Depression and Vitamin D in Pregnancy

By

Amy Rebekah Lamb

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in

Nursing Science

August, 2014

Nashville, Tennessee

Approved:

Melanie Lutenbacher, Ph.D.

Ken Wallston, Ph.D.

Shelagh Mulvaney, Ph.D.

Lavenia Carpenter, M.D.

To my Savior, Jesus Christ, for apart from Him I am nothing

and

My precious family, both immediate and extended,

My infinitely supportive husband David and my children Levi, Lark, and Luke whom I love dearly.

ACKNOWLEDGEMENTS

This work would not have been possible without the financial support of the Vanderbilt Clinical and Translational Science Award from the National Center for Advancing Translational Sciences, and the Vanderbilt University Graduate School Dissertation Enhancement Grant. I am especially indebted to Dr. Calvin Hobel, Dr. Melanie Lutenbacher, Associate Professor of Nursing and Medicine (Pediatrics), and Dr. Ken Wallston, Professor of Psychology and Nursing, who have believed in me and who worked actively to support the advancement of my research.

I am grateful to all of those with whom I have had the pleasure to work during this and other related projects. Each of the members of my Dissertation Committee and my mentor has provided me extensive personal and professional guidance and taught me a great deal about both scientific research and life in general. I would especially like to thank Dr. Melanie Lutenbacher, the chairman of my committee. Over the course of many years and various projects she has continued to support and encourage me and for that I am very grateful. And to Dr. Calvin Hobel, my mentor, he has taught me more than I could ever give him credit for here. He has shown me, by his example, what a good scientist (and person) should be.

I acknowledge my Lord and Savior Jesus Christ for with God all things are possible. Nobody has been more important to me in the pursuit of this project than my family. I would like to thank both my parents whose love and guidance are with me in whatever I pursue. Most importantly, I wish to thank my strong and supportive husband, David, and my three wonderful children, Levi, Lark and Luke, who are the great lights of my life.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Statement of the Problem

Depression in pregnancy is a serious and growing problem affecting 8-27% of women worldwide (CDC, 2007; Bansil et al., 2010; Gaynes et al., 2005; Ko, Farr, Dietz, & Robbins, 2012; NSDUH 2012; Villegas, McKay, Dennis, & Ross, 2011). A woman experiencing depression during her pregnancy is at increased risk for pregnancy complications. She is also at higher risk for inter-relational conflict with her partner that may include partner violence (Roberts, Bushnell, Collings, & Purdie, 2006). Maternal depression during pregnancy also places the developing fetus at risk for complications (2004-2006 NHIS; Milgrom et al., 2008; Pilowsky, 2008; Pesiah et al., 2004; Weissman et al., 2006). The disease burden of depression in pregnancy leads to increased healthcare costs and negative sequelae for society, healthcare, and individuals (Lin, Lin, Hsiao, & Li, 2009; Pesiah et al., 2004; Seto et al., 2005; WHO, 2012).

Potentially compounding the development of depression in pregnancy are low levels of Vitamin D. Growing evidence suggests that many pregnant women have low vitamin D levels despite taking prenatal vitamins (Brannon & Piccano 2011; Bodnar et al., 2007; Holmes et al., 2009; Marwaha et al., 2011). Vitamin D deficiency may be an important risk factor for depression in pregnancy (Brandenbarg, Vrijkotte, Goedhart, & van Eijsden, 2012; Cassidy-Bushrow, Peters, Johnson, Li, & Rao, 2012), but this evidence remains inconclusive (Nielsen et al., 2013). In order to further the science in this area, this dissertation study was undertaken to examine the longitudinal association

between depressive symptoms and vitamin D in a sample of pregnant women. An overview of depression in pregnancy and vitamin D deficiency, the significance of these problems individually and collectively and the purpose of the study is presented below.

Definition of Terms

Depression

Globally, depression is the leading cause of disability with over 350 million people suffering worldwide (WHO, 2012). Untreated depression can lead to suicide, which accounts for 1 million lives lost each year (WHO, 2012). Women are more likely than men to be affected by depression with depression being the most common mental disorder in women (WHO, 2012). Depression affects approximately 1-2 out of every 10 mothers within the first year after giving birth (Banti et al., 2011; WHO, 2012).

Depression in pregnancy increases the risk for pregnancy complications (2004-2006 NHIS; Milgrom et al., 2008; Pilowsky, 2008), may heighten the potential for inter-relational conflict increasing the partner's risk for mental disorders and violence (Roberts, Bushnell, Collings, & Purdie, 2006) and creating increased healthcare costs (Lin, Lin, Hsiao, & Li, 2009). In addition, depression in pregnancy has been associated with impacts on the developing fetus such as later cognitive and behavioral disabilities (Pesiah et al., 2004; Weissman et al., 2006). Depression is a syndrome characterized by depressed mood and psychomotor agitation for more than 2-weeks with impairment in functions of daily living (Pratt & Brody, 2008; U.S. Department of Health and Human Services (HHS), 2007; Kendall, Hollon, Beck, Hammen, & Ingram, 1987). Symptoms of depression may include the following: sad feelings, fatigue, sleep disturbances, changes

in appetite, physical pain, trouble focusing, and an inability to enjoy pleasant activities (NIMH, 2008).

Timing of depression can vary in the perinatal period, with onset in the antenatal period, occurring in pregnancy prior to giving birth, or during the postpartum period for up to 12 months after giving birth (Gaynes et al., 2005). Antenatal depression may be phenotypically different from depression that has its onset in the postpartum period (Altemus et al., 2012). Women who experience depression in pregnancy are more likely to cite poor social support, abuse, an unplanned pregnancy, to have a history of depression, and to have recently stopped taking antidepressants (Altemus et al., 2012; Mora et al., 2009; Stowe, Hostetter, & Newport, 2005). Women with depression onset postpartum are more likely to be experiencing their first episode of depression, to cite infant medical problems as a stressor, and to suffer severe features such as intrusive violent thoughts and psychosis (Altemus et al., 2012; Mora et al., 2009; Stowe et al., 2005). There are, however, no differences in rates of suicidal ideation during the two time periods (Altemus et al., 2012). Despite potential mechanistic differences antenatal and postpartum depression are highly correlated (Milgrom et al., 2008) and comprehensive meta-analysis findings suggest that the incidence in both periods is approximately the same (Gaynes et al., 2005).

Along with timing, the severity of depression can vary from mild to severe. Mild depression is characterized by the presence of depressive symptoms but does not meet the standard criteria for major depression (Gaynes et al., 2005). Major depression as defined by *DSM-IV-TR* criteria is the demonstration of specific depressive symptoms with regularity and significant impairment for at least 2 weeks (APA, 2000). Severe

depression is major depression that is causing severe impairment (e.g., catatonia) and that may develop into psychotic depression (Gaynes et al., 2005). Since the primary interest in depression is its relationship to vitamin D, all forms of depression are relevant to better understanding this link.

Several risk factors for depression in pregnancy have been identified in previous studies. These include: anxiety, life stress, history of depression, unplanned pregnancy, inadequate social support, domestic violence (Bunevicius et al., 2009; Lancaster et al., 2010; Milgrom et al., 2008; O'Hara, 1986), financial stress (Grote & Bledsoe, 2007), obesity, low educational attainment, poverty, a history of poor obstetrical outcome (Lancaster et al., 2010; Murphy, Mueller, Hulsey, Ebeling, & Wagner, 2010), obesity, and physical inactivity (Shivakumar et al., 2010). Recent evidence also suggests that underlying inflammation may be an important risk factor and possible biological mechanism for depression in pregnancy (Blackmore et al., 2011; Cassidy-Bushrow et al., 2012; Christian et al., 2009; Leonard & Maes, 2012; Maes, Ruckoanich, Chang, Mahanonda, & Berk, 2011). Despite growing knowledge on risk factors for depression in pregnancy preventative interventions have not been identified and the problem is growing.

Over the last decade, rates of maternal depression have been on the rise. In a large study of 32,156,438 hospital deliveries, the rate of maternal depression increased from 2.73 per 1,000 deliveries in 1998 to 14.1 per 1,000 deliveries in 2005 ($p < 0.001$) (Bansil et al., 2010). Some posit that the rising rates of depression may be linked to an emerging modern lifestyle (Hidaka, 2012). Recent years have seen advances in the maternal age of pregnant women (Mathews & Hamilton, 2009), increased fatigue

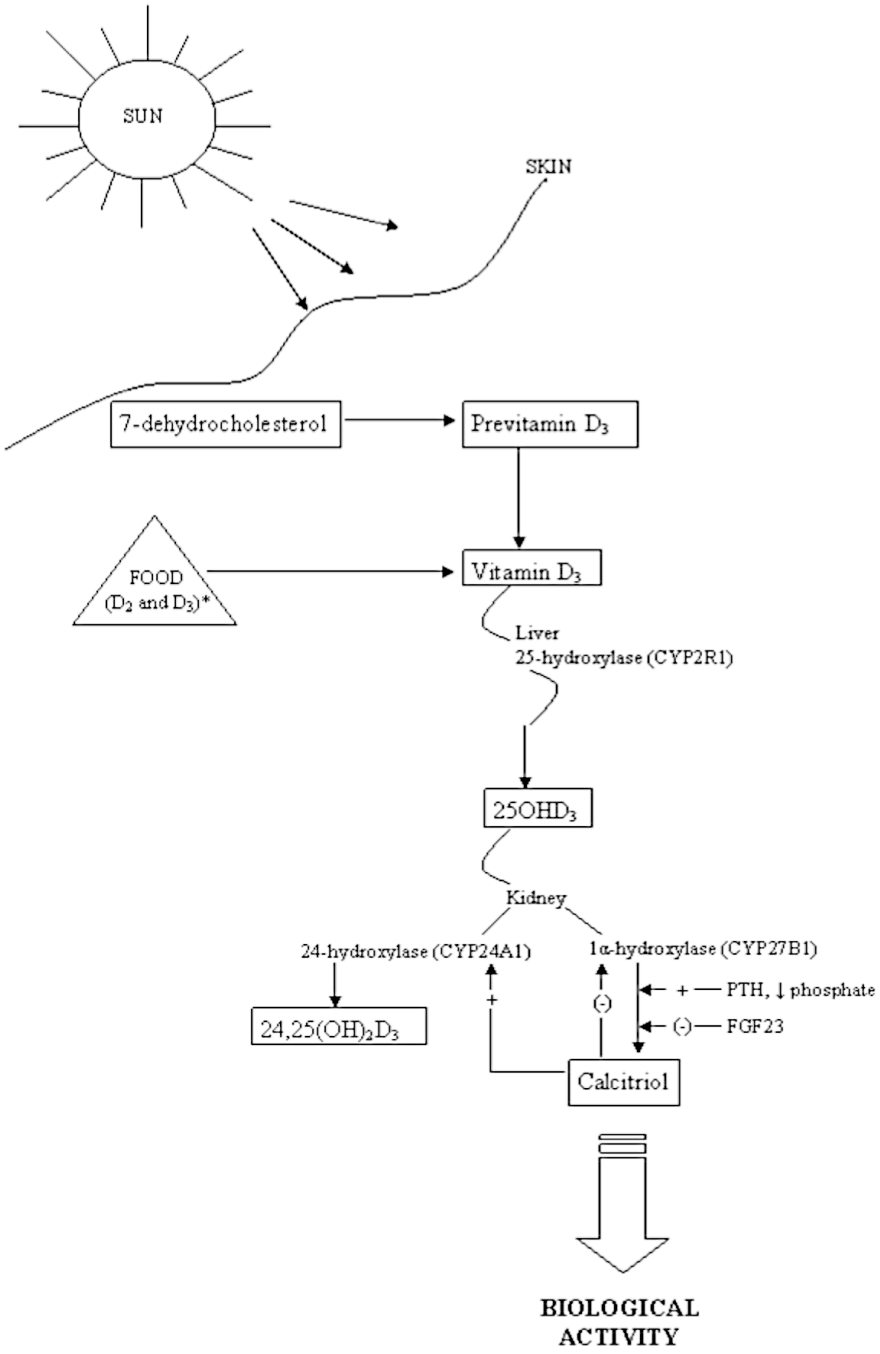
(National Sleep Foundation, 2009), higher rates of obesity (Orelind et al., 2012), more sedentary lifestyles (Gerdhem et al., 2005; Jorde et al., 2008), and increased social isolation (McPherson et al., 2006; Hidaka, 2012). For many women, current lifestyle has led to decreased time spent outside, increased sunscreen use, and increased exposure to pollutants (Agarwal et al., 2002; Hosseinpanah et al., 2010) resulting in a decrease of their bodies' ability to synthesize vitamin D. Current literature suggests that low vitamin D levels may increase the risk for depression in pregnancy and thus may explain some of the rise in the incidence of depression in pregnancy (Brandenbarg et al., 2012; Brannon & Picciano, 2011; Cassidy-Bushrow et al., 2012; Murphy et al., 2010; Robinson et al., 2014). But this relationship is not clearly established (Nielsen et al., 2013).

Vitamin D

Vitamin D is both a nutrient and a pro-hormone (Institute of Medicine (IOM), 2011) synthesized in the skin as a result of exposure to sunlight. Skin cells make 7-dehyrdocholesterol. When these cells are exposed to sunlight 7-dehyrocholesterol is converted to vitamin D_3 (Glossmann, 2010). Along with synthesis in the skin, vitamin D is found in some foods and animal products. A synthetic, fat-soluble form can be taken as a supplement or through fortified foods (e.g., milk). Vitamin D_3 is the form made within the body as a result of exposure to sunlight, and vitamin D_2 is historically thought of as the supplemental form; however supplementation with D_2 is phasing out and most foods and supplements today contain D_3. Despite the availability of supplements and fortified foods, adequate amounts of vitamin D cannot be achieved through supplementation alone. Endogenous vitamin D through exposure to sunlight is critical (IOM, 2011).

Vitamin D_3 and vitamin D_2 are metabolized in the same way and are both useful at equal doses for treating vitamin D deficient disease (e.g. rickets) (IOM, 2011; Jurutka et al., 2001). Both forms of vitamin D are initially biologically inactive and are transformed into their active forms through two hydroxylations. The first takes place in the liver converting both endogenous and exogenous forms of vitamin D to 25-hydroxyvitamin D (25OHD). The second hydroxylation takes place primarily in the kidneys where 1α-hydroxylase catalyzes conversion of 25OHD to its active form 1,25 dihydroxyvitamin D [$1,25(OH)_2D$] also known as calcitrol. Calcitrol production is increased by the up regulation of parathyroid hormone (PTH) and low levels of serum phosphorus, and decreased by fibroblast-like growth factor-23 (FGF23) (Bergwitz and Juppner, 2010; Galitzer et al., 2008; IOM, 2011; Prie & Friedlander, 2010). Calcitrol exerts its biological actions by binding to vitamin D receptors (VDRs) located throughout the body (IOM, 2011; Jones et al., 1998). Figure 1 depicts vitamin D synthesis and uptake (IOM, 2011).

Figure 1. Overview of vitamin D synthesis and uptake



(IOM, 2011)

The interaction between calcitrol and VDRs plays an important role in the up regulation of cathelicidin an antimicrobial peptide (Borella, Nesher, Israeli, & Shoenfeld,

2014). Vitamin D has been found to have a role in T cell regulation and other immunomodulating pathways (van Etten & Mathieu, 2005). However a recent study of young vitamin D deficient women (N=131) noted no increase in mRNA expression of cathelicidin after 6 months of vitamin D supplementation (Das, Tomar, Sreenivas, Gupta, & Goswami, 2014). Much still remains to be discovered about the role of vitamin D in immunity.

Beyond immunity, the primary biological action of vitamin D is maintaining a balance of calcium and phosphorous in the body, thus protecting skeletal health. However, vitamin D may have many other functions within the body and its function has been implicated in both immune system regulation (as noted above) and cell production (Adams & Hewison, 2010; Hayes et al., 2003; IOM, 2011). Vitamin D has been implicated in brain function (Harms et al., 2011; Kesby et al., 2011; Eyles et al., 2009) and vitamin D receptors have been found in neurological tissue (Jones et al., 1998). Vitamin D may also have an important role in pregnancy as VDRs have been found in ovarian, mammary, and placental tissue and vitamin D has been shown to affect fertility in animal models (Jones et al., 1998).

Since the primary source of vitamin D is endogenous synthesis through skin exposure to sunlight, risk factors for vitamin D include anything that inhibits this process. Proper sunscreen use (Matsuoka, Ide, Wortsman, MacLaughlin & Holick, 1987) and dark skin pigmentation (Clemens, Adams, Henderson, & Holick, 1982) can independently reduce synthesis of vitamin D by as much as 99% (Holick & Chen, 2008). Older adults are also at increased risk for vitamin D deficiency because of decreases in levels of 7-dehyrdocholesterol with advancing age (Holick, 2007). Similar to depression, obesity has

been linked to a vitamin D deficiency. Vitamin D is fat-soluble and can be sequestered by large stores of fat (adipose) cells (Wortsman, Matsuoka, Chen, Lu, & Holick, 2000). In addition anticonvulsant and anti-retroviral use (Zhou et al., 2006) has been linked to vitamin D deficiency due to their catabolic action on 25OHD. Some forms of cancer (e.g. lymphoma) (Adams & Hewison, 2006) and hyperparathyroidism can lead to vitamin D deficiency due to accelerated metabolism of 25OHD to calcitrol (Grey et al., 2005). As previously described a modern lifestyle may help explain why vitamin D deficiency remains an important public health issue and is relevant for pregnant women.

There is great debate about the incidence of vitamin D deficiency. The incidence of vitamin D deficiency in the form of childhood rickets dropped significantly in the 1930s and 1940s with the fortification of milk (National Research Council, 2003; Quick & Murphy, 1982). However, as previously mentioned, risk factors for vitamin D deficiency persist. Current estimates of the incidence of low vitamin D in a perinatal population range widely from 5% to 84% (Brannon & Picciano, 2011). This wide range may be attributed to the ongoing debate about what level of vitamin D should be considered adequate. Despite debates on exact levels, the literature indicates that pregnant women have lower levels of vitamin D than non-pregnant women despite taking prenatal vitamins (Brannon & Piccano 2011; Bodnar et al., 2007; Holmes et al., 2009; Marwaha et al., 2011).

