b missing 119, c missing 7.

Table 2. Distribution of independent variables by the presence and absence of HDP

Variable		Total (n=803) %	HDP (n=197) %	No HDP (n=609) %	χ^2 P-value
Demographic Variables					
Age	*15-20 yrs*	14.7	17.01	13.69	
	21-35 yrs	54.8	55.15	54.68	0.4631
	36-44	30.5	27.84	31.36	
Race/Ethnicity	*Non-Hispanic White*	44.7	49.5	43.2	
	Non-Hispanic Black	16.8	14.4	17.6	0.2795
	Other Racial Groups	38.5	36.1	39.2	
Low Vitamin D Status	*Yes*	42.3	40.2	43.0	0.490
	No	57.7	59.8	57.0	
Annual Household Income	*Less than $20,000*	123.9	24.31	23.8	
	$20,000.00 - $55,000	40.3	38.6	40.9	0.8646
	$55,000+	35.7	37.0	35.3	
Education Level	*Less than High School*	26.2	24.2	26.8	
	High School Some College	48.5	50.9	47.8	0.752
	College Grad	25.3	24.8	25.4	
Independent Variables					
Low Vitamin D Level/Status	*Low*	42.34	40.2	43.0	
	Not Low	57.66	59.8	57.0	0.4895
[b]BMI	*Underweight*	29.51	23.71	31.36	
	Normal Weight	32.75	30.41	33.50	
	Overweight	21.17	22.16	20.85	**0.0099**
	Obese	16.56	23.71	14.29	
Liver Disease	*Yes*	1.62	2.1	1.5	
	No	98.38	97.9	98.5	0.5264
Diabetic Status	*Yes*	7.04	10.4	6.0	**0.035**
	No	92.96	89.6	94.0	
Use of Supplements	*Yes*	83.9	89.2	82.3	
	No	16.1	10.8	17.7	**0.0225**

Table 3. Distribution of independent Variables by the Vitamin D status

Variable		Total (n=803) %	Vitamin D Deficient (n=197) %	Vitamin D Sufficient (n=609) %	χ^2 P-value
Demographic Variables					0.6293
Age	*15-20 yrs*	14.69	15.12	14.12	
	21-35 yrs	54.79	53.35	56.76	
	36-44	30.51	31.53	29.12	
Race/Ethnicity	*Non-Hispanic White*	38.48	42.33	47.94	0.1249
	Non-Hispanic Black	44.71	16.20	17.65	
	Other Racial Groups	16.81	41.47	34.41	
Annual Household Income	*Less than $20,000*	23.94	24.31	23.83	0.8646
	$20,000.00 - $55,000	40.34	38.67	40.87	
	$55,000+	35.71	37.02	35.30	
Education Level	*Less than High School*	26.17	24.22	26.77	0.7520
	High School Some College	48.54	50.93	47.80	
	College Grad	25.29	24.84	25.43	
Independent Variables					
[b]**BMI**	*Underweight*	29.51	31.75	26.47	0.2247
	Normal Weight	32.75	33.26	32.06	
	Overweight	21.17	20.09	22.65	
	Obese	16.56	14.90	18.82	
Liver Disease	*Yes*	1.62	1.08	2.35	0.1578
	No	98.38	98.92	97.65	
Diabetic Status	*Yes*				
	No				
Use of Vitamin D Supplements	*Yes*	83.94	80.35	88.82	**0.0012**
	No	16.06	19.65	11.18	

Table 4. Association with Low vitamin D and the selected independent variables with HDP.

Variables	p-values	OR	95% CL
Low Vitamin D	0.4896	1.123	(0.808, 1.56)
Non-Hispanic White	0.1110	1.246	(0.874, 1.776)
Non-Hispanic Black	0.3344	0.893	(0.866, 2.248)
BMI	0.0015	1.040	(1.015, 1.066)
VitaminD	0.9296	1.001	(0.983, 1.019)
Vitamin D vs Deficiency	0.4936	0.853	(0.517, 1.405)
Vitamin D vs Insufficiency	0.9456	0.934	(0.577, 1.513)
DM	0.0378	0.545	(0.307, 0.966)
Liver Disease	0.5762	0.712	(0.217, 2.339)

OR = Odds ratio; CL = confidence limits

Table 5. Shows results of OR estimation for different vitamin D states and HDP

Description	OR	95% CI
Vitamin D deficiency vs vitamin D sufficiency	0.853	(0.517, 1.405)
Vitamin D insufficiency vs vitamin D sufficiency	0.934	(0.577, 1.513)

Table 6. Results of stepwise logistic regression analysis of factors that are associated with HDP

	Odds Ratio Estimates		
Variable	OR	95% Wald Confidence Limits	
BMI	1.040	1.015	1.066

Chapter V - Discussion

In this study the association between vitamin D status and HDP in a representative sample of the US population was investigated. Further, the risk of HDP in NHB women in the population was examined. The results showed that pregnant women who were vitamin D deficient were 1.123 times more likely than vitamin D sufficient women to have HDP; however this association was not significant (95%CI: 0.808-1.56). NHB women were 0.893 (95%CI: 0.866, 2.248) times less likely to have HDP; this association was also not significant. The only covariate that remained statistically significant after stepwise logistic regression was BMI (OR=1.040; 95%CI 1.015-1.066).