Studies also indicate low levels of vitamin D increase the risk for serious complications during pregnancy (Baker, Haeri, Camargo, Espinola, & Stuebe, 2010; Baker, Haeri, Camargo, Stuebe, & Boggess, 2012; Merewood, Mehta, Chen, Bauchner, & Holick, 2009; Ota et al., 2014), and negatively impact the health and development of

children born to vitamin D deficient mothers (Belderbos et al., 2011; Camadoo, Tibbott, & Isaza, 2007; Kalra et al., 2012; McGrath, Burne, Feron, Mackay-Sim, & Eyles, 2010). A better understanding of the association between vitamin D and depression in pregnancy may be the key to reducing risk, developing effective interventions, and improving birth and postpartum outcomes for women and children.

Significance of Depression and Vitamin D Deficiency in Pregnancy

The negative impact of depression and vitamin D deficiency in pregnancy extends to society, individuals, healthcare, and nursing. As a society we see the effects of depression and vitamin D deficiency in pregnancy by the impaired ability of women, their children, and their partners to contribute to society, coupled with the financial weight of disability (Grant, 2011; Grant, Schwalfenberg, Genuis, & Whiting, 2010; Murray et al., 2011; Seto et al., 2005; Stewart, Ricci, Chee, Hahn, & Morganstein, 2003; WHO, 2008). Depression in pregnancy is significant to both healthcare and nursing because it leads to great disease burden and increased healthcare costs (Bansil et al., 2010; Berto, D'Ilario, Ruffo, Di Virgilio, & Rizzo, 2000; Greenberg et al., 2003; Kalra et al., 2012; WHO, 2012). Nurses are poised to take an important role in emerging research about vitamin D deficiency and depression in pregnancy and in raising awareness and improving treatment. A review of the significance of depression in pregnancy is critical to understanding the need for further research in this field.

Significance to Society

Perinatal depression has been brought to the social forefront through public personas like Brittany Spears and horrific tragedies such as the case of Andrea Yates. These cases have spawned national attention and social responses to depression in pregnancy and postpartum such as charities and help lines. Funding for screening and care of women with postpartum depression was included in the Patient Protection and Affordable Care Act as part of the March 2010 health reform legislation.

Despite growing social awareness only about 4 out of 10 women with perinatal depression receive treatment (Witt, 2009). In general women who do not receive treatment for their depression may be more likely to be divorced, have lower incomes, less education, higher substance abuse disorders, and more financial problems (Seto et al., 2005). Not only does perinatal depression have negative influences on women, but also partners of women with depression have a higher incidence of depression, aggression, and other psychological problems (Roberts, Bushnell, Collings, & Purdie, 2006).

Children of depressed mothers are at risk for experiencing negative sequelae to their health and psychological well-being. Longitudinal studies of children born to mothers with depression found that these children display higher incidences of anxiety disorders, substance abuse, disruptive behaviors, and psychological diagnoses (Pesiah et al., 2004; Weissman et al., 2006) as compared to children of mothers without depression. Higher depressive symptoms during the perinatal period have been associated with cognitive disability (Sohr-Preston & Scaramella, 2006), poor language development (Stein, Malmberg, Sylva, Barnes, & Leach, 2008), autisim (Rai et al., 2013), and a

greater incidence of mental illness later in life (Murray et al., 2011). Perinatal depression not only affects the development of healthy members of society, it clearly impairs those who are suffering. Depression generally costs about $83.1 billion dollars annually. Of this $51.5 billion dollars is due to lost productivity of those individuals with depression as compared to those without depression (Stewart, Ricci, Chee, Hahn, & Morganstein, 2003).

Similar to depression, low levels of vitamin D have significant impact on society. The World Health Organization (WHO) notes that vitamin D deficiency may be linked to diseases associated with up to 67% of the annual deaths for women globally (Grant, 2011; WHO, 2008). In children, low vitamin D levels have long been known to cause rickets (IOM, 2011) whereas in adults low vitamin D levels have been linked to an increased risk for fractures (Cummings et al., 2005; IOM, 2011), falls (Flicker, 2003; Sambrook et al., 2004), diabetes (Brock et al., 2011), cancer (Lappe et al., 2007; Wactawski-Wende et al., 2006), and depression (Hoogendijk et al., 2008; Jorde et al., 2008).

Significance to Healthcare

Depression and vitamin D deficiency are very significant issues within our health care delivery system and present many challenges to the care of pregnant women. Depression in pregnancy has been associated with a caesarean delivery, preterm birth (Staub et al., 2012), anemia, diabetes, preeclampsia (Bansil et al., 2010), infant death, low birth weight, and congenital malformations (2004-2006 NHIS; Schneid-Kofman et al., 2007). Perinatal depression represents a unique challenge to the healthcare community

because it often negatively affects innate defenses, causes worsening of other illnesses (Pratt & Brody, 2008), and may prevent patients from taking an active role in their healthcare due to the inherent feelings of helplessness or apathy in a depressed patient.

The American College of Obstetrics and Gynecology (ACOG) strongly encourages obstetrical providers to screen women during and after pregnancy for depression (ACOG, 2010). However on average less than half of women in the perinatal period are screened and even fewer practitioners report using validated screening tools (Delatte et al., 2009; Hatton et al., 2007). As a result many cases of depression in pregnancy go undetected and untreated (Delatte et al., 2009; Hatton et al., 2007). This is despite current evidence suggesting that pregnant women choose treatment as much as 93.4% of the time when treatment is offered to them (Dietz et al., 2007).

Anti-depressants are the most commonly used treatment for depression in the U.S. (Lawrence et al., 2012). Unfortunately, use of anti-depressants in pregnancy may increase the risk for miscarriage (Kjaersgaard et al., 2013; Nakhai-Pour et al., 2010; Nikfar et al., 2013), lower birth weights (Klieger-Grossmann et al., 2011; Ross et al., 2013), preterm birth (Wisner et al., 2009), persistent pulmonary hypertension (PPH) of the newborn (Grigoriadis et al., 2014; t Jong, Einarson, Koren, & Einarson, 2012), and autism (Rai et al., 2013). However, research on comorbidities and anti-depressant use in pregnancy is limited. Further studies are needed to validate these associations. The need for more research to improve understanding of risk factors and underlying mechanisms to facilitate early identification and safe effective therapy for perinatal depression is great.

The presence of depression can significantly complicate and heighten the level of medical care needed for these women, which often results in significant medical costs. Estimates

suggest that prenatal care costs for women with depression may be increased by as much as 44% (Lin et al., 2009) and postpartum costs may increase by almost 20% as compared to women without depression (Petrou, Cooper, Murray, & Davidson, 2002). Limited information is available related to specific healthcare costs for perinatal depression, however it has been estimated that the U.S. spends approximately $26.1 billion in direct healthcare costs for depression; 67% of these costs are accounted for by hospitalization and $5.4 billion are suicide-related (Berto, D'Ilario, Ruffo, Di Virgilio, & Rizzo, 2000; Greenberg et al., 2003). A more recent report by the WHO noted that general depression represents about 4.4% of the global disease burden, on par with the impact of diarrheal illnesses and ischemic heart disease (WHO, 2012; Chisholm, Sanderson, Ayuso-Mateos, & Saxena, 2004).

The impact of vitamin D deficiency in pregnancy on healthcare is evidenced by an increased risk for miscarriage (Ota et al., 2014), anemia (Bener, Al-Hamaq, & Saleh, 2013), pre-eclampsia (Baker et al., 2010; Bener et al., 2013; Haugen et al., 2009; C. J. Robinson, Alanis, Wagner, Hollis, & Johnson, 2010; C. J. Robinson, Wagner, Hollis, Baatz, & Johnson, 2013), the development of gestational diabetes (Baker et al., 2012; Bener et al., 2013; Ramos-Lopez et al., 2008; Soheilykhah, Mojibian, Rashidi, Rahimi-Saghand, & Jafari, 2010; Zhang et al., 2008), and primary cesarean delivery (Merewood et al., 2009). However, studies investigating these associations are limited and inconclusive. For example, some study findings do not show a significant correlation between low vitamin D and gestational diabetes (Farrant et al., 2009) or pre-eclampsia (Powe et al., 2010; Shand, Nassar, Von Dadelszen, Innis, & Green, 2010).

Neonates born to mothers with low vitamin D levels may be at increased risk for respiratory syncytial virus (Belderbos et al., 2011), childhood asthma (Devereux et al., 2007), schizophrenia (McGrath et al., 2010), hypocalcemic seizures (Camadoo et al., 2007), congenital rickets (ACOG, 2011; Bodnar et al., 2007; Dijkstra et al., 2007), and infant heart failure (Maiya et al., 2008). Not only do low levels of maternal vitamin D increase infant morbidities, but vitamin D supplementation in pregnancy, in addition to prenatal vitamins, has been associated with greater infant birth weight, length, and head circumference (Kalra et al., 2012), and higher one and five minute APGAR scores (Hossain et al., 2014).

In light of the negative role that vitamin D deficiency plays in pregnancy and infant development it has been estimated that treatment of vitamin D deficiency could reduce obstetrical healthcare costs by 10% or more (Grant, Schwalfenberg, Genuis, & Whiting, 2010). In 2004 Grant et al. (2005) estimated the economic burden for vitamin D deficiency in the U.S. was approximately $40-56 billion annually (Grant, Garland, & Holick, 2005). More recent literature indicates that economic burden resulting from vitamin D deficiency could be reduced by 6.9% or $14.4 billion dollars by increasing vitamin D levels in Canada (Grant et al., 2010).

Significance to Nursing

Depression in pregnancy holds particular significance for nursing the largest healthcare occupation in the U.S. accounting for 2.6 million jobs nationally (U.S. Labor Department, 2009). National nursing organizations clearly recognize the significance of depression. Major national nursing organizations such as the Association of Women's

Health, Obstetric, and Neonatal Nurses (AWHONN) and the American College of Nurse-Midwives (ACNM) have position statements that support the role of nurses in the prevention, screening, education, and treatment of women with depression or postpartum mood disorders and call for further research to better understand these phenomena (AWHONN, 2008; ACNM, 2003).

In addition, findings from studies of nurses underscore the potential and actual roles of nurses. For example, in a survey of 520 nurses assessing their perception of nursing roles in postpartum depression care, 93.7% of the respondents agreed that nurses should have a role in postpartum depression screening and treatment (Segre et al., 2010). Better understanding of perinatal depression is not only significant to nursing in these specific ways, but it is also represented in nursing's overall goal to prevent illness, optimize health, and secure treatment for those who are ill (ANA, 2003). Societal and healthcare impacts of depression in pregnancy are immense and warrant greater effort towards intervention development on the part of the scientific community.

As previously noted the profession of nursing is large and well situated, although perhaps unequipped, to educate pregnant women about the risks of vitamin D deficiency as well as depression. Recent research investigating the association between depression and vitamin D in pregnancy includes both nurses and nurse-midwives (Cassidy-Bushrow et al., 2012; Murphy et al., 2010). However, one study that surveyed nurses and midwives conducting community health visits in the United Kingdom showed that only 52% were aware of current recommendations for vitamin D supplementation (Locyer, Porcellato, Gee, 2011). With the important role that nurses play in research, education,

and public health more efforts are needed to involve the profession of nursing with the issue of vitamin D deficiency in pregnancy.

Purpose of the Study

Depression in pregnancy and vitamin D deficiency are serious diseases with widespread implications for society and science. Research suggests shared risk factors and inflammatory mechanisms in both depression and vitamin D deficiency. Learning more about the association between depressive symptoms and vitamin D levels in pregnancy may increase our understanding of the mechanisms underlying these diseases. If vitamin D deficiency can be identified as a risk factor for depression in pregnancy then this may be an important step towards early identification and prevention of depression among women in pregnancy and beyond. In order to further the science in this area, this dissertation study was undertaken to examine associations between depressive symptoms and vitamin D levels in a sample of women during the course of their pregnancy. The following research questions and hypothesis guided the study:

Research Questions

1. Is there a difference between early and late pregnancy vitamin D levels?
2. Is there a difference between early and late pregnancy depressive symptoms?
3. Is there a relationship between vitamin D levels and depressive symptoms during pregnancy?

Hypothesis

There is an inverse association between vitamin D and depressive symptoms in this sample of pregnant women. As vitamin D levels decrease, depressive symptoms will increase during pregnancy.

CHAPTER II

LITERATURE REVIEW AND THEORETICAL FRAMEWORK

In an effort to further understand the association between vitamin D and depression a review of relevant literature was conducted. Knowledge derived from the literature was used to construct a theoretical framework for describing hypothesized associations between depression and vitamin D in pregnancy. An understanding of current literature and a theoretical framework were used to develop this dissertation study, which explored associations between vitamin D and depression in pregnancy.

Historical Perspective

The hypothesis and study of a relationship between vitamin D and depression is a more recent phenomenon than the study of the individual illnesses. Depression predates vitamin D in historical literature. Hippocrates describes depression or melancholy humor as a prolonged "fright or despondency" in his *Aphorisms* from approximately 400 BC (Hippocrates, 400 BC). He also wrote about depression as it relates to the pregnant woman describing postpartum depression as "puerperal fever," believing that unexpressed bodily fluids were being shunted to the head and causing mood changes in the woman (Thurtle, 1995). Currently, both postpartum and antenatal depression though widely acknowledged are not defined as unique diagnoses from general major depression in the *DSM-IV-TR* (APA, 2000), but the timing of the depression as occurring after birth or during pregnancy can be specified when the diagnosis is confirmed.

Soranus of Ephesus may have been the first to describe vitamin D deficiency in the form of "distorted limbs" and "weak bones" (rickets) in 1st and 2nd Century A.D. (Soranus & Temkin, 1956). An early perinatologist, he attended the famous medical school in Alexandria and noted that this condition of bone deformities was particularly common in Rome, as compared to Greece, and attributed it to premature weight bearing and poor maternal care of infants (Soranus & Temkin, 1956). However, rickets was not formally described until 1645 by Daniel Whistler in his medical school dissertation. It was more thoroughly detailed by Francis Glission in his Latin treatise "De Rachitide" later in 1650 (Hess, 1929; Ruhrah, 1925). Then in 1919 Sir Edward Mellanby found that giving cod liver oil to dogs raised without exposure to sunlight prevented the development of rickets and noted that this must be due to a "vitamin" the dogs lacked in their diets (Mellanby & Cantag, 1919). In 1922 E.V. McCollum et al. separated vitamin A from vitamin D (McCollum, Simmonds, Becker & Shipley, 1922) and in 1923 Goldblatt and Soames discovered that skin exposed sunlight synthesized "a fat soluble vitamin", vitamin D (1923).

Early theories on a relationship between depression and vitamin D perhaps began with Hippocrates who noted in 400 BC that seasonal changes often lead to the development of disease (Hippocrates, 400 BC). Later, in 200 AD, the Greek physician Aretaeus wrote on a possible link between mood and vitamin D, noting 'lethargics are to be laid in the light and exposed to the rays of the sun (for the disease is gloom)'(Aretaeus & Adams, 1856). The potential immunomodulating and anti-bacterial properties of vitamin D have been noted since the turn of the century as tuberculin patients were often treated in open air sanatoriums with "sunbaths" and "heliotherapy" and marked

improvement was noted as compared to traditional treatments (Cook, 1999; Kibler & Watson, 1935). Most of the research supporting a possible link between vitamin D and depression within pregnancy has been conducted within the last 5-10 years (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Murphy et al., 2010; Nielsen et al., 2013; M. Robinson et al., 2014). A detailed review of current and associated literature follows.

Literature Review

Current literature related to vitamin D and depressed mood were reviewed. Included in the review of the literature were studies examining relationships between the following: vitamin D and seasonal affective disorder (SAD), vitamin D and depression or depressive symptoms in non-pregnant populations, and randomized controlled trials that evaluated vitamin D supplementation in pregnant women. This synthesis of literature provides a helpful context for understanding the current state of the science related to between vitamin D and depression/depressive symptoms in the perinatal period.

Vitamin D and SAD

Several studies have explored the relationship between levels of vitamin D and SAD in a variety of populations. All of the studies reviewed involving SAD were prospective in design and interventional in nature, assessing for improvement in mood and/or vitamin D levels following treatment with light and/or vitamin D supplementation (Dumville et al., 2006; Gloth et al., 1999; Harris& Dawson-Hughes, 1993; Oren et al., 1994; Partonen et al., 1996). Overall, findings from these studies did not support a relationship between vitamin D and SAD. For example, Dumville et al. randomized 1621

elderly women to either take 800 IU of vitamin D daily or receive an educational handout (2006). Level of depressive symptoms was measured at baseline and again 6 months later. There were no significant differences between the two groups (*p=0.262)* (Dumville et al., 2006). However, the researchers did not measure vitamin D levels in their sample prior to or after supplementation, a major limitation in trying to understand if the women in the treatment group were more or less deficient than the women not receiving treatment, or if supplementation successfully raised their vitamin D levels. Current research indicates that in order to significantly raise blood levels of vitamin D participants must take approximately 2,000 international units (IU) of Vitamin D daily for a least one month (Hollis et al., 2011).

Methodological issues limited many of the studies exploring interactions between vitamin D and SAD. In addition non-random designs, small sample sizes, and low levels of supplementation (e.g. ≤ 800 IU of vitamin D) were commonly used (Dumville et al., 2006; Gloth et al., 1999; Harris& Dawson-Hughes, 1993; Oren et al., 1994; Partonen et al., 1996). The only study with findings supporting an association between level of depressive symptoms and vitamin D levels (i.e., a decrease in depressive symptoms was significantly correlated with increased vitamin D levels of those with SAD), tested vitamin D supplementation of 100,000 IU over the course of a month (Gloth et al., 1999). A major limitation of this study was the small sample size (n=15). Given the numerous methodological issues noted above, the association between vitamin D levels and SAD seems probable but remains inconclusive.

Vitamin D and Depression In Non-Pregnant Populations

Studies that specifically examined depression and vitamin D in a non-pregnant population were reviewed (Eskandari et al., 2007; Hoang et al., 2011; Hoogendijk et al., 2008; Jorde et al., 2008; Wilkins et al., 2006). For example, Jorde et al., randomized overweight men and women to two groups, either receiving a placebo or receiving vitamin D supplementation (20,000 IU and 40,000 IU/week). Participants were followed for one year to assess levels of depressive symptoms (2008). Investigators observed increases in vitamin D levels and decreases in depressive symptoms as compared to the placebo group ($p<0.05$; Jorde et al., 2008). Similarly, in a large population based study (n=1,282), researchers found vitamin D levels were 14% lower in participants with a depression diagnosis as compared to those without ($p<0.01$; Hoogendijk et al., 2008). Findings from a study of premenopausal women (N=116) indicated depressed women had lower vitamin D levels of as compared to non-depressed women (25OHD 27 vs. 34 ng/ml; $p=0.002$; Eskandari et al., 2007). Confirming this association was a noted increase in depressive symptom sum scores on the Beck Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) among older adults with vitamin D deficiency (OR: 11.69, $p=0.02$; Wilkins et al., 2006). From these studies, a probable association between depression/levels of depressive symptoms and vitamin D levels has been established.

Vitamin D Supplementation In Pregnancy

Relevant literature assessing associations between vitamin D and depression in pregnancy and postpartum periods were reviewed; including four randomized controlled

trials (RCTs) examining the outcomes of supplementation of vitamin D in pregnancy (Dawodu et al., 2013; Hossain et al., 2014; Mallet et al., 1986; Wagner et al., 2013). None of these RCTs measured depression or depressive symptoms of participants (Dawodu et al., 2013; Hossain et al., 2014; Mallet et al., 1986; Wagner et al., 2013). Two of the studies examined the safety and efficacy of supplementation with vitamin D of up to 4,000 IU a day in pregnancy (Hollis et al., 2011; Dawodu et al., 2013). Both studies noted a significant rise in 25OHD levels in the 4,000 IU a day groups as compared to placebo groups and did not find any adverse effects of supplementation (Hollis et al., 2011; Dawodu et al., 2013). Wagner et al. conducted a trial comparing two different levels of vitamin D supplementation. Women were randomized into two groups (N=200 each group), one group of women received 2,000, IU of vitamin D and the second group received 4,000 IU of vitamin D. Women assigned to the 4,000 IU group tended to have fewer comorbidities, but the association did not reach statistical significance (Wagner et al., 2013). They did find that women with lower vitamin D levels (<32 ng/ml) had an increased incidence of comorbidities in pregnancy as compared to women with normal 25OHD levels (67.3% vs. 55.3%, $p=0.006$; Wagner et al., 2013).

Supplementation of women with vitamin D in RCTs has been found to be safe and effective at raising levels of vitamin D at high doses (e.g., 4,000 IU/daily). The impact of supplementation with vitamin D on pregnancy is difficult to assess in these RCT's because vitamin D deficiency was not an inclusion factor in these studies and thus these studies do not assess treatment of vitamin D deficiency. None of the RCT's examined depression or depressive symptoms.

Associations Between Depression and Vitamin D

To date this author has identified five studies that examined the relationship between depression and vitamin D in the perinatal period (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Murphy et al., 2010; Nielsen et al., 2013; M. Robinson et al., 2014). The studies can be clustered by the period during which the association was assessed: the pregnancy period (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012), the postpartum period (Murphy et al., 2010), and the relationship of the pregnancy period to the development of depression or depressive symptoms postpartum (Nielsen et al., 2013; M. Robinson et al., 2014).

In a cross-sectional study, Cassidy-Bushrow and colleagues (2012) examined total 25OHD levels and levels of depressive symptoms (using the Center for Epidemiologic Studies Depression Scale (CES-D)) in early pregnancy in a sample (N=178) of African American women. Study findings indicated a significant inverse association between 25OHD and high depressive symptoms (CES-D scores ≥ 16) (adjusted OR 0.54, 95% confidence interval [CI] 0.29-0.99, p=0.046). This association remained stable even when controlling for age, education, marital status, and season. They evaluated a linear association and noted that for every 2.72 ng/mL increase in 25OHD, the odds for having a CES-D scores ≥ 16 decreased by 46% (Cassidy- Bushrow et al., 2012). Several limitations were noted in this study and include: very high rates of vitamin D deficiency in their sample (82.6%) and physician prescribed supplementation, external to the study protocol, with vitamin D (50,000 IU/week $25OHD_2$) after time of 25OHD measurement in the study and prior to depression screening (Cassidy-Bushrow et al., 2012).

In a larger nested cross-sectional study (N=4,101), the Amsterdam Born Children and Their Development Cohort Study, investigators observed an increased risk for high levels of depressive symptoms among pregnant with low first trimester 25OHD levels in the summertime (adjusted OR 1.66 (95% CI, 1.06-2.59), however this association was not significant during winter months (OR, 1.24; 95% CI, 0.86-1.78) (Brandenbarg et al., 2012). Both studies conducted during the pregnancy period were limited by cross-sectional designs, gaps in timing between measurements of vitamin D (3-11.4 weeks between measures), and homogenous samples (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012).

Murphy et al. (2010) examined relationships between vitamin D and depression in the postpartum period. In their exploratory descriptive study, they used a longitudinal time series design to examine differences in mean depression scores among postpartum women (N=56) with low vitamin D as compared to postpartum women (N=41) with adequate levels. They noted that women with low vitamin D levels had higher depressive symptom scores (Δ =0.8 ±0.3, t (388) = 2.3, p = .02; N=97) (Murphy et al., 2010). These findings are limited by a convenience sample taking supplementation as part of a larger trial, exclusion of women with pre-existing diabetes, and exclusion of women whose infants were both breast and bottle-fed.

Finally, two of the studies explored the association between vitamin D status in pregnancy and the development of postpartum depression (Nielsen et al., 2013; M. Robinson et al., 2014). Robinson et al (2014) conducted a secondary analysis of serum samples from Caucasian women (N=706) collected between 1989 and 1991 as part of the Western Australian Pregnancy Cohort. They noted an association between low vitamin

D levels (<47nmol/L) at 18 weeks gestation and increased depressive symptoms at three days postpartum (prior to hospital discharge) (*b*=0.93, 95%CI = 0.27, 1.58). Women with vitamin D deficiency were more likely to experience elevated depressive symptoms as compared to women with higher (≥ 70 nmol/mL) vitamin D levels (OR= 2.19, 95%CI =1.26, 3.78) (Robinson et al., 2014). This study was limited by the large amount of time between measurements of vitamin D and screening for depression (~20 weeks), use of an abbreviated Edinburgh Postnatal Depression Scale, and measurement of depressive symptoms within the immediate postpartum period only.

In contrast, Nielsen et al. (2013) in their retrospective case-control study of Danish women, 605 cases of women who filled prescriptions for anti-depressants within the first year of giving birth vs. 875 controls who did not, noted significant associations between both low (<49 nmol/mL) and *high* (>79 nmol/L) levels of vitamin D in pregnancy and postpartum anti-depressant use as compared to women with moderate levels of vitamin D (50-79 nmol/L; p = 0.08). However, the overall association between vitamin D levels in pregnancy and postpartum anti-depressant use was not significant (*p*=0.10) (Nielsen et al., 2013). They noted that abnormally high levels of vitamin D might lead to degradation of the active form of vitamin D, $1,25(OH)_2D$, allowing for dysfunction of vitamin D-dependent brain processes leading to depression (Nielsen et al., 2013).

This study was limited by the case-control design (e.g., does not establish incidence, is unable to examine temporal precedence, and subject to sampling bias), and a lack of a standardized measure for assessing depression. The study designated case or control based on whether a prescription for antidepressants was filled in the first year

postpartum or not, but this fails to take into consideration depressed women who did not receive treatment and women taking anti-depressants for reasons besides depression (Nielsen et al., 2013). Other limitations included the exclusion of women with antidepressant use and mental illness prior to pregnancy, long gaps of time between measurement of vitamin D levels and depression, and a wide variation in gestational age at time of vitamin D measurement (Nielsen et al., 2013).

An overview of studies that explored associations between depression/depressive symptoms and vitamin D in the perinatal period and its associated literature revealed only a small number of studies. Most notably, these studies were limited by their study designs, poor measurement techniques, timing of measures, sample selections and conflicting findings. A more detailed review of specific methodological issues in relevant literature exploring associations between depression/depressive symptoms and vitamin D in the perinatal period is provided below.

Methodological Assessment

A review of methods used in studies that investigated the relationship between vitamin D and depression in pregnancy is critical to a thorough understanding of this link. Key research methods reviewed were sample selection, study design, measures, and analysis techniques. This review was necessary to identify strengths in prior works, challenges to conducting related research and gaps in our understanding of methodological issues. Information gained from this review guided the development of this dissertation study.

Sample Selection

Selecting a sample and choosing a setting are important steps in the research process. A review of samples and settings found in studies that examine the relationship between vitamin D and depression in pregnancy served to inform the development of the dissertation study. Considering the range of studies reviewed, the largest sample noted was a nested cohort of 4,101 pregnant women in the Amsterdam Born Children and Their Development Cohort Study (Brandenbarg et al., 2012). The smallest sample noted was 97 postpartum women in a longitudinal study examining vitamin D and depression in the postpartum period (Murphy et al., 2010). Given the difficulty of enrolling and retaining large prospective samples of pregnant women it is not surprising that the only available longitudinal study had the smallest sample size.

All studies assessing an association between vitamin D and depression in the perinatal period employed non-probability sampling methods, with convenience and purposive sampling being the most common selection strategies. Use of non-probability sampling is also not surprising considering the nature of the population of interest. The only available longitudinal study reported a retention rate across all 7 postpartum visits of 57% (Murphy et al., 2010).

There are a wide variety of reasons why enrollment and retention of pregnant and postpartum women in research is so difficult (Macklin, 2010; Wisner, Appelbaum, Uhl, & Goldkind, 2009; Merkatz, 1998). Partly the explanation lies with pregnant women themselves as there is a great deal of stigma surrounding research participation, and pregnant women often feel protective of themselves and their fetuses, which may make them reluctant to participate in research. Pregnancy and delivery also may lead women to

make transitions (i.e. relocate, leave their jobs, etc.) that can lead to difficulties with study retention. Pregnancies are also vulnerable to natural and elective mortality leading to study attrition and often preventing or deterring researchers from recruiting women in early pregnancy, thus underrepresenting them in samples. Miscarriage is the most common complication of early pregnancy, with 8-20% of all pregnancies less than 20 weeks resulting in miscarriage (Wang et al., 2003).

Barriers to enrollment and retention of pregnant women also lie within the scientific community. The National Institutes of Health [NIH] recognizes pregnant women and their fetuses as an official vulnerable population (NIH, 2001) and, as such, there are special research requirements and ethics that apply to them, which are important for their protection in research but present unique challenges to investigating this population. Study enrollment is a traditionally difficult research task and adding the unique challenges of a pregnant population makes it particularly difficult to enroll and retain large samples of pregnant women. Both study attrition and non-random sampling introduce bias and limit the generalizability of study findings.

Reviewed samples noted women with high depressive symptoms and vitamin D deficiency tended to be younger, less likely to be married, have less education, higher BMI's, lower incomes, less social support, unplanned pregnancy, and were more likely to smoke and use alcohol (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Murphy et al., 2010; Nielsen et al., 2013; M. Robinson et al., 2014). Similar trends were noted in all studies, however not all differences were statistically significant in all studies. The mean age range in reviewed studies was 28.9 (5.5) to 31 (4.8) years old (Mean (SD); Brandenburg et al., 2012; Murphy et al., 2010), however not all studies reported mean or

median age for their overall samples. On average participants with depression (Brandenbarg et al., 2012; Cassidy-Bushrow et al. 2012) and low vitamin D levels tended to be younger by 1.5 years (25.8-29.9 vs. 26.9-31.4) (Murphy et al., 2010). However, two studies noted no significant difference in age between women with depression or vitamin D deficiency and non-depressed women and women with adequate vitamin D levels (Nielsen et al., 2013; Robinson et al., 2014). It is not surprising that studies noted slightly younger ages in women with depression. Very young and older aged women may be at greater risk for depression. The noted finding of the association between younger aged women and a vitamin D deficiency is somewhat curious. Possible explanations for the observed younger age in the study by Murphy et al. (2010) is the high prevalence of vitamin D deficiency in their sample (58%) as the age of women in the deficient group closely approximates the mean age for the overall sample (28.1 vs. 28.9).

The reviewed samples, although overall diverse individually, are largely homogenous with primary representation of White non-Hispanic/Caucasian women (Nielsen et al., 2013; Robinson et al., 2014) or all Black non-Hispanic/African American women (Cassidy-Bushrow et al., 2012). The two studies with heterogeneous samples (Brandenbarg et al., 2012; Murphy et al., 2010) noted ethnic disparities for both vitamin D deficiency and depression. Murphy et al. (2010) reported black non-Hispanic women were significantly more likely to be deficient than white non-Hispanic women (N=97; $\chi^2 = 25.3$, $p < .0001$). Brandenbarg et al. (2012) confirmed this disparity noting a deficiency incidence of 60% (vs. 42% overall) in non-western women and a higher incidence of depression (44% vs. 23%) among this group as compared to western women in their sample. Samples that only represent one ethnicity control for differences in the sample as

a result of skin pigmentation to some degree, however limit the generalizability of their findings. Race/ethnicity may be a probable moderator between diabetes and depression. This underscores the need for diverse samples to fully understand the role of race/ethnicity in the association between depression and vitamin D.

Most studies only include women receiving regular prenatal care. Women who are not getting regular prenatal care are significantly underrepresented in the available research despite their increased risk for depression (Milgrom et al., 2007). These women are also less likely to be screened for depression and vitamin D deficiency and may have undiagnosed illness; thus, even in large samples (e.g., hospital discharge data), they may remain hidden. This is a significant limitation of prior studies.

The majority of the studies reviewed were conducted at one site in large urban medical centers or clinics in developed countries. An urban setting in a developed country (e.g., USA) is an ideal location for examining the link between depression and vitamin D deficiency, since rates of both diseases are higher in these particular areas (Hosseinpanah et al., 2010; Villegas et al., 2011). However, having only one site in an urban location limits the generalizability of study findings and makes application to rural and underdeveloped regions and populations difficult. While all the sampling plans reviewed have some weaknesses, the study by Brandenbarg et al. (2012) has a particularly strong sample. Those authors examined women prospectively as part of the Amsterdam Born Children and Their Development Cohort Study. The major strengths of this sample are it's large sample size (N=4101), ethnic diversity, a broad age range, population based, a variety of care providers, different insurance types, and enrolled women in their first trimester (Brandenbarg et al., 2012). The major weakness of this

sample was women self-selected to be in the study or not and authors noted that, despite efforts to reach non-Dutch speaking women, a bias towards more educated Dutch speaking women may have existed within the sample.

Future research should include the strongest sample plans possible to facilitate the collection of high quality data and further the understanding of the relationship between depression and vitamin D in pregnancy. Important factors to consider would be sample size, ethnic diversity, study location, and carefully planned inclusion/exclusion criteria. These factors and prior research were taken into consideration in the planning of this dissertation study.

Designs

Prior studies investigating the link between depression and vitamin D in pregnancy used a variety of designs. All of the studies reviewed that investigate the link between depression and vitamin D were observational in nature (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Murphy et al., 2010; Nielsen et al., 2013; M. Robinson et al., 2014). Study designs seen in the reviewed research regarding vitamin D and depression in pregnancy consist of prospective longitudinal (Murphy et al., 2010; Robinson et al., 2014), cross-sectional (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012), and case-control (Nielsen et al., 2013). Understanding the strengths and weaknesses of designs previously used was useful in developing the dissertation study.

A prospective longitudinal cohort design allows researchers to investigate changes in characteristics across time in the sample and assess the relationship between risk factors on outcomes of interest. Specific to the topic at hand, this type of design

facilitates a better understanding of the degree to which vitamin D and depression are risk factors for one another and any other variables that may contribute to their relationship. For example, Murphy et al. (2010) noted women with vitamin D deficiency at visit 1 had higher depressive symptoms at time 7 as compared to women with sufficient vitamin D, suggesting chronic low levels of vitamin D may lead to increases in depressive symptoms over time. In further support of this direction of the relationship between depression and vitamin D Robinson et al. (2014) reported a significant association between low vitamin D levels in pregnancy and the development of depression postpartum. However, they did not measure depression in pregnancy or vitamin D levels postpartum, thus limiting their ability to distinguish the direction of the relationship (Robinson et al., 2014).

Brandenbarg et al. and Cassidy-Bushrow et al. used cross-sectional designs to examine vitamin D and depression during pregnancy (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012). Participant response rates for both studies were similar and ranged from 31.1% (203/652) to 35.5% (4,389/12,373) of approached women. A great strength of cross-sectional studies is that they are able to control for a large number of covariates and have limited to no study attrition. Accounting for the influence of covariates gives information about the explanatory power of the primary variables of interest and may reveal important mechanisms underlying the link between depression and vitamin D deficiency in pregnancy. This understanding could be vital in the development of future interventional studies. Both studies demonstrated a significant association between low levels of depression and elevated depressive symptoms in pregnancy, however due to their design they were unable to examine direction of the association.

Case-control designs (Nielsen et al., 2013), like longitudinal designs, can provide some information on the predictive nature of vitamin D deficiency for depression (and vice-versa). Case-control designs provide useful information about character differences between the two groups. A nested case-control design is strengthened by its inclusion in a cohort, which typically results in a greater capacity to control for extraneous variables than in a non-nested design. In a nested case-control analysis of a prospective cohort study (Danish National Birth Cohort), Nielsen et al. (2013) compared a single vitamin D level drawn in pregnancy between women who filled anti-depressants in the first year postpartum (cases) to women who did not (controls). They excluded women with anti-depressant use or a registered mental illness diagnosis in the year prior to delivery and women hospitalized for mental illness at any time (Nielsen et al., 2013). As previously noted they found an increased risk for anti-depressant use among women with very low vitamin D levels (<15nmol/L, OR 1.68) and women with very *high* vitamin D levels (≥100nmol/mL, OR 2.03; p=0.03).

In this case, the main weakness in the case-control design was that the groups were created by selecting women already diagnosed with depression, which prevented the study from being able to address the predictive nature of depression for vitamin D and from establishing causality (Langer & Langer, 1994; Mautner et al., 2009). For example, the study by Nielsen et al. (2013) was limited in its ability to demonstrate the predictive nature of vitamin D (high or low) for depression. Although they attempted to exclude women with depression in pregnancy, vitamin D levels were not measured in the postpartum period. Additionally, women with depression in pregnancy may have had a different pathology than women with new onset depression postpartum (Altemus et al.,

2012; Mora et al., 2009; Stowe et al., 2005) and more likely to have chronic low levels of vitamin D. It is also possible that some controls were depressed despite not filling prescriptions for anti-depressants. Finally, there is evidence that anti-depressant use lessens bone mass and increases the risk for fractures (Richards et al., 2007), suggesting that women taking anti-depressants (case group) may be predisposed to lower not higher levels of vitamin D further threatening the validity of the findings in this case-control study (Nielsen et al., 2013).