Since there are no comparable studies of vitamin D status and HDP, these finding are compared to studies examining vitamin D status and preeclampsia as previously mentioned. These findings are in contrast to other studies that show a significant positive association between low vitamin D status and preeclampsia. Bodnar and colleagues, in a nested case control study involving 55 nulliparous women, showed that women who ultimately developed HDP were 2.5 time more likely to have vitamin D deficiency in early pregnancy after adjusting for confounders (Bodnar et al., 2010). In addition a large cohort study by Haugen et al., 2009, a large cohort study of over 23,000 nulliparous women who had a higher vitamin D intake, had a lower rate of preeclampsia when compared to those who had a lower intake (15-20µg/day verses <5µg/day, OR 0.76, 95%CI 0.6-0.95) after adjusting for confounders.

A number of explanations for this discrepancy exist. This study examined HDP which is comprised of multiple disorders in pregnancy (preeclampsia, eclampsia, and gestational as well as chronic hypertension). In order to determine the presence or

absence of these conditions, a number of surrogate measures were used. For instance the presence of chronic or gestational hypertension was determined by a positive answer to the question 'has a doctor ever told you, you have hypertension?' This method is clearly subjective and prone to reporting bias. Additionally in the nested case control study mentioned above, serum 25(OH)D concentrations were measured at a mean 10 weeks of gestation. In this study the length of gestation was not known. The study by Haugen et al., 2009, suggested that vitamin D supplementation in early gestation was beneficial.

Another unexpected finding was that a larger than expected prevalence of HDP was observed in this study population (24%) than accepted prevalence in the general population (10 to 15%). Again the imprecise definition of HDP could have contributed to this discrepancy.

An increased risk of HDP observed was expected since it is known that women with hypertensive disorders have lower circulating 25(OH)D3 levels compared to normotensive pregnant women (Bodnar et al., 2007;Halhali et al., 200; Robinson et al., 2010). What was not expected was the lack of significance in these findings. This could have been due to lack of sufficient power as a result of the relatively small sample size of the data, even after combining multiple years of the NHANES survey. In addition the distribution of serum 25(OH)D concentration in the study population was skewed to the left, hence sample size and effect size could have been affected, interfering with conclusion validity. Another contributor could have been the imprecise definition of HDP due to the nature of the secondary data.

Another unexpected finding was that non-Hispanic Black women did not show an increased risk for HDP compared to Non-Hispanic White as predicted. In fact, it showed

a potential protective effect vitamin D deficiency although not significant (OR: 0.82; 95%CI: 0.321 – 2.098). A larger sample size of NHB women may determine the true extent of this relationship. The results did not confirm an increased risk of HDP among Non-Hispanic Black women as seen in other studies (Bodnar et al, 2007; Bodnar et al, 2010). In addition, a disproportionately high rate of HDP was not observed in this population as reported in current literature. These findings could have been due to the relative small sample size of the NHB population in this study. In addition, the distribution of the independent variable across the subpopulation of non-Hispanic Blacks, as well as the other two groups in this study, was skewed toward lower vitamin D levels. Therefore differences due to high or low vitamin D levels would be difficult to ascertain.

Currently there are no set guidelines for concentration of 25(OH)D that inform vitamin D deficiency or sufficiency; only recommendations by the IOM (CDC.gov – NCHS Data Brief 59, 2011). Interestingly 84% of the study population used supplement presumed to contain vitamin D, however 42% remained vitamin D deficient. Guidelines for vitamin D supplementation are still in debate at this time.

Similarities and Differences with Past Research

With respect to the total population, the results of this study were not consistent with the majority of other studies that demonstrated a relationship between low vitamin D status and preeclampsia, a hypertensive disorder of pregnancy. An example of this can be found in a nested case control study by Baker et al., 2010 that found a 4-fold increase in severe preeclampsia in women who had <50nmol (20ng/ml) 25(OH)D$_3$.

Conversely the finding of no association between low vitamin D status and non-Hispanic Black population is not consistent with a majority of current literature on vitamin D and preeclampsia.

Other evidence of vitamin D's association of to preeclampsia exists. In addition to reports from observational studies of the seasonal variation of preeclampsia, lower levels of serum 25(OH)D are more prevalent in preeclamptic women. Current knowledge insists that adequate vitamin D intake is during pregnancy is optimal for maternal and child health (Shin et al., 2010). Research has shown that the placenta produces and responds to vitamin D, which in turn modulates implantation, cytokine production and the immune response to infection (Shin et al., 2010).