The science concerning the relationship between vitamin D and depression in pregnancy is still in the developing stages with efforts focused on clearly establishing or refuting the existence of an association. Thus, carefully planned observational studies are the most appropriate design at this time. While, cross-sectional and case-control designs may continue to play a role, moderate to large longitudinal cohort designs are critical for exploring the natural history of interactions between vitamin D and depression in pregnancy. This type of design when feasible would be ideal for answering the question about a relationship between vitamin D and depression in pregnancy.

Measures

Measuring the relationship between vitamin D and depression in the most reliable and valid way is a critical component of the research process. Reliable measures consistently measure the same thing and valid measures are those that accurately measure the concept they were designed to measure. Therefore, a tool that is both reliable and valid consistently measures the concept it was created to assess. There are a wide variety of ways to measure both vitamin D and depression. The following assessment of most

commonly used measures will include a general review of their reliability and validity. An assessment of the usefulness of the measure for examining the association between vitamin D and depression in pregnancy is provided. Using the most sensitive and specific tool for measuring a particular phenomenon ensures the best possible understanding of concepts, associations, and outcomes. A review of the strengths and weaknesses of currently used tools for assessing the link between depression in pregnancy and vitamin D deficiency follows, and facilitates the identification of the best measures to be used in future research.

Vitamin D. There is currently great debate surrounding screening for vitamin D deficiency in pregnancy. The American College of Obstetrics and Gynecology (ACOG, 2011) and the IOM (2011) do not recommend routine screening and supplementation of vitamin D in pregnancy beyond what is in prenatal vitamins. However, despite recommendations against routine screening, this author has observed that in practice many prenatal providers routinely screen and treat low vitamin D levels in pregnant women, because of emerging evidence and growing public concern. No studies were found examining current provider practices for routine vitamin D screening, but authors in one reviewed study noted routine screening for vitamin D deficiency was standard of care at their institution (Cassidy-Bushrow et al., 2012).

All forms of vitamin D are metabolized in the same way and are useful for treating vitamin D deficiency disease (IOM, 2011; Jones et al., 1998). The main circulating form of vitamin D and the form most useful for indicating adequate levels is 25-hydroxyvitamin D (25OHD). 25OHD is the most valid measure of vitamin D because it includes both what is made in the skin and ingested forms after processing through the

liver and prior to conversion to its active form $1,25(OH)_2$ vitamin D (IOM, 2011). 25OHD is also highly stable, unlike $1,25(OH)_2$, and able to represent vitamin D status over a period of approximately 3 weeks (Wootton, 2005). 25OHD also represents the most cost effective way to assess for vitamin D status (IOM, 2011; Wootton, 2005; Zerwekh, 2008). All of the reviewed studies that assessed vitamin D levels included 25OHD and most studies only reported total 25OHD levels. One study did report only 25OHD3, noting 25OHD2 levels were insignificant in their sample (Nielsen, et al. 2013).

There is still a great deal of variation within methods of screening, diagnostic values for levels of vitamin D, and time of testing. This discordance in measurement of vitamin D makes comparison of study findings difficult and clouds the relationship of vitamin D with depression and other variables. Until a consensus related to the measurement of vitamin D is reached, an assortment of methods will continue to be used. The most common methods, values, and screening times currently in use are reviewed below in light of recent literature and recommendations.

Method of screening. The two main methods for obtaining 25OHD are assay kits and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Assay kits can be divided into five basic categories: radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), competitive protein binding (CPB), high-performance liquid chromatography (HPLC), and chemiluminescence (Zerwekh, 2008). As a result of the wide variety of assays and complexities with measurement the Vitamin D External Quality Assessment Scheme (DEQAS) was formed and monitors inter and intra laboratory validity. Assay kits have improved over time and have received approval by the FDA for use in research, assay kits are affordable and easy to use, but are prone to

greater variation in results and less accurate than LC-MS/MS in differentiating between $25OHD_2$ and $25OHD_3$ (Zerwekh, 2008). Vitamin D is very stable and can be left at room temperature without degradation for up to three days (Lissner, Mason, & Posen, 1981). Both serum and plasma can be used for analysis of 25OHD and should be stored at -20° Celsius (Zerwekh, 2008).

LC-MS/MS is considered the "gold standard" for 25OHD measurement and is highly accurate for measuring both $25OHD_2$ and $25OHD_3$. Disadvantages to LC-MS/MS are the risk of ion suppression, which can lead to the sample not yielding results, and the need for expensive equipment (Zerwekh, 2008). In reviewed studies the general procedure for processing 25OHD was as follows: whole blood was collected and centrifuged, serum was then aspirated off, the samples were frozen in cryotubes at -20° to -80° C then thawed and analyzed for 25OHD. The use of Chemiluminescence (Cassidy-Bushrow et al., 2012), RIA (Murphy et al., 2010), ELISA (Brandenbarg et al., 2012), and LC-MS/MS (Nielsen et al., 2013) were all noted in the literature. Robinson et al. (2014) used an ELISA kit and cross-validated a subset of samples with LC-MS/MS.

Diagnostic values. Variations in the values used for diagnosing vitamin D deficiency are widespread and impede consistency in the identification of women with the disease for both researchers and practitioners. Until an agreement is reached, the debate remains regarding which values are the most sensitive and specific for diagnosing vitamin D. The IOM in late 2010 defined vitamin D deficiency as 25OHD < 30 nmol/L (< 12 ng/ml), insufficiency as 30-50 nmol/L (12-20 ng/ml), adequate as ≥ 50 nmol/L (≥ 20 ng/ml), and unsafe levels as > 125 nmol/L (> 50 ng/ml) (IOM, 2011). These values are set based primarily on the amount of vitamin D needed to maintain skeletal health as the

IOM deemed current data about vitamin D and other disease inconclusive (IOM, 2011). In the summer of 2011 the Endocrine Society defined deficiency as 25OHD < 20 ng/ml (50 nmol/L) and insufficiency as 21–29 ng/ml (52.5–72.5 nmol/L)(Holick et al., 2011). In support of this, several studies present findings that bring into question the credibility of the IOM ranges particularly as they relate to women (Marwaha et al., 2011) and pregnancy (Hollis et al., 2011; Lau et al., 2011).

In the reviewed literature most studies evaluated vitamin D deficiency based approximately on current IOM recommendations (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Nielsen et al., 2013). However, both Murphy et al. (2010) and Robinson et al. (2014) set their levels closer to those recommended by the Endocrine Society (Holick et al., 2011). Murphy et al. (2010) reported insufficiency as less than 32 ng/ml and Robinson et al. (2014) used four cut points to create quartiles with the lowest quartile set at less than 47 nmol/L. The ongoing issue of acceptable parameters for vitamin D levels is a challenge for research in this area. Going forward, clear articulation of the ranges used within studies is important for the comparison of findings between and across studies.

Timing of screening. Timing of vitamin D measurement varied across studies. Current evidence regarding changes in vitamin D across trimesters of pregnancy indicate that $1,25(OH)_2$ rises significantly from the first trimester to term, but levels of 25OHD remain largely unchanged (Brannon & Picciano, 2011; Marwaha et al., 2011). Two studies, however, did note a slight increase of 25OHD in the third trimester (Cross et al., 1995; Sanchez et al., 1996). With the recent advent of supplementation of pregnant women with vitamin D, the prescription of prenatal vitamins in the first trimester, and

growing popularity of vitamin D supplements, measurement of 25OHD in the first trimester may reflect the best baseline value.

Assessment of measures for vitamin D. The complexity involving measurement of vitamin D makes the study of this disease difficult. With multiple methods and values for determining vitamin D deficiency researchers face problems with measure selection, secondary analysis, and comparison of findings. The body of literature is growing in this area but difficulties will still remain as researchers attempt to conduct retrospective analyses and compare previous study findings with current findings. Clearly articulated and careful measurement of vitamin D, using the most evidenced based methods and values, is a critical component in understanding the relationship between vitamin D and depression and should be employed in future studies in this area.

Depression and depressive symptoms. Diagnosis for depression is based on previously described DSM-IV criteria and requires skilled clinical assessment. It is often not feasible or appropriate to screen for depression by direct clinical assessment so researchers often screen using symptoms scales or other indicators (e.g. filling a prescription for an anti-depressant) for depression instead (Nielsen et al., 2013). Depressive symptoms and markers for depression are proxy measures for estimating the prevalence of depression. Symptom scales are typically used in prospective studies and markers are often seen in retrospective or secondary analysis designs. Accurately measuring depression means identifying a valid tool that is both specific and sensitive for a perinatal population. A measure is considered specific if it correctly identifies those without disease as being without disease and it is sensitive when it finds those with disease as having the illness of interest. While both specificity and sensitivity are

important in the case of depressive measures it is most critical that the tools are sensitive, because the result of untreated depression in the perinatal period can result in serious morbidity and mortality as previously described.

The American Congress of Obstetrics and Gynecology (ACOG) currently recommends that all pregnant women be screened for depression at least once every trimester and in the postpartum period (ACOG, 2006). However, in current practice, most women are only screened during the postpartum period, if at all (LaRocco-Cockburn, Melville, Bell, & Katon, 2003). A large number of depressive symptoms scales exist and have been shown to be appropriate for use in pregnant and postpartum populations. The Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977) and the Edinburgh Postnatal Depression Scale (EPDS) (Cox, Holden, & Sagovsky, 1987) were the most commonly used tools for depressive symptoms in the reviewed studies. Another commonly used measure for depressive symptoms is the Beck Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). The BDI is not well represented in the studies reviewed. Due to its high prevalence of use generally, it will be included in the review of measures. Of note the Postpartum Depression Screening Scale (PDSS) and the Patient Health Questionnaire (PHQ-9) were not included in the review due to their relative absence from studies concerning depression and vitamin D in pregnancy. An assessment of the strengths and weaknesses of the BDI, CES-D, EPDS, and depression markers is detailed below.

Beck Depression Inventory. The Beck Depression Inventory (BDI) was designed and published in 1961 by Beck, Ward, Mendelson, Mock, and Erbaugh, revised in 1971, and copyrighted in 1978 (Beck, Steer, & Garbin, 1988). The BDI was designed to

identify depth and intensity of depression in a depressed population (Beck et al., 1988). According to Beck there is no formal theoretical foundation for the scale, but he did note that it reflects many aspects of depression theory present during the time of development (Beck et al., 1988; Shafer, 2006). Beck and colleagues developed the items for the scale based on behaviors and emotions that he noticed were unique to patients with depression (Shafer, 2006). The scale is commonly used in the general population to screen for depression or depressive symptoms (Beck et al., 1988; Kendall, Hollon, Beck, Hammen, & Ingram, 1987; Shafer, 2006). The BDI was originally designed to be administered by researchers, but is now often self-administered (Beck et al., 1988; Kendall et al., 1987; Shafer, 2006).

There are two major forms for the BDI: a long form and a short form (Beck et al., 1988). The short form consists of 13 items. It was noted to have a strong correlation with the long form of the BDI (α =0.96) in one large study by Beck, Rial, & Rickels (1974). The long form of the scale has three versions. The original 1961 version was revised for the DSM-III diagnostic criteria in 1971 (BDI-IA) and copyrighted in 1978 (Beck et al., 1988). This same BDI was again revised to reflect DSM-IV criteria in 1996 as the BDI-II (Beck, Steer, Ball, & Ranieri, 1996). The BDI-II is comprised of 21 individual items that examine both psychological and physical depressive symptoms (Beck et al., 1996). The BDI-II also asks about depressive symptoms over the last two weeks instead of the past week (Beck et al., 1996) as in previous versions. Each of the items represents a particular aspect of depressive symptomatology and has four potential options ranging from zero to three with increasing numbers corresponding to increasing depressive symptomatology (Beck et al., 1988; Kendall et al., 1987; Shafer, 2006). To calculate a total score,

responses for each of the items are tallied together, total scores range from 0 to 63 (Beck et al., 1988; Kendall et al., 1987; Shafer, 2006).

A wide variety of optimal cut-off values for the BDI have been reported. although not diagnostic, in an average population sample, a cut-off score of 21 for the BDI tends to indicate depression. For a sample with diagnosed depression a BDI score of greater than 17 indicates current intensity of moderate to severe depression (Beck et al., 1988; Kendall et al., 1987; Shafer, 2006). Typically the cut-off of 21 is used in a perinatal population as well; however, it has been suggested that lower cut-off scores may be more accurate for this population (Chaudron et al., 2010). The BDI can classify the intensity of depression into subcategories, but it does not have any formal subscales (Beck et al., 1988; Kendall et al., 1987; Shafer, 2006).

The BDI is both a valid and reliable scale with over 1,000 studies representing its use (Beck et al., 1988; Kendall et al., 1987; Shafer, 2006). Beck et al. (1988) analyzed 25 studies looking at internal consistency for the BDI. They found that for groups with mental illness the mean coefficient alpha was 0.86, and for samples without psychiatric disease the mean alpha was 0.81 (Beck et al., 1988). The BDI was found to be moderately correlated with the CES-D in a sample of adolescent mothers ($r = 0.58$) (Wilcox, Field, Prodromidis, & Scafidi, 1998), although it is surprising that a greater correlation was not found. The BDI and the BDI-II have been shown to be correlated with the EPDS (0.68-0.82) in postpartum populations (Teissedre & Chabrol, 2004; Lee, Yip, Chiu, Leung, & Chung, 2001) and is noted to have moderate to high (0.58-0.79) concurrent validity with most depression scales (Richter, Werner, Heerlein, Kraus, & Sauer, 1998).

Consistent with higher rates of depression, women tend to have higher scores on the BDI than men (Beck et al., 1988). For the population of pregnant and postpartum women, both the BDI and the BDI-II have been shown to be moderately sensitive (BDI 82%) (BDI-II 57-74%) and highly specific (BDI 89%) (BDI-II 83-97%) (Beck & Gable, 2001; Chaudron et al., 2010; Lee et al., 2001; Su et al., 2007). In general the literature indicates that the BDI and BDI-II tend to perform well in a perinatal population, but may not be as sensitive or as acceptable as other scales in this population (specifically, the EPDS) (Gjerdingen & Yawn, 2007; Hewitt 2009; Lee et al., 2001; Su et al., 2007; Wilcox et al., 1998).

Harrington and Greene-Harrington (2007) noted that when the BDI is used with other depressive screens it allows for a more dynamic assessment of depression because the BDI indicates both presence and severity of depression. Strengths of the BDI and BDI-II are its ability to measure severity of depression and its broad use in a variety of populations. Additional strengths of the BDI-II include its close approximation to DSM-IV criteria. Weaknesses of the BDI include a lower sensitivity as compared to other measures in a perinatal population and items that include some symptoms that may be a normal part of the perinatal period.

Center for Epidemiologic Studies-Depression Scale. The Center for Epidemiologic Studies Depression Scale (CES-D) was developed and refined by Lenore Sawyer Radloff (1977) at the Center for Epidemiologic Studies within the National Institutes for Mental Health (Eaton, Muntaner, Smith, Tien & Ybarra, 2004). Radloff (1977) developed the CES-D to screen for depression among the general public (Shaffer, 2006). The theoretical underpinnings and components of the scale were derived from

popular depression screens and theories existing at the time of its development (Eaton et al., 2004; Radloff, 1977; Shaffer, 2006).

Some of the most prominent scales Radloff employed to develop the CES-D were the Beck Depression Inventory (BDI), the Raskin Depression Rating Scale, the Minnesota Multiphasic Personality Inventory (MMPI), and the Zung Self-Rating Depression Scale (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961; Radloff, 1977; Eaton et al., 2004; Raskin, Schulterbrandt, Reatig, & McKeon, 1969). The CES-D was developed originally to be administered by interviewers, but has since evolved to be self-administered in a variety of formats (i.e., paper-and-pencil, phone, electronic, etc.) (Eaton et al., 2004). The CES-D is now one of the most widely used depression screens in the U.S. and has been tested among many different settings, ages, races, and languages (Eaton et al., 2004; Shaffer, 2006).

The 20-item CES-D primarily measures emotional and physical attributes of depression (Eaton et al., 2004; Radloff, 1977; Shaffer, 2006). It evaluates for depressed mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance (Eaton et al., 2004; Radloff, 1977; Shaffer, 2006). For each of the 20 items there is an ordinal response scale ranging from zero ("rarely or none of the time") to three ("most or all of the time"), representing the number of times in the past week the person taking the scale has felt or experienced what is described in the item (Radloff, 1977).

The items are scored by tallying the total number on each of the items, except items 4, 8, 12, and 16 which are reverse scored because they are positive affect items; the

range of possible total scores is 0 to 60 (Radloff, 1977). One drawback to the combination of both positive and negative items is that it can create falsely elevated CES-D scores as a result of misunderstanding or item confusion on the part of the respondent or the researcher may miscalculate the score (Shafer, 2006). Although this is a potential problem for any scale designed in this way, the CES-D has been noted to be particularly problematic, possibly due to the positively worded items being dispersed throughout the scale as opposed to grouped together.

Total CES-D scores greater than 16 have been positively correlated with diagnosed depression (Radloff, 1977) although the CES-D is not diagnostic for depression. It was primarily designed to measure depressive symptomatology and depressed mood (Eaton et al., 2004; Radloff, 1977). Radloff (1977) originally reported that scores of 16 or higher were useful for distinguishing a psychiatric population, many with clinical depression, from the general population. Since her original work there has been much discussion on the ideal cut off score, with some suggesting that a cut off of 16 is too low and leads to high false positive rates (Zich, Attkisson, & Greenfield, 1990). However, the value of 16 or higher remains the established standard for use in most populations (Thomas, Jones, Scarinci, Mehan, & Brantley, 2001).

Reliability of the CES-D is evident in many populations with a general test-retest reliability of about 0.4 to 0.7 and Cronbach's alpha coefficients of 0.85 to 0.90 (Radloff, 1997; Eaton et al., 2004). Christian, Franco, Iams, Sheridan, and Glaser (2009) noted the CES-D is an appropriate measure in a sample of low-income pregnant women. The CES-D has been shown to be moderately correlated with the EPDS (0.63 to 0.77) (Anderson,

2010; Logsdon & Myers, 2010; Logsdon, Usui, & Nering, 2009) and the BDI as noted above.

The CES-D was used in two of the reviewed studies regarding the link between vitamin D and depression in pregnancy (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012). It seemed to be a valid measure of depressive symptomatology and a useful tool for better understanding the relationship of interest. Both studies reported using a cut-off score of 16 to distinguish between women with high depressive symptoms and those with low depressive symptoms and reported a high reliability coefficient ($\alpha = 0.90$) (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012). Specificity and sensitivity have been reported as high (0.92-0.97) and low (0.43-0.60) in a perinatal population (Campbell & Cohn, 1991; Gaynes et al., 2005). And in a study of adolescent mothers (Wilcox et al., 1998) the CES-D was found to be more acceptable by participants over the BDI. The CES-D is a reliable and valid scale that has been widely used in many populations. Although it is acceptable for use in a perinatal population it may not be as sensitive as other screens in identifying pregnant or postpartum women with depression.

The Edinburgh Postnatal Depression Scale. The Edinburgh Postnatal Depression Scale (EPDS) was developed by Cox, Holden, and Sagovsky and published in 1987. They designed the scale because they observed there was no valid tool to screen for postpartum depression in the general community (Cox et al., 1987). They built the items in the EPDS scale through close examination of the Depression and Anxiety Scale (Snaith et al., 1978), the Hospital and Anxiety Scale (Zigmond & Snaith, 1983), and the Anxiety and Depression Scale (Bedford & Foulds, 1978). Cox et al. did not note a specific theoretical basis for their scale development beyond their review of scales and

their own research in which they discovered a high prevalence of untreated depression in postpartum women (Cox et al., 1987).

Initially the scale had 21 items derived from the reviewed existing scales and from the researchers' own ideas surrounding traits of postpartum depression (Cox et al., 1987). They piloted items and after an extensive factor analysis, identified 10 items to be most specific and valid for measuring postpartum depression (Cox et al., 1987). EPDS items do not include physical symptoms that may be considered physiologically normal during the perinatal period, such as those in the CES-D (e.g., "my sleep was restless"). Factor analysis of the scale reveals the most parsimonious model is the two factor model with depression and anxiety as the two factors (Phillips, Charles, Sharpe, & Matthey, 2009). Interestingly, depression loads on seven of the 10 items and anxiety loads on the other three items creating a small anxiety subscale of the EPDS, the EPDS-3A (Matthey, 2008). Evidence exists to support its use as a measure for anxiety in both pregnant and postpartum populations (Matthey, Fisher, & Rowe, 2013).

The EPDS is usually self-administered or given orally by a general practitioner and takes approximately 5 minutes to complete (Cox et al., 1987). For each of the 10 items there are four possible responses on an ordinal response scale ranging from zero to three and correlate with the intensity of depressive symptomatology (Cox et al., 1987). The total score is calculated by tallying each of the items, with items 3, 5, 6, 7, 8, 9, and 10 being reverse scored (Cox et al., 1987). The total possible score for the EPDS ranges from 0 to 30 with cut-off scores listed in the literature anywhere from 9 to 15 and/or positive suicidal ideation correlating with depression (Cox et al., 1987; Murray & Carothers, 1990; Sit & Wisner, 2009). There is great debate surrounding the appropriate

cut-off values to use. A cut off value of 10.5 captures approximately 90% of women with depression, but with a high false positive rate (Cox et al., 1987; Murray & Carothers, 1990).

Most research on the EPDS notes a cut-off of 13, which yields a broad range of reported sensitivity (65-100%) and specificity (77%-92%) (Cox et al., 1987; Gaynes et al., 2005; Su et al., 2007; Lee et al., 2001). In a large meta-analysis examining several different measures for perinatal depression, the EPDS was found to have higher sensitivity for detecting major depression than other scales (Hewitt et al., 2009). When used, the EPDS is generally well accepted by practitioners and participants (Hewitt et al., 2009). The EPDS was originally developed to be used to screen for depression in the postpartum period (up to eight weeks postpartum), but has also been tested and validated among all periods of pregnancy and in non-pregnant populations (Cox, Chapman, Murray, and Jones, 1996; Lee et al., 2001; Murray & Carothers, 1990; Su et al., 2007; Yonkers, Smith, Gotman, & Belanger, 2009). The EPDS has demonstrated good convergent validity and has been shown to be moderately correlated with both the BDI and CES-D.

The EPDS has several advantages. It has higher sensitivity than other scales in identifying major depression in a pregnant population (Gaynes et al., 2005). It is brief and designed specifically for the perinatal period (Cox et al., 1987), and highly acceptable to women when surveyed and is widely used to screen for perinatal depression (Chaudron et al., 2010; Gjerdingen & Yawn, 2007; Hewitt et al., 2009). Noted disadvantages of the EPDS are that it measures depressive symptoms as a state. This can lead to variation in the scores over time. Without repeat testing it is difficult to know if

the scores are fully representative of the emotional state of the participant over time (Cox et al., 1987; Murray & Carothers, 1990). The EPDS more accurately identifies major depression than both major and minor depression as compared to other commonly used depressive measures (Hewitt et al., 2009).

The EPDS was used in reviewed literature examining vitamin D and depression in the perinatal period (Murphy et al., 2010; Robinson et al., 2014). Murphy et al. (2010) used a cut-off score of greater than 9 to include all women with possible depression, both major and minor. They did not report a reliability coefficient for their study. Robinson et al. (2014) used a modified six questions version of the EPDS to assess for "anxiety, sadness, mood fluctuation, tearfulness, appetite changes, and sleep disturbances not related to caring for the baby." They did not specifically indicate, which items they included. They summed the six items on the same four-point scale as the original EPDS to create a "blues" score and used a cut-off score of 6 or higher to indicate elevated depression. They also did not report a reliability coefficient for their study (Robinson et al., 2014).

Markers for depression. Another method to assess for existing depression is to use a variety of markers for depression. For example, markers for depression in the perinatal period may include abstraction of a diagnosis from medical records (Schneid-Kofman et al., 2007), *ICD-9* codes for depression (Bansil et al., 2010; Kozhimannil et al., 2009), or filling a prescription for an anti-depressant medication (Nielsen et al., 2013). Depression markers such as these may be less reliable and less valid than direct measurement of depression. They may be vulnerable to variation in method, criteria, diagnostician or coder, and interpretation (e.g., anti-depressant medications can be taken

for reasons other than depression). These vulnerabilities can be a threat to the internal validity of the study. However, where it is not possible to use direct measures (e.g., secondary analysis) efforts can be made to minimize these threats. Some ways to minimize threats to internal validity when using markers are to use more than one marker for depression, confirm *ICD-9* codes with medical records, and check for validity by examining diagnostic and coding processes.

When using markers for depression it should also be taken into consideration that depression is classically under diagnosed and therefore actual prevalence in the sampled population may be much higher than indicated by markers (Rasmussen-Torvik, & Harlow, 2010). The study by Nielsen et al. (2013) used filling a prescription in the first year after giving birth as inclusion for their case group. Although antidepressant use as a marker for depression may be specific, most women taking antidepressants are depressed. It's sensitivity as a marker has been reported at approximately 50% meaning many depressed women are not on antidepressants (Thielen et al., 2009).

Assessment of depression and depressive symptom measures. Accurately measuring depression is a critical part of developing an understanding of the link between depression and vitamin D. Use of a depressive symptom scale to screen for depression is superior to using markers for depression. Depressive symptom scales are typically higher level data, provide direct assessment of symptoms in a controlled and standardized manner, and provide dynamic information about depression beyond just its presence or absence.

The BDI, CES-D, and EPDS are all widely used and valid in a perinatal population. Of these scales, the EPDS appears to be the most widely used to measure perinatal depression and has the highest sensitivity of the three in a perinatal population. The EPDS also has the additional value of a small anxiety subscale. Most sources agree that more research with large diverse samples is needed before a gold standard for depressive symptom measurement in pregnancy can be established (Gjerdingen & Yawn, 2007).

Analysis

Statistical analysis is a test of the study hypothesis (Trochim, 2001) through an examination of collected data. Analysis methods are based on sample, design, measures, and data collected. The reviewed studies used a variety of univariate, bivariate and multivariate statistical analyses including the following: descriptive statistics, chi-square, student's *t*-test, correlational methods (e.g., point-biserial correlations), analysis of variance, and multivariate linear and logistic regressions. Descriptive statistics were used to describe samples and chi-square or *t*-test to examine differences between groups; for example, depressed and non-depressed or deficient and sufficient women (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Murphy et al., 2010; Nielsen et al., 2013; Robinson et al., 2014).

Regressions, especially multivariate hierarchical models, allow researchers to test multiple hypotheses and adjust for the influence of other variables on the association. Both regressions and correlations allow for a two-tailed assessment looking for both positive and negative associations. Examining both positive and negative aspects of associations between vitamin D, depressive symptoms, and significant covariates can be

useful as we develop a better understanding of the relationship between vitamin D and depression. Primary issues to consider when using regressions are the level of data, number of cases (sample size), inter-correlations between variables, and multicollinearity. Level of statistical significance used is also an important component in evaluating analysis and findings. Three of the reviewed studies used logistic regression dichotomizing women as depressed vs. non-depressed or having insufficient vs. sufficient vitamin D levels (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Nielsen et al., 2013). Nielsen et al. (2013) added a fractional polynomial of the degree 3 citing the need for greater flexibility in light of debate around appropriate vitamin D levels.

A generalized linear mixed models (GLMM) approach can be applied to a longitudinal design and allows for dependent repeated measures (within subjects) analysis. The GLMM also allows for the "control" of covariates. The GLMM gives individual participant level data (intercept and slope), which can be used to predict participant level patterns. This may be a useful tool for dealing with missing or incomplete data. A GLMM is considered mixed because it includes both "random" and "fixed" factors. The GLMM addresses questions about the longitudinal relationship between the vitamin D and depression in pregnancy. Two studies reported using general linear models (Murphy et al., 2010; Robinson et al., 2014) to analyze their data. All reviewed studies use a statistical significance level less than 0.05. This level of significance is appropriate for research attempting to establish a relationship between GDM and PD.

Many of the reviewed studies analyzed their data using logistic or linear regression analyses. It was noted in review that vitamin D levels and depression scores

were not normally distributed and required transformation or grouping. Several studies opted to group their findings into depressed or not depressed and/or adequate vs. inadequate vitamin D levels. Many of the studies attempted to control for known covariates. Many of the studies failed to give a complete report of statistical findings including: a clear indication of the level of measurement used for analysis, inter-correlation values, report of R-squared change, and an evaluation of residuals.

Synthesis of Methodological Knowledge

A review of research methods currently used to examine the association between vitamin D and depression in pregnancy was conducted. Several issues emerged as important to consider in planning future studies. Despite potential challenges, efforts to enroll and retain a moderate to large ethnically diverse groups of women across the perinatal period is important. Ideally, samples selected should include women who are not regularly receiving prenatal care in order to obtain a true representation of the pregnant population. Lastly, when sampling and enrolling within this vulnerable population, protections should be in place to prevent violations of research ethics.

An assessment of designs demonstrated that a prospective longitudinal cohort design may be highly useful for answering questions about the link between vitamin D and depression in pregnancy, because it can assess concurrent risk and provide a natural history of the illnesses. Regarding designs generally, knowledge regarding the association between vitamin D and depression in pregnancy is in the developmental stages where observational designs tend to be more appropriate than interventional ones. As this

understanding develops, interventional studies will be critical in the translation of knowledge to treatment.

A critical evaluation of currently used measures for vitamin D and depression in pregnancy revealed that direct measurement of the illness (e.g., depression scales) is superior to indirect quantifying by using markers for illness (e.g., filling an antidepressant prescription). For measuring vitamin D either IOM or Endocrinology recommendations can be used, although emerging evidence makes a strong case for the second. For method of measurement first trimester measurement of 25OHD using mass spectrometry appears that the most accurate. A review of direct measures for depression in pregnancy suggests that measuring depressive symptoms using a validated screen is more feasible than clinical evaluation and more sensitive than using proxy markers for depression. There is no consensus on the ideal measure for depression in pregnancy but use of the EPDS with screening in each trimester holds high promise for examining the relationship between depression and vitamin D in pregnancy. Statistical analysis should allow for the detection of both negative and positive associations; a thorough report of statistical findings should be included; and both non-statistically significant observed trends as well as statistical significance of study findings should be considered.

Theoretical Framework

Methods are important building blocks in the development of future research. Theories and concepts can provide a framework upon which to build our understanding of the phenomenon of interest. None of the studies reviewed for this project included a

clear theoretical model beyond descriptions of biological mechanisms (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Murphy et al., 2010; Nielsen et al., 2013; Robinson et al., 2014). However, a broader review found that concepts for the theory of maternal role attainment have been used to guide the examination of depressed mood and other pathology (e.g., diabetes) in the perinatal period (Chazotte et al., 1995).

Additionally, many studies reviewed noted that life stress and anxiety might have a role in the association between depression and vitamin D (Berk et al., 2007; Berk et al., 2013; Murphy et al., 2010). The concept of life stress on disease pathology and coping fits well with the transactional model of stress and coping, which emphasizes the importance of appraisal or perception of the stressor(s) (Lazarus & Folkman, 1984). Stress and coping in non-pregnant women with depression have been examined through the lens of the transactional model and the concepts of the model have been applied in several diseases processes and study designs (Rao, 2009). An overview of these theories and their usefulness is detailed below.

Maternal Role Attainment

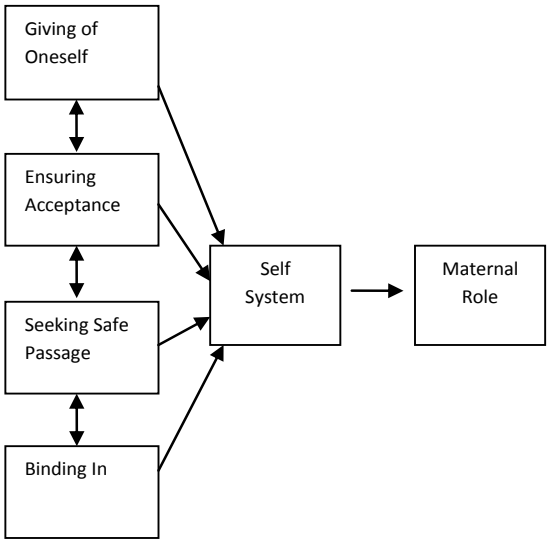
Maternal role attainment theory concepts have been applied in research examining the link between gestational diabetes mellitus and postpartum depression (Chazotte et al., 1995). Maternal role attainment is the theory of maternal identity described by Rubin and Mercer (Rubin 1967; Mercer, 1981). The original model by Rubin is the adaptation of the feminine identity to describe a maternal identity by moving through four maternal tasks within the self-system (ideal self, body image, self image; Rubin, 1967; 1984) (see Figure 2). Rubin based her theory on Theodore Sarbin's and George Mead's theories

about role attainment (Sarbin, 1954; Mead, 1934). Rubin's own ideas developed from her initial dissertation work to the publishing of models of the theory (Rubin, 1967). Continued work yielded a more parsimonious version of the theory (1976) that was eventually documented in detail in *Maternal Identity and the Maternal Experience* (1984). Rubin's (1967) model was later adapted by Mercer (1981) to look at the role of maternal role attainment in the first year of motherhood and the processes that women move through with their infant during that time.

Maternal role attainment as described by Rubin is distinctive because of its multi-relational linkages, specific tasks for pregnancy, and focus on the maternal identity. The concept of maternal identity is the adaptation of self as a mother and all the desired characteristics, emotions, and behaviors that accompany motherhood (Rubin, 1984). This process of incorporating a view of self as mother is described as three images of self and four maternal tasks. Replication is part of the change in self-perception and occurs when the woman observes behaviors that correlate with her belief of motherhood and copies them within herself (e.g., wearing maternity clothes prior to needing them). This "taking-on" or copying of others does not mean that these characteristics or behaviors have been incorporated into the woman's self, but she is feeling them out to see if they will eventually mesh with her (1984). It is not only critical that the woman achieve "a functional equilibrium of self in a dynamic system" to obtain maternal identity, but she must also move through a series of four maternal tasks: seeking safe passage, acceptance by others, binding-in, giving of oneself (Rubin, 1984 p.52). It is these tasks and the focus on maternal identity that make this theory unique.

Also the importance of binding-in or bonding between mother and her newborn has been affirmed in the literature (Klaus & Kennell, 1998). Examples of the research topics that have resulted from Rubin's theory include maternal and infant bonding (Klaus & Kennell, 1998), postpartum depression (Beck, 1995), and teen or high-risk pregnancies (Mercer, 1980). Mercer and others have used the concepts of role, self-identity, and attachment from Rubin's theory to explore aspects of vulnerable pregnant populations and possible interventions to improve their outcomes (Mercer, 1981; Beck, 1995; Ament, 1990). Maternal role attainment theory predicts the outcome of emotional stability or instability based on the completion of the maternal tasks and the integrity of the self-system (Rubin, 1984).

Figure 2. Model of maternal role attainment



Transactional Model of Stress and Coping

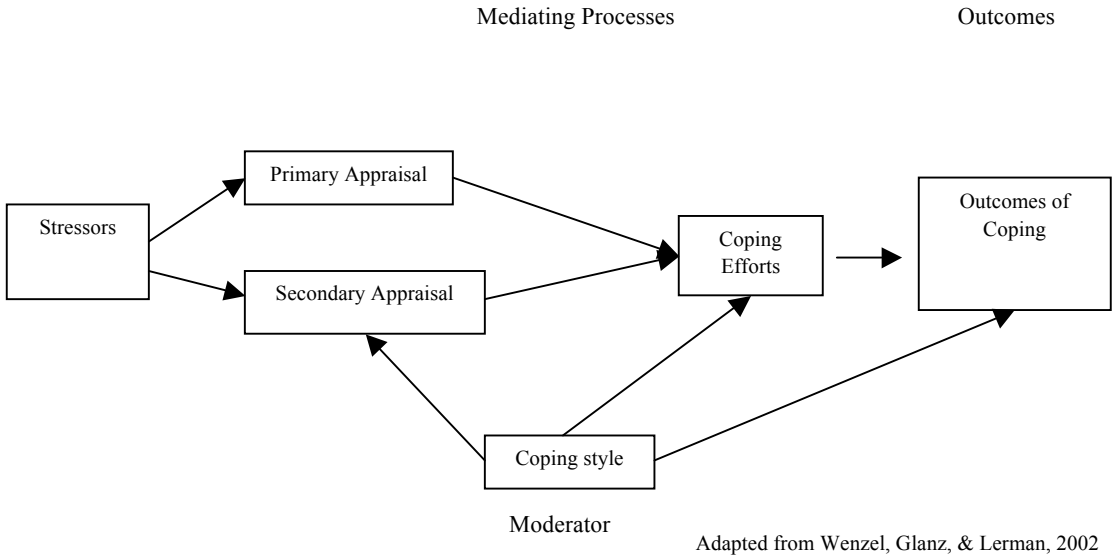
The transactional model of stress and coping was developed by Lazarus and Folkman (1984) to describe stress as both internal and external events that have negative effects (e.g., vitamin D deficiency) on the person experiencing them and are ameliorated or reduced by the person's innate ability and available resources to help them cope (see Figure 3). The transactional model of stress and coping begins with a stressful stimulus, which is then processed by the individual through primary and secondary appraisal (Lazarus & Folkman, 1984). Primary appraisal is one's interpretation of a stressor as a threat, and secondary appraisal involves analyzing one's ability to control the stressor (Lazarus & Folkman, 1984). Glanz et al. (2002) further describes stressors as "demands made by the internal or external environment that upset balance or hormones…" (p. 211). There are both psychosocial stressors (e.g. poor social support) and biological stressors (e.g. vitamin D deficiency), which are "demands" that can be categorized as stressors in pregnant women.