Studies examining how genetic variability contribute to the disparity of maternal outcomes in NHB and NHW women suggest that the difference in the race-specific allelic frequencies in the vitamin D receptor (VDR) gene, is potentially involved in these health disparities. Vitamin D's direct influence on molecular pathways involved in the pathogenesis of preeclampsia and the high prevalence of its deficiency in Non-Hispanic Black women (Bondar et al., 2007; Dror, 2011); together suggests a role for vitamin D in preeclampsia and possible HDP. Finally the high prevalence of vitamin D deficiency is, may have been thought to explain the increasing rates of HDP.

This finding is consistent with a few studies that do not find a positive relationship between vitamin D deficiency and hypertension in pregnancy. A study in British Columbia Canada found that there is no difference in the rates of preeclampsia,

gestational hypertension, or adverse pregnancy outcome in a group of 221 pregnant women who were largely vitamin D deficient (Shand et al., 2010).

Several factors affecting conclusion validity need to be taken into account. They include sample size, effect size and power. Threats to conclusion validity in this study could result from low reliability of measures. Sources of low reliability of measures could result from questionnaire wording and recall bias and the change in the vitamin D formulations. Relying on self reported measures in the NHANES survey could have been subjected to recall or reporting bias, which could also affect conclusion validity. Quality control studies by the CDC estimated that the mean serum 25(OH)D in the NHANES 2003-2004 and NHANES 2005-2006 either were over or underestimated by 10% (CDC.gov, 2010;Yetley et al.,2010; Looker et al., 2008). Assay reformulation by the manufacturer and lot-to-lot variation in calibration was most likely responsible for this variation in serum 25(OH)D between surveys (CDC.gov, 2010;Yetley et al.,2010; Looker et al., 2008). As a result, an advisory note by the NCHS that the adjusted rather than unadjusted data should be used for all analyses utilizing serum 25(OH)D. This study utilized the adjusted data (CDC.gov, 2010). Therefore the validity of the vitamin D measurements is not believed to be compromised. Violated assumptions of statistical test may have compromised conclusion validity since almost of the study population was vitamin D deficient. Another threat to conclusion validity is the question of whether or not the study population was too heterogeneous, and a small percentage of the study population being NHB. The effect of this would be that any differences experienced in the NHB population would become small relative to the larger group.

Strengths and Limitations

This study has strengths which add to its reliability. One major strength was the national representativeness of the data source funded by the National Center of Health Statistics. The sample size was relatively large at 803 subjects. This study also attempts to determine the association between vitamin D and HDP in nationally representative sample, which had been done before. However one limitation of this study was the imprecise definition of HDP through surrogate measures obtained from the NHANES survey questionnaires. The imprecise nature of the definition of HDP may have lead to a distortion of the relationship of vitamin D and HDP. For example the question 'have you ever been told you have hypertension assumes that the study participant has hypertension now. If persons who are vitamin D sufficient are recorded as having HDP, then it becomes more difficult to detect the relationship between vitamin D deficiency and HDP.

Recommendations

This study attempted to find the relationship between vitamin D deficiency and HDP. Given the maternal and fetal burden that is caused by HDP; pregnant women should still adhere to current vitamin D requirements in an effort to optimize their vitamin D status. The limitations in this study should inform what difficulties future studies should avoid such as a more reliable definition of HDP. Other factors to be considered are sample size and heterogeneity of the sample. For example a large sample of non-Hispanic black

The results of this study highlighted the need for additional studies with a larger sample size that will improve power of the findings. In addition future research should

focus on a large enough size of only pregnant non-Hispanic Black women since there are

not many studies on this population (Bodnar et al., 2007).

Improving the instrument – Nationally representative datasets of only pregnant women

could be used henceforth, once information on vitamin D status of the participants is

available. This way, matters of sample size and adequate power would be accounted for

in addition to precision of HDP measures.

Conclusion

This study attempted to determine whether pregnant women with low vitamin D

had an increased risk of HDP, and further, if NHB women had an even higher risk, based

on low vitamin D status. The results of this study show that low maternal vitamin D

status does not significantly increase the risk of HDP in neither pregnant NHW women

nor in NHB women. Only increased BMI showed an increased risk for HDP. This

finding is consistent with findings from many studies showing a positive association

between BMI and increased risks of preeclampsia and HDP (Tsai et al., 2012; Saeed et

al., 2011;Anderson et al., 2012). These results may not be generalizable to the general

population because of the discrepancy of HDP rates study population and in the overall

general population as reported. In light of previous findings of a positive association

between low vitamin D status and HDP and the biological plausibility of the association;

this finding needs to be tested in a larger sample size. In addition studies focusing on

NHB women alone should be carried out to determine the true nature of the association

between low vitamin D status and disparity of HDP rates in this population. Such an approach would diffuse the effects of a vastly heterogeneous population.

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