The transactional model is linear, general in nature, and describes a coping process (Glanz et al., 2002). Coping with stress is described by Lazarus (1993) as being viewed as style or process. Style refers to the person's personality or personal characteristics and process refers to steps and behaviors or actions to improve coping. Coping as a style is hierarchical, and derived from psycho-developmental stages and traits based in popular psychology (Lazarus, 1993). Coping as a process is what Lazarus employs and describes as "something that changes overtime in accordance with the situational context in which it occurs" (Lazarus, 1993). He proposes that coping as a process is examined separately from outcomes, so that thoughts and behaviors are not

biased by the outcome of their use. According to Lazarus (1993), coping must always be examined within the context within which it is occurring. Lazarus says it is very important to assess "what the person is thinking and doing" both in relation to themselves and others (intra- and inter-personal dynamics). Continued use or adoption and consolidation of coping methods should also be investigated (Lazarus, 1993). It is also important to analyze both "problem-focused" and "emotional" coping, particularly in the context of relationships.

The concept of emotional coping as a response to stress is most relevant for pregnant women as they cope with the stress of their pregnancy. The feelings and emotions they experience will drive their ability to cope well and successfully reduce their stress. This model has been used to evaluate the stress that women experience in pregnancy and how their ability to cope affects their health and the health of their developing infant (Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2002). The concepts of stress and outcomes as mediated by coping in the transactional model are useful for developing a framework for better understanding the association between vitamin D and depression in pregnancy.

Figure 3. Transactional Model



Adapted from Wenzel, Glanz, & Lerman, 2002

Synthesis of Conceptual and Theoretical Knowledge

The transactional model and the theory of maternal role attainment have commonalities which allow them to both describe, in part, an association between vitamin D deficiency and depression in pregnancy. A review of the shared strengths and relevant weaknesses of the two models for describing the link between vitamin D and depression in pregnancy lays a foundation for designing a framework that incorporates these strengths and addresses significant gaps. The transactional model and the model for maternal role attainment have shared ground in personal perception of the problem (appraisal and self-system), addressing stressful stimuli (coping with stress and moving through maternal tasks), and specific outcomes (outcomes of coping and maternal identity). Also both theories describe a process, have a goal of personal well-being, depict the individual as vulnerable to stress, and include involvement with personal

characteristics (Glanz et al., 2002; Rubin, 1984). They are both influenced by personal attributes. In the transactional model the appraisal process is heavily influenced by personal attributes, and the self-system in the maternal role attainment theory (Glanz et al., 2002; Rubin, 1984). The models stem from behaviorism ideology with both symbolic interactionism and social cognitive theory originating from a behaviorist background and both theories being mediated by behaviors (coping and maternal tasks) (Graham, 2010). Finally, they both propose to predict outcomes from stressors based on behaviors.

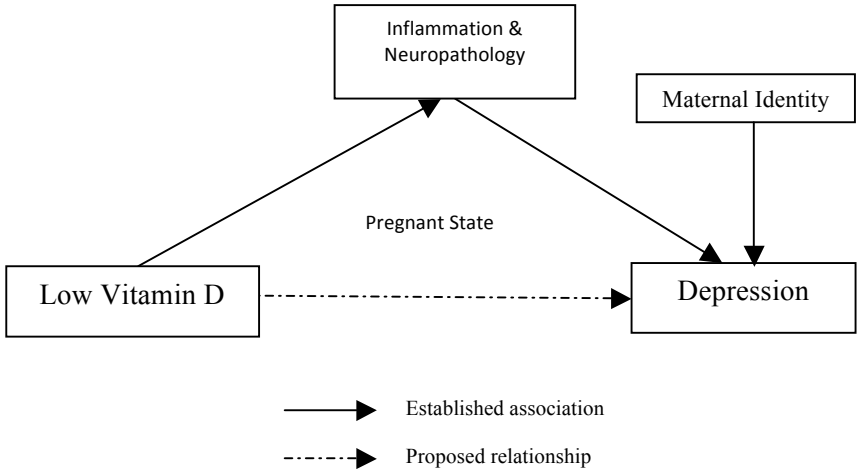
Proposed Model for Dissertation Study

The model designed by Rubin has high relevance for pregnant women, but lacks simple usefulness for designing a study to assess the link between depression and low vitamin D in pregnancy. The transactional model lacks specific relevance for pregnant women, but poses a clear framework for understanding stress, response, and outcome. Since, neither of the models found in the literature pertaining to the relationship between vitamin D deficiency and depression in pregnancy provide a sufficient framework for explaining the association, a new model is hereafter described.

The proposed framework (see Figure 4) is derived from the transactional model where vitamin D deficiency is the primary stressor and depression is the possible outcome. The framework is further modified by the incorporation of the concept of maternal identity as a possible moderator on depression and inflammation as the mediator, a form of biological "coping", on the association between vitamin D and depression in pregnancy. Moderators and mediators are distinct pieces of the theoretical framework. The difference between these two concepts has been stated by Barron and Kenny (1986), "Whereas moderator variables specify when certain effects will hold,

mediators speak to how or why such effects occur" (p.1176). Moderators act independently on the outcome of interest and mediators explain the interaction between the two variables (Barron & Kenny, 1998). It is important to note is that the concepts have been placed within in the framework as mediators or moderators, but since the association between vitamin D and depression is still under investigation these roles are only hypothetical for this proposed framework. The proposed model presents a broad view of the hypothesized relationship between vitamin D deficiency and depression in pregnancy.

Figure 4. Theoretical Framework for depression and vitamin D in pregnancy



Pregnant state. The pregnant state is the condition in which the woman finds herself immediately after conception until completion of delivery. This state is marked by dramatic physiological changes within the maternal environment in order to accept and sustain the developing fetus. These changes have an effect on the entire woman and constitute an increased demand on all body systems. For this reason, the state of pregnancy can be considered a stressful stimulus or stressor since it places unique

demands and strains on a woman's body that have been identified as both psychologically and physiologically stressful (Williams, 2003).

The changes that a woman undergoes during pregnancy are too numerous to be reviewed here. There are specific changes, however, that have particular relevance in understanding the link between depression and vitamin D. In pregnancy many women normally display changes that are often thought to be signs of depression in the non-pregnant female. These include changes in sleep, increased fatigue, changes in appetite, reduced sex drive, cognitive changes, and physical symptoms such as back pain. Pregnant women experience serious sleep disturbances with increases in sleep in the first trimester, decreases in their sleep in the third trimester, more frequent night awakenings, and reductions in deep periods of sleep throughout the pregnancy period (Gabbe et al., 2007). Often these changes in sleep coupled with increasing metabolic demands, hormonal changes, and a rising cardiovascular demand will make women feel fatigued (Gabbe et al., 2007).

Many women also experience changes in appetite during pregnancy with a possible decrease in the first trimester related to feelings of nausea and increases in the second and third trimesters (Gabbe et al., 2007). Many women experience reductions in sex drive secondary to hormonal changes, physical discomforts, or body image concerns. Cognitive changes in pregnancy have been reported by women and noted by researchers with probable explanations relating to sleep disturbances, hormonal changes, and preoccupation with fetal well-being and delivery (Rendell & Henry, 2008). Women also have physiologic alterations in metabolism, and all pregnant women experience some glucose intolerance (Gabbe et al., 2007). Pregnant women tend to have a more dramatic

response to both fasting and feeding that has been linked to human placental lactogen, estrogen, cortisol, progesterone, and human placental growth hormone (Gabbe et al., 2007). It is generally accepted that overall levels of vitamin D in pregnant women tend to be lower than in the general population (Holmes et al., 2009). Most studies also note 25OHD tends to be stable across pregnancy, but that the active form of vitamin D (1,25OHD) is dramatically higher in pregnant women and rises over the course of pregnancy (Brannon & Picciano, 2011; Marwaha et al., 2011). These normal alterations in the maternal physiology as a result of the pregnant state are an important to consider when investigating the association between vitamin D and depression within the context of pregnancy.

The relationship between vitamin D and depression. Vitamin D acts as a neurosteroid with direct affects on brain development and function (Eyles et al., 2009; Eyles, Smith, Kinobe, Hewison, & McGrath, 2005; Harms, Burne, Eyles, & McGrath, 2011; Kesby, Eyles, Burne, & McGrath, 2011). Both vitamin D receptors and 1α-hydroxylase have been found in the brain suggesting low levels of vitamin D may be linked to neuropathology (Eyles et al., 2005). There is evidence in animal models that developmental vitamin D deficiency can lead to brain abnormalities that closely mimic those observed in individuals with schizophrenia (e.g. enlarged ventricles) (Eyles et al., 2009) demonstrating that vitamin D may be critical for normal cognitive development and function.

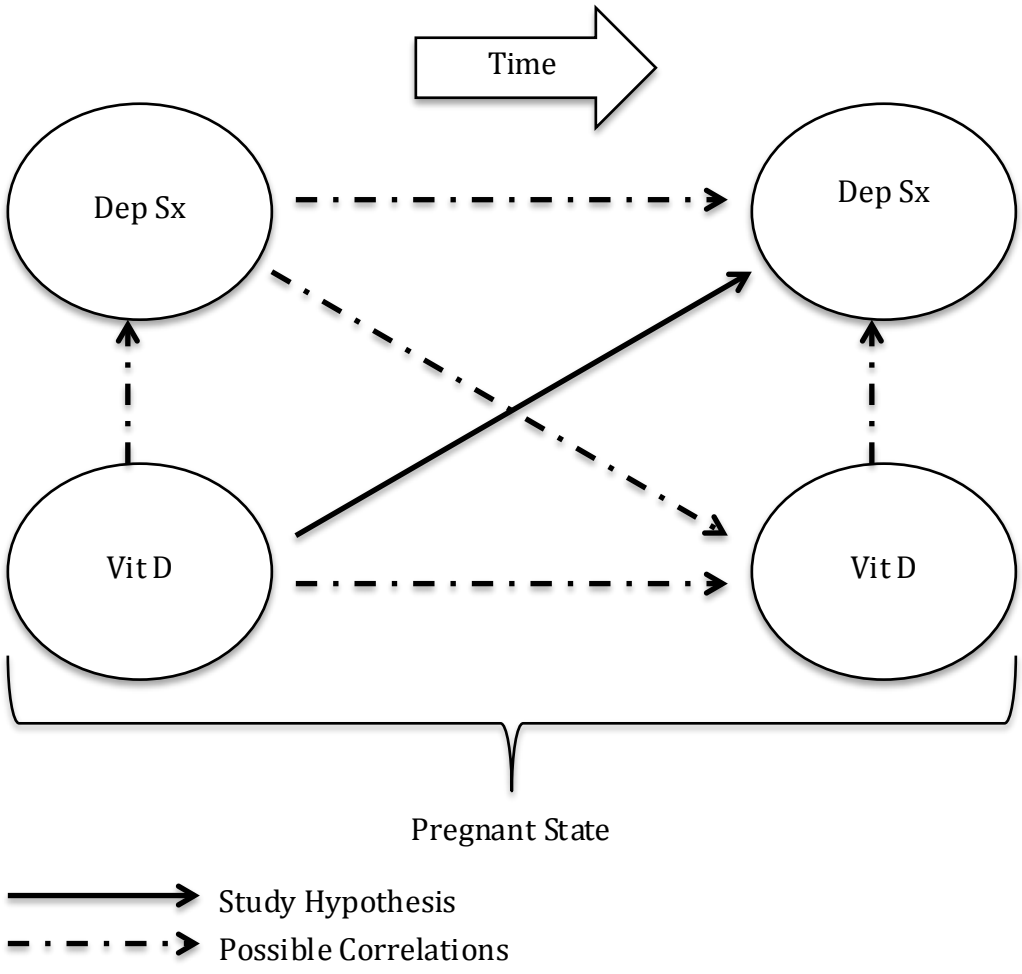
Typically, in theoretical frameworks, depression is categorized as an outcome that occurs when the person is unable to cope effectively and depressive symptoms are the outcome variable in this study. In light of the strong biological role vitamin D plays in

immune regulation, neurologic function, and reproductive systems vitamin D is hypothesized to be the independent variable leading to an outcome of depression in the theoretical framework for this study. The hypothesized direction of the relationship between low levels of vitamin D and increased depressive symptoms is that low vitamin D may be predictive of depression. As previously discussed two studies found lower vitamin D levels in early pregnancy were associated with depression later in pregnancy or postpartum (Cassidy-Bushrow et al., 2012; M. Robinson et al., 2014), however these studies were limited by designs and were unable to demonstrate causality.

Therefore, it is entirely possible that the relationship between vitamin D and depression is bidirectional. However, as a result of pathological development it is often difficult to discern cause-effect timing of depression and vitamin D deficiency. For example vitamin D deficiency may not be noted until the initial prenatal visit at 10 weeks gestational age. However, the vitamin D levels of the woman prior to pregnancy are often unknown. This same issue with timing and progressive development is also true with depression. Further research is needed to validate the association between vitamin D and depression in pregnancy and investigate its direction.

In light of the complexities of potential associations between vitamin D and depression in pregnancy a conceptual model describing possible relationships is included (see Figure 5). A greater understanding of inflammation as the underlying biological mechanisms may begin to elucidate the nature of the direction of the relationship between depression and vitamin D in pregnancy.

Figure 5. Conceptual model of possible relationships between vitamin D (Vit D) and depressive symptoms (DepSx).



Inflammation. The mechanism underlying the hypothesized association between depression and low vitamin D levels may be at least in part be mediated by inflammation. There is sufficient evidence to suggest that inflammation may be an important mediator in the pathophysiology of depression (Leonard & Maes, 2012; Maes et al., 2011). Recent literature demonstrates increased oxidative and nitrosative stress and immune dysfunction in depression (Leonard & Maes, 2012). And two randomized controlled trials have demonstrated that the administration of endotoxins in order to induce an inflammatory

state lead to the expression of depressive like symptoms (Grigoleit et al., 2011; Reichenberg et al., 2001).

Vitamin D may act as an anti-inflammatory in the brain keeping harmful proinflammatory cytokines in check, and vitamin D deficiency has been linked to proinflammatory disease (Harms et al., 2011; Raedler, 2011). It is also possible that as a result of vascular changes in pregnancy the maternal cerebral environment is more vulnerable to inflammation perhaps explaining in part the link between vitamin D and depression in pregnancy (Cipolla, 2013). In light of the inflammatory nature of depression and the strong role of vitamin D as an anti-inflammatory and immunomodulator, inflammation may mediate the relationship between vitamin D and depression in pregnancy (Figure 4) (Arora, C. 2011; Arora et al., 2011; Guillot et al., 2010; Hoeck & Pall, 2010; Lumeng & Saltiel, 2011; Cassidy-Bushrow et al., 2012). study. These hypotheses remain only theoretical and the biological explanation for the link between depression and low vitamin D levels is currently unknown.

Pregnant women are experiencing an increased incidence of depression in the perinatal period. A concern over vitamin D deficiency in pregnancy is growing. Recent evidence suggests an association exists between depression and low vitamin D levels (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Eskandari et al., 2007; Hoang et al., 2011; Hoogendijk et al., 2008; Jorde et al., 2008; Murphy et al., 2010; Wilkins et al., 2006) but due to many methodological issues in prior works the evidence is limited and inconclusive. Given the current state of the science in this area, a study exploring the relationship between depression and vitamin D in pregnant women was undertaken for this dissertation. Using the proposed framework as a guide, this study explored

longitudinal associations between vitamin D and level of depressive symptoms across pregnancy. The primary hypothesis of the study was: *There is an inverse association between vitamin D and depressive symptoms in a sample of pregnant women.* As vitamin D levels decrease, depressive symptoms will increase during pregnancy.

CHAPTER III

METHODOLOGY

In light of the disease burden of depression and vitamin D deficiency in pregnancy, the current evidence and related significance of a possible association between vitamin D and depression in pregnancy, a longitudinal cohort study investigating the association between levels of vitamin D and depressive symptoms in pregnancy was conducted. A detailed description of the methodology is described in this chapter. Key aspects of study methodology are reviewed and include: study design, setting, sample, procedures, instruments, and analysis.

Research Design

An observational longitudinal cohort design was used for this study.

Description of Research Setting

The research setting for participant recruitment and data collection was Cedars-Sinai Medical Center in Los Angeles, California. Cedars-Sinai Medical Center is a large Jewish urban non-profit tertiary health care facility located in Los Angeles California adjacent to the city of Beverly Hills. Cedars-Sinai has a large ethnically diverse obstetric population. The Cedars-Sinai Medical Group outpatient obstetric and gynecologic care clinic and the obstetrical unit at Cedars-Sinai Medical Center were the sites used during the study. The Obstetric and Gynecology Clinic is staffed by the Cedars-Sinai Medical Group obstetricians/gynecologists (from the Cedars-Sinai Department of Obstetrics and

Gynecology) and a physician assistant, and is within one mile of the Cedars-Sinai Medical Center.

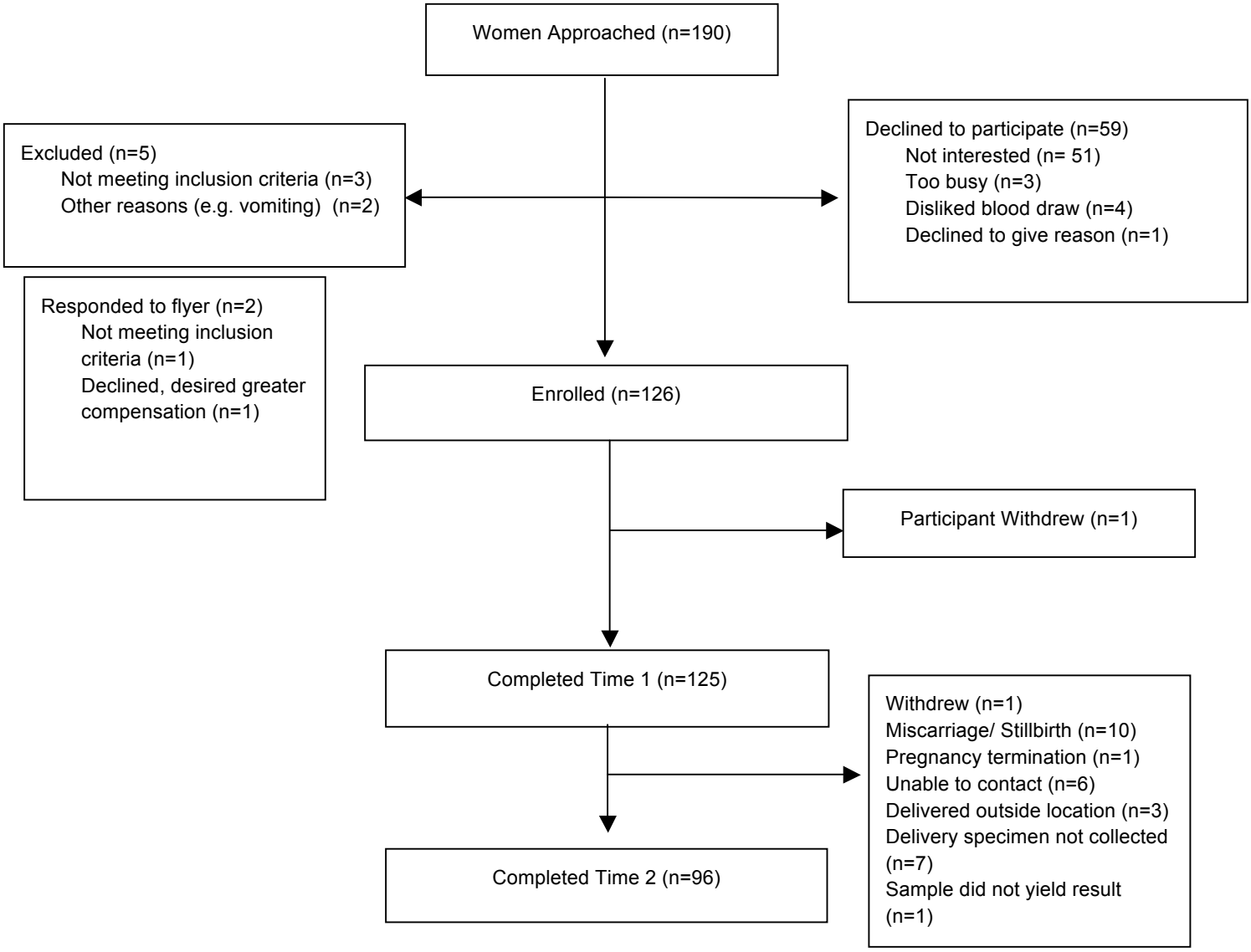
Sample and Sampling Plan

Nature and Size of Sample

A total of 126 participants were enrolled in the study between January 2013 and April 2013. One hundred and twenty-five (N=125) women completed baseline data collection (time 1). One participant completed the informed consent process, but did not complete time 1 data collection and subsequently requested to be withdrawn completely from the study. She noted her husband did not want her to participate.

A total of 96 women completed all data collection points. Figure 6 depicts the recruitment and retention process of study participants. Following time 1, a second participant withdrew citing she was too busy to continue participation, one woman decided to terminate her pregnancy, three women delivered at an outside location, six women were unable to be contacted for the time 2 EPDS, and seven women did not have their time 2 blood collected for a variety of reasons (e.g., precipitous delivery). Additionally, ten women were removed from the sample following miscarriage or stillbirth in light of the strong influence this would likely have on their mood. Finally, one sample failed to yield a 25OHD result upon laboratory analysis.

Figure 6. Recruitment and Retention Process



Sample size was estimated based on a review of available literature examining associations between vitamin D and depression, and an understanding of the clinical importance of an association between vitamin D and depression. Sample size was estimated to detect a Pearson correlation of 0.25 between vitamin D levels and depressive symptoms scores. It was felt that a correlation of 0.25 indicated a valuable finding in

regards to the association between depression and vitamin D and that a correlation of less than 0.25 would not be large enough to represent clinical importance. A one tailed test estimate based on a correlation of 0.25 indicated a sample size of 100 participants would yield ≥ 80% power at a 0.05 significance level. For a two-tailed test correlations of 0.25 indicated a sample size of 125 participants would yield ≥80% power at a 0.05 significance level. A goal was set and achieved to enroll 125 women in order to detect a small correlation and allow for two-tailed analysis and some attrition. However, only 96 of the 125 women completed data for all time points.

Criteria for Sample Selection

To be eligible for study participation, women had to be 18 years of age or older, with a confirmed pregnancy at less than 25 weeks gestation, and receiving prenatal care at the Cedars-Sinai Medical Group obstetric and gynecology clinic. Women were excluded from the study if they had parathyroid disease, uncontrolled thyroid disease, or severe pre-existing mental illness other than depression. Exclusion of women with parathyroid and uncontrolled thyroid disease was necessary because these women may have low vitamin D levels due to disease pathology. Women with severe pre-existing mental illness other than depression (e.g., schizophrenia) were excluded. Severe pre-existing mental illness other than depression can make it difficult to distinguish this disease from depressive symptoms. Women with or without existing depression were included. The presence or absence of depression or taking an antidepressant in a potential participant did not impact a potential participant's eligibility for the study.

Study Staff

The study team for this research project included Amy Lamb, Dr. Calvin Hobel, two research nurses, two anesthesia obstetric fellows, and four research assistants. This author is principal investigator for this study and is listed as such at Vanderbilt University. Due to restrictions on nurse researchers at Cedars-Sinai Dr. Hobel is listed as the principal investigator at Cedars-Sinai and this author is listed as Co-investigator. Dr. Calvin Hobel is a NIH funded senior researcher at Cedars-Sinai with experience in maternal child research and research involving vitamin D. Both of the research nurses are experienced in conducting maternal and child research and have current California registered nursing licenses. Two anesthesiologists currently in their obstetrical fellowship at Cedars-Sinai Medical Center participated in chart abstractions. Research assistants involved in the study had bachelors' level degrees or higher and met requirements for research assistants working or volunteering at Cedars-Sinai Medical Center, including ethical human research orientation and training.

All study staff members underwent ethical research training through the online collaborative IRB training initiative (CITI) program, included on the IRB application as key study personnel, and familiarized with the study. All study staff members, except the two anesthesiologist who did not directly interact with participants, were trained on administering the Edinburgh Postnatal Depression Scale to pregnant women and were instructed one-on-one by this PI as to the protocol for responding to suicidal participants.

Methods for Subject Recruitment

Several strategies were used to recruit study participants. IRB approved flyers announcing the study were placed in the waiting room and exam rooms at the obstetric and gynecologic offices of the Cedars-Sinai Medical Group. Obstetric providers were informed of the study and given information to refer interested patients to the study. An announcement of the study was also placed on the Cedars-Sinai Medical Center Obstetrics and Gynecology Research website. Only two women responded to flyers; one was found to be ineligible and one declined to participate. Neither flyers nor physician referral resulted in the recruitment of study participants.

To facilitate recruitment, potentially eligible women were identified using patient appointment lists in the electronic medical record (Epic) CS-Link at Cedars-Sinai obstetric clinics. The appointment lists indicated gestational age based on a self-reported last menstrual period or expected delivery dated (EDD) defined by first or second trimester ultrasound, maternal date of birth, and visit type (e.g., first obstetric visit). A clinic nurse provided an initial introduction to the study and then asked women if they would be interested in talking with a research nurse representing the study about further study information. All women who expressed an interest in further study information were approached at the clinic and invited to participate if determined to meet eligibility criteria for the study. Due to clinic and investigator concerns regarding protecting private patient information, study staff did not retain data on women who declined to participate in the study.

A trained research nurse or this principal investigator reviewed a complete description of the study with all eligible women who demonstrated interest in the study.

All participants were fully informed both verbally and in writing regarding the study purpose, risks and benefits, alternatives, and right to refuse or leave. All women agreeing to participate completed a written informed consent process. A trained department research nurse or this principal investigator obtained written informed consent for all enrolled participants. The consent form was reviewed and women were given the opportunity to ask questions and all questions were answered. For consenting women the consent form was signed by both the participant and witnessed by the consenting study staff member. A copy of the consent form was given to all participants and a copy retained for the study records. For non-English speaking potentially eligible women an IRB approved short form consent form and an interpreter were available. However no non-English speaking women were identified during the recruitment process.

Data Collection Methods

Study Procedures

Table 1 provides an overview of data collection procedures and time-points. Data collection began immediately after women completed the informed consent process with completion of a study questionnaire, the Edinburgh Postnatal Depression Screen (EPDS) and a drawn blood sample (Time 1). A study nurse was available to answer participant questions. One woman had her blood drawn at her follow-up clinic visit 3 days later due to needing to leave for personal reasons at her enrollment visit.

Data collection for Time 2 occurred between 12 to16 weeks from the time of enrollment in the study. Participants received a phone call from a study nurse or this investigator to complete both the time 2-study questionnaire and a second EPDS during

the phone call. Despite efforts to reach all participants by phone prior to delivery, nine women had their second study questionnaire and EPDS collected during their hospital admission for delivery or immediately postpartum. At time of admission for delivery of their infant, a second maternal blood sample was collected (Time 2 blood). Following study completion and using a standardized clinical abstraction form, participants' medical records were abstracted and data stored. Details involving chart abstractions are described in the instrument section of this chapter.

Most women (53%, N=66) were enrolled into the study in their first trimester (≤ 13.9 weeks) and had their Time 2 data questionnaire and EPDS in their third trimester (Mean (SD) 31.5 (6.3) weeks (wks)). Time between depressive symptom screens (EPDS) was approximately 16 weeks between Time 1 and Time 2 (16.8 (4) wks). Time between blood draws for vitamin D level measurements was on average 16 weeks (16.2 (4.7) wks) between Time 1 and Time 2. Average time between EPDS at Time 2 and vitamin D level at Time 2 was 7 weeks (7 (5.9) wks).

Table 1. Study Procedures and Time Points

Procedures	**Time 1** **<25 wks gestation** **(N=125)**	**Time 2** **EPDS 12-16 wks from Time 1 and Blood at Delivery**
Study Questionnaires that included the EPDS and Demographic and Clinical Information	X	X (N=110)
Blood draw/serum collection to measure Vitamin D (25OHD)	X	X (N=109)
Chart abstraction		X (N=125)

Human Subjects Protections and Data Safety

Human subjects protection. The population of interest was pregnant women who are considered to be a vulnerable group by the Department of Health and Human Services (DHHS) (DHHS, 2009). The ethical principles of beneficence, justice and respect for persons guided the development of several strategies to protect participants' from any harm. Overall, the study posed minimal risk to participants and their fetus. Study procedures posed no risks greater than women would encounter as part of their routine prenatal care. Some minor risks were considered.

The minor risks involved in the study concerned screening women for depressive symptoms, blood sampling, and protecting personal health information. Screening for postpartum depression is the current standard of care. In addition, the American Congress of Obstetricians and Gynecologists has stated that prenatal screening for depression should be strongly considered by providers (ACOG, 2010). Depression screening raises the ethical question of not placing undue distress on participants and having safety plans and referral mechanisms in place should participants report thoughts/intentions for self-harm (Chaudron et al., 2007). In this study women were considered positive or at risk for suicidal ideation if they reported anything besides "never" to item 10 "the thought of harming myself has occurred to me" on the EPDS.

Study staff identified two women who screened positive or at risk for suicidal ideation at the clinic or the hospital. Both women had their primary obstetrician notified immediately, and they were referred to the Cedars-Sinai emergency department for assistance. Additionally, another woman noted positive suicidal ideation during a phone interview. Study staff immediately notified her obstetrician and she was referred to the

Cedars-Sinai emergency department. All of the women who screened positive for suicidal ideation denied immediate thoughts of self-harm at that time and none of them had a plan. Women at risk for suicidal ideation were also given the number of the National Suicide Prevention Lifeline (1-800-273-TALK), which is a free accredited crisis hotline. No adverse events occurred in this study.

A small risk existed related to the blood draws to measure vitamin D levels. Screening for blood levels of vitamin D is not currently standard of care and the data concerning the value of knowing vitamin D status is conflicting. Whenever possible blood was drawn at the same time that other labs were being collected as part of standard prenatal care; however, separate blood draws were conducted when the sample was not collected as part of standard care. Registered nurses at the clinic and hospital as well as the research nurse drew blood using standard venipuncture methods.

A small risk related to patient privacy was considered. Participant protected health information was collected via self-report on study questionnaires and chart abstraction; however efforts were made to minimize risk involved with collecting and accessing protected information. Further details regarding data management/safety are described in the section below.

IRB approval for the study was received from Cedars-Sinai Medical Center and Vanderbilt University. The informed consent process clearly outlined to participants that they could still receive their regular prenatal care regardless of study participation and that the study was not part of routine prenatal care. Subjects were given a $10 gift card at the time of enrollment and another $10 gift card at the time of study completion. All study activities with participants were conducted in a private area in order to protect

participants' privacy and maintain confidentiality. All study protocols approved by the IRB were followed.

Data Management and Safety Monitoring Plan. To ensure participant privacy, blood samples and questionnaires were labeled with the participants' study identification numbers (ID) instead of their names. All databases with study ID numbers and patient names were encrypted and kept locked in the research office at Cedars-Sinai East Tower, suite 1001. Study ID numbers were used as much as possible in place of participants' names. Collected data were entered into a password protected and encrypted REDCap database. No identifiable health information was stored on personal computers, laptops, or external drives. Only trained and IRB approved study staff had access to participant information. Access to identifying information was restricted and only accessed by necessary personnel. Physical documents containing data on research participants were stored in locked file cabinets in locked research offices. Research computers were encrypted and password secured. A data safety monitoring plan/committee was not required by the IRB for this study.

Instruments

Study Questionnaires. The questionnaires included socio-demographic and prenatal questions related to maternal age, ethnicity, marital status, annual income, years of education, height, pre-pregnancy and current weight, if the pregnancy was planned or not, and current medications/supplements they were using (Appendix A). Study staff recorded the season during which the questionnaires, EPDS, and blood samples were

collected. Seasons were categorized as Summer/Fall including the months of July to December and Winter/Spring including January to June based on the reported number of clear sunny days in Los Angeles city (Western Regional Climate Center, 2010).

Edinburgh Postnatal Depression Scale. The 10-item Edinburgh Postnatal Depression scale (EPDS; Cox, Holden, & Sagovsky, 1987) was used to measure depressive symptoms (Appendix B). Originally, the EPDS was designed to screen for depression in the postpartum period (up to eight weeks postpartum; Cox et al., 1987), but has subsequently been validated for use during pregnancy (Cox, Chapman, Murray, and Jones, 1996; Lee et al., 2001; Murray & Carothers, 1990; Su et al., 2007; Yonkers, Smith, Gotman, & Belanger, 2009).

The EPDS was self-administered or given orally by a trained study staff member if the interview was conducted over the phone. Respondents were asked to rate each of the 10 items using four possible responses that were subsequently scored 0 – 3. Each response corresponded to a level of intensity of depressive symptomatology (Cox et al., 1987). A total score was calculated by tallying responses for each of the items, with items 3, 5, 6, 7, 8, 9, and 10 being reverse scored (Cox et al., 1987). The total possible score for the EPDS ranges from 0 to 30 with cut-off scores listed in the literature between 9 to 15 and/or a positive response to item 10, suicidal ideation, correlating with depression (Cox et al., 1987; Murray & Carothers, 1990; Sit & Wisner, 2009). Many studies note a cut-off score of 9, which yields a broad range of reported sensitivity (68-80%) and specificity of 77% (Murray & Carothers, 1990; Lawrie, Hofmeyr, de Jager, & Berk, 1998) to distinguish between depressed and non-depressed women. In order to identify all women

with possible major or minor depression a cut-off score of 9 was employed in this study (Cox et al., 1987).

The EPDS was chosen for this study for its accuracy and ease of use. In a large meta-analysis examining several different measures for perinatal depression the EPDS was found to have higher sensitivity for detecting major depression than other scales (Hewitt et al., 2009). The EPDS can be completed quickly and is generally well accepted by participants (Cox et al., 1987; Hewitt et al., 2009). The EPDS demonstrates good convergent validity and is moderately correlated with both the BDI and CES-D. The calculated Cronbach's alpha for the EPDS in the current study was α=.82. Other studies have employed the EPDS to examine associations between vitamin D and postpartum depression (Murphy et al., 2010; M. Robinson et al., 2014).

25OHD. 25-hydroxyvitamin D (25OHD) was used to measure vitamin D levels in maternal blood samples. Trained nurses drew maternal blood samples using standard venipuncture methods. Whole blood was collected into serum separator tubes. Blood samples were spun and frozen or refrigerated and then spun and frozen within an average of 48 hours from time of collection. Some degree of mild to moderate hemolyzation was noted for 54 samples. Hemolysis at moderate levels has been shown to not influence test results, however at severe levels it made lead to slight under estimation of vitamin D levels (Nowak et al., 2011). After being centrifuged for a minimum of 15 minutes serum was aspirated and alliqoted to 1-2 ml cryotubes. Cryotubes were frozen at -20° C and then hand carried packed in dry ice to Endocrine Sciences, Esoterix Inc., a part of LabCorp Specialty Testing Group in Calabasas Hills, CA, 91301. At the lab, samples were thawed and analyzed by LC-MS/MS using a Thermo® ARIA® TX-4 HPLC system

with Agilent® 1200SL pumps and a Sciex® API5000 triple quadrupole mass spectrometer. A Supelco® PFP analytical column was used (100 x 2.1mm, 2.7um, 100A) with a water:methanol gradient to achieve full baseline chromatographic separation of epimer and non-epimer forms of each analyte. Independent calibration curves were prepared for all metabolites. Calibration was performed using UV/Vis spectrophotometry and was further verified using reference materials from NIST as well as 3rd party vendors. Sample preparation consisted of isotope dilution using the internal standards 25OHD2-2H3, 25OHD3-2H6, protein precipitation and liquid-liquid extraction. Both $25OHD_3$ and $25OHD_2$ were measured in this study, however similar to the study by Nielsen et al (2013) $25OHD_2$ values were found to be non-contributory and therefore only $25OHD_3$ values are reported. Vitamin D levels were processed at the end of the study in order to keep study staff and participants blinded to the levels during the study.

Chart Abstraction. Additional maternal and infant data were abstracted from the participant's electronic medical record stored on CS-Link (Epic) system. Abstracted data included: delivery information such as infants' gestational age at time of delivery, mode of delivery, and neonatal outcomes. This PI and the two anesthesiology fellows working with the study completed the chart abstractions. Collected data were then entered into the password protected REDCap database for management and storage.

Data Analysis

Descriptive and inferential statistics were used for analysis of study data. Descriptive statistics (e.g., means, standard deviations, interquartile ranges, percentages) were used to describe the sample characteristics. Women who enrolled in the study were

dichotomized into groups by sufficient (25OHD ≥30ng/ml) and insufficient (25OHD <30ng/ml) vitamin D levels to help describe the study sample, and independent t-tests and chi-square values are reported for significant differences between groups for bivariate analyses. Paired *t*-test and Wilcoxon Signed Rank tests were used to evaluate differences between early and late pregnancy vitamin D levels and depressive symptom scores within the total sample group. Correlations between $25OHD_3$ and EPDS sum scores were analyzed for the total sample. Both Pearson product moment and Spearman's rho correlations were evaluated. Correlations are reported for all time points.

In order to test the hypothesis of whether low vitamin D levels lead to an increase in depressive symptoms, a two-step linear regression was conducted. Two analyses were analyzed to help determine the temporal precedence of the association (i.e., did early low levels of vitamin D predict higher levels of depressive symptoms or vice versa?). The first model was analyzed using the EPDS Time 2 sum scores as the dependent variable; then, Time 1 EPDS sum scores and Time 1 vitamin D levels were added in a stepwise fashion. To assess for the obverse of the hypothesis (i.e., high depressive symptoms lead to low vitamin D), a second regression analysis was analyzed. The second two-step linear regression included vitamin D Time 2 levels as the dependent variable, and then introduced Time 1 vitamin D levels on step 1 and Time 1 EPDS sum scores on step 2.

Additionally, a two-step hierarchical multiple linear regression model was evaluated to control for the influence of possible confounding variables. This model used Time 1 EPDS scores as the dependent variable. Four possible confounders (BMI, years of education, ethnicity, and unplanned pregnancy) were added as independent variables in step 1 followed by $25OHD_3$ Time 1 levels in step 2. SPSS 22.0 was used for all

statistical analyses, and a two-tailed $\alpha = 0.05$ level of significance was used for all statistical tests.

Missing Data

Missing and incomplete data were present in this study. Several (N= 30) women did not complete all data collection points. Three women did not answer one or two of the questions on the EPDS. For these three EPDS questionnaires computational means based on other items from the same screen were used to impute missing values. These women were included in the final analysis. Using the computational mean as a substitute for the missing values when there are a minimal number of missing values can be an effective method of addressing missing data in summative rating scales, although it may introduce some bias (Raaijmakers, 1999).

CHAPTER IV

RESULTS

Statistical analysis of data yielded a number of important study results. Findings from the longitudinal cohort study on the association between vitamin D and depression in pregnancy are discussed in regards to sample characteristics, study questions, and study hypothesis. Additional findings not directly related to the primary hypothesis and questions are briefly reviewed.

Sample Characteristics

Table 2 depicts the clinical and demographic characteristics for the total sample and for subgroups of women by vitamin D level (i.e., sufficiency or insufficiency). Overall, this ethnically diverse sample of 125 women tended to be older, overweight or obese, upper class, well educated, married, having planned their pregnancy, and working. Most women in the study delivered at term and no women in the sample smoked (N=0). Approximately, one fourth of women (N= 32) reported elevated depressive symptoms (25.6% with mean EPDS sum score ≥ 9) and approximately two-thirds (N = 83) had vitamin D insufficiency (66.4% at $25OHD_3 < 30$ ng/ml) in the total sample. The characteristics of the final sample of 96 women (i.e. those with complete data for both time points) were similar to the total sample (N=125). Women who were excluded from the final sample (N=29) due to incomplete data for both time points for any reason (e.g., fetal demise, miscarriage, withdrew, delivered at outside of hospital) were more likely to be unmarried ($\chi^2 = 8.95$, $p = 0.030$) and of ethnic minority ($\chi^2 = 11.5$, $p = 0.042$) as compared to the final sample (N=96).

Table 2. Participant characteristics for total and final samples

Variable	Total (N= 125) Median (IQR)	Total (N= 96) Median (IQR)
Age (yrs)	33 (31-36.5)	33 (31-36)
BMI	25 (23-29)	25 (23-28)
Normal/Underweight (≤ 24.9)	39.2% (49)	41.7% (40)
Overweight (25-29.9)	38.4% (48)	37.5% (36)
Obese (≥ 30)	22.4% (28)	20.8% (20)
Annual Household Income ($) (104)	103,295k (50k-120k)	87.5k (50k-150k)
Education (years)(117)	16.4 (2.5)[a]	16.4 (2.5)[a]
Parity	2 (1-3)	2 (1-3)
Estimated Gestational Age (EGA) at time of Delivery	39.4 (38.4-40.1)	39.6 (38.7-40.3)
EPDS Sum Score[b]	5.7 (4.1)[a]	5.6 (3.5)[a]
Vitamin D Level ($25OHD_3$)[b]	26.1 (9.2)[a]	25.8 (9.1)[a]
	Percent (N)	**Percent (N)**
Marital Status		
Single	3.2% (4)	2.1% (2)
Partner (Not Cohabitating)	1.6% (2)	2.1% (2)
Living with Partner	16% (20)	11.5% (11)
Married	79.2% (99)	84.4% (81)
Divorced	------	------
Ethnicity		
Asian	16.7% (21)	13.5% (13)
Black/AA	19% (24)	17.7% (17)
Caucasian	32% (41)	37.5% (36)
Hispanic	21.4% (27)	24% (23)
Indian	4.8% (6)	2.1% (2)
Mixed	4.8% (6)	5.2% (5)
Employment		
No	26.4% (33)	28.1% (27)
Yes	73.6% (92)	71.9% (69)
History of Depression		
No	91.2% (115)	92.7% (89)
Yes	8.8% (11)	7.3% (7)
Illicit Drug Use		
No	97.6% (122)	97.9% (94)
Yes	2.4% (3)	2.1% (2)
Do you currently drink alcohol?		
No	96.7% (117)	94.8% (91)
Yes	3.3% (4)	3.1% (3)
Amount (drinks/week)	1.19 (1.88)[a]	1.6 (2.1)[a]
Planned Pregnancy		
No	28.8% (36)	27.1% (26)
Yes	71.2% (89)	72.9% (70)

[a] Mean (SD); [b] For the total sample (N=125) only time 1 values are reported, but for the final sample (N=96) the mean of Time 1 + Time 2 is reported.

Analysis of Research Questions and Hypothesis

Study findings are organized by research questions and study hypothesis.

Research Questions

Is there a difference between early and late pregnancy vitamin D levels? Time 1 and Time 2 vitamin D levels were moderately correlated in this study (r=0.67, p <0.001). A statistically significant difference between Time 1 vitamin D levels and Time 2 vitamin D levels (Mean $25OHD_3$ Time 1=25.8 vs. Time 2= 34.1 ng/ml; paired t= 10.04, p <0.001) emerged from the paired t-tests analysis. Late pregnancy (Time 2) vitamin D levels were higher than vitamin D levels in early pregnancy (Time 1). A significant difference between early and late pregnancy vitamin D levels was observed in this sample.

Is there a difference between early and late pregnancy depressive symptoms? Early (Time 1) and late pregnancy (Time 2) EPDS sum scores were also moderately correlated in this sample (r=0.63, p <0.001). A paired t-test was used to evaluate for differences between Time 1 and Time 2 depressive symptoms scores. There was no statistically significant difference found (Mean EPDS sum scores Time 1 = 5.5 vs. Time 2 = 5.7; paired t=*0.51*, p=0.611). No differences between depressive symptoms in early and late pregnancy were observed in this sample.

Is there a relationship between vitamin D levels and depressive symptoms during pregnancy? Results of a correlational analysis that examined the level of association between EPDS scores and vitamin D levels at all time points are depicted in Table 3. Time 1 EPDS sum scores and Time 1 vitamin D levels showed inverse associations for both the total original sample (N=125; r=-0.152, p=.092) and the final reduced sample

(N=96; r=-0.236, p=.020), but the association was only significant in the final sample. Significant inverse associations were noted between Time 2 EPDS scores and Time 2 vitamin D levels (r = -.321; p=.001). Also, a significant inverse association was observed between overall mean EPDS sum scores (5.6) and overall mean vitamin D levels (29.9) in the final sample (N=96; r =-.334; p=.001). In addition women with insufficient vitamin D tended to have higher mean depressive symptom sum scores (6.7 (3.6) vs. 4.8 (3.5); t=-*3.02, p* = .003) and women with elevated depressive symptoms had lower mean $25OHD_3$ levels (26.4 vs. 30.9 ng/ml; p=*.047*). Therefore, a significant relationship between vitamin D levels and depressive symptoms during pregnancy was observed in this sample.

Table 3. Correlations between levels of depressive symptoms and vitamin D by time point for the final sample (N=96).

	EPDS Time 1	**EPDS Time 2**	**$25OHD_3$ Time 1**	**$25OHD_3$Time 2**
			r (p-value)	
EPDS Time 1	1	.658 (< .001*)*	-.236 (*.020)*	-.289 (*.004)*
EPDS Time 2	-----	1	-.267 (*.009)*	-.321 (*.001)*
$25OHD_3$ Time 1	-----	-----	1	.668 (< .001*)*

Predictive Hypothesis

A regression analysis was conducted to test the primary hypothesis that low vitamin D levels may lead to higher depressive symptoms. A two-step regression was analyzed using EPDS Time 2 as the dependent variable, controlling for EPDS at Time 1. The addition of baseline vitamin D to the regression model on step 2 did not add a statistically significant amount of explanatory power (R^2 = .392 model 1, R^2 = .409 model

2, Δ R^2 =*.017*, *p*= 0.102). Thus, this finding did not support the hypothesis that low levels of vitamin D lead to increased depressive symptoms.

A second regression was conducted to assess the reverse hypothesis (i.e., increasing depressive symptoms lead to decreases in vitamin D levels), and yielded similar null results (R^2 = .446 model 1, R^2 = .456 model 2, Δ R^2 = 0.010, *p*= 0.202.

Adjusting for Covariates

A final regression model was evaluated to control four possible confounding variables: BMI, years of education, ethnicity, and whether the pregnancy was planned or unplanned. Those variables were chosen based on prior literature and significant associations with either EPDS or 25OHD in this sample. Time 1 EPDS and $25OHD_3$ values for all 125 women in the initial sample were used for this regression. The correlation between Time 1 $25OHD_3$ and Time 1 EPDS sum scores for the total sample (N = 125) was significant in the regression model as a one-tailed test (*r* = -.152; *p* = .046), but when covariates were included in the model, $25OHD_3$ only accounted for a small, non-significant increase in explanatory power (1%) for EPDS sum scores (R^2 = .030 model 1, R^2 = .039 model 2, Δ R^2 =*.010*, *p=.272)*.

Other Findings

Women were dichotomized into two subgroups, sufficient and insufficient vitamin D levels ($25OHD_3 \geq 30$ng/ml vs. $25OHD_3 < 30$ng/ml), based on mean vitamin D levels aggregated across both time points (Table 4). Women with insufficient vitamin D tended to have higher BMIs (*t=-2.03, p =.045*), fewer years of education (*t=2.04, p =.044*), were more likely to have darker skin tones (e.g., Black, Indian, and Hispanic) (χ^2

=8.29, *p=0.008),* and have an unplanned pregnancy (χ^2 =5.28, *p=0.022)* as compared to women with sufficient vitamin D levels. In the final sample (N=96) women with elevated depressive symptoms were more likely to report a history of depression (20% vs. 4%; *p=.014*), and be unemployed (50% vs. 22.4%; *p=.014*). There were no statistically significant differences in women with elevated depressive symptoms in the final or total sample for BMI, ethnicity, unplanned pregnancy, and years of education. There were also no differences between women who had their Time 2 EPDS collected at time of delivery as compared to those who were screened at 12-16 weeks from Time 1 (*t=-2.93,* *p*=0.770).

Some additional findings of interest were noted related to gestational age and blood loss at time of delivery. Vitamin D levels were positively correlated with gestational age at time of delivery for both mean (*r=0.28, p*= .006) and Time 2 vitamin D values (*r=.247, p*= .015). Women who hemorrhaged at delivery (blood loss ≥ 500 cc; N = 22) had significantly lower $25OHD_3$ levels at Time 2 (delivery) (M = 29.6) as compared to women who did not hemorrhage (M = 35.4; *t*=-2.23, *p*= 0.03*)*.

Table 4. Participant characteristics by sufficient and insufficient vitamin D levels

Variable	Vitamin D Sufficiency $25OHD_3$ ≥30ng/ml (N=48)	Vitamin D Insufficiency $25OHD_3$ <30ng/ml (N=48)	*p-value*
Age (yrs)	33.8 (4.2)	32.7 (5.4)	.256
BMI	25.2 (3.9)	27.1 (5.4)	.045*
Annual Household Income ($) (82)	126k (121k)	98k (84k)	.226
Education (years)	16.9 (2.7)	15.9 (2.1)	.044*
Parity	2.2 (1.5)	2.5 (1.4)	.425
Estimated Gestational Age (EGA) at time of Delivery	39.6 (1.5)	38.9 (1.7)	.055
Mean EPDS sum scores	4.5 (3.4)	6.6 (3.4)	.003*
	% (N)	**% (N)**	
Marital Status			
Single	0% (0)	4.2% (2)	.181
Partner	2.1% (1)	2.1% (1)	
Living with Partner	6.3% (3)	16.7% (8)	
Married	91.7% (44)	77.1% (37)	
Divorced	------	------	
Ethnicity			
Asian	12.5% (6)	14.6% (7)	.008*
Black/AA	10.4% (5)	25% (12)	
Caucasian	52.1% (25)	22.9% (11)	
Hispanic	16.7% (8)	31.3% (15)	
Indian	2.1% (1)	2.1% (1)	
Mixed	6.3% (3)	4.2% (2)	
Employment			
No	25% (12)	31.3% (15)	.496
Yes	75% (36)	68.7% (33)	
History of Depression			
No	93.8% (45)	91.7% (44)	.695
Yes	6.3% (3)	8.3% (4)	
Planned Pregnancy			
No	16.7% (8)	37.5% (18)	.022*
Yes	83.3% (40)	62.5% (30)	

$* p \leq 0.05$

CHAPTER V

DISCUSSION

This study examined associations between levels of vitamin D and depressive symptoms at multiple time points in a sample of pregnant women. Overall study findings partially support the study hypothesis and study questions. An inverse association between depressive symptoms and vitamin D emerged; however, the hypothesized direction of the association could not be established. Results suggest that vitamin D insufficiency may be an important risk factor for depression in pregnancy, but we can't rule out the alternative hypothesis that depressed mood might contribute to lower Vitamin D levels. Study results are discussed within the context of current evidence and proposed relationships. Study limitations and implications are presented and recommendations for future research are discussed.

The sample of women in this study were well-educated, upper-middle class, and ethnically diverse. Differences in socio-demographic characteristics between women with sufficient and insufficient vitamin D were similar to those observed in other studies (Murphy et al., 2010; M. Robinson et al., 2014) and consistent with risk factors for hypovitaminosis D. The incidence of depression in this sample was slightly higher than expected (25%), however still in keeping with estimates from other studies of this nature (Cassidy-Bushrow et al., 2012; M. Robinson et al., 2014; Villegas et al., 2011). The incidence of vitamin D insufficiency was high in both the total (original) and final (reduced) samples (66.4% and 50%). Levels were similar to other studies investigating

vitamin D and depression in pregnancy (Cassidy-Bushrow et al., 2012; Murphy et al., 2010; M. Robinson et al., 2014) and may be due to using the higher Endocrine Society cut-off of 30 ng/ml for insufficiency (Endocrine Society, 2011). Similar to other studies, women with higher depressive symptoms were more likely to have a history of depression and to be unemployed (Greenberg et al., 2003; Stewart et al., 2003).

Discussion of Findings in Relation to Research Questions and Hypothesis

Vitamin D Levels Over the Course of Pregnancy

The study sought to answer research questions about changes in vitamin D levels and depressive symptoms over the course of pregnancy. Current research suggests that 25OHD values tend to be stable across all trimesters of pregnancy (Brannon & Picciano, 2011; Marwaha et al., 2011). However, some studies have noted a rise in 25OHD in the third trimester (Cross et al., 1995; Sanchez et al., 1996). In this study, Vitamin D levels at the time of delivery, were noted to be significantly higher than those assessed during early pregnancy (i.e., less than 25 weeks gestation). Similar to the study by Cross et al. (1995) a rise in 25OHD from early pregnancy to time of delivery may be explained by season at time of measurement. Baseline vitamin D levels were measured during Winter/Early Spring (January to April), and most delivery vitamin D levels were obtained in the Summer (July to September) when there are more sunny days based on the average number of clear sunny days in Los Angeles and the temperatures are warmer (Western Regional Climate Center, 2010). More sun and higher temperatures can lead to women wearing less clothing. Both factors can contribute to increased skin exposure to sunlight thus increasing the synthesis of vitamin D with a concurrent rise of vitamin D levels in

the summer months. Another possible explanation for the rise in vitamin D levels from early to late pregnancy could be initiation of prenatal vitamins. However, this explanation is questionable since the limited amount of vitamin D present in standard prenatal vitamins has been shown to be inadequate for raising 25OHD levels in pregnant women (Hollis et al., 2011).

Level of Depressive Symptoms Over Time

Consistent with other studies (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012), levels of depressive symptoms were correlated between early and late pregnancy. Prior research suggests that elevated depressive symptoms in early pregnancy are a good predictor for late pregnancy and postpartum depression (Milgrom et al., 2008). Findings from this study were consistent with the literature; women with elevated depressive symptoms were more likely to have a history of depression. This finding provides further support for the chronic nature of depression. Findings from the current study underscore the stability of depressive symptoms across time. One unique feature of this study in regards to depressive symptoms was the repeated measures of vitamin D and depressive symptoms during pregnancy. Prior studies examining these variables have only measured depressive symptoms at a single time point.

Vitamin D and Depressive Symptoms in Pregnancy

Results of this study indicated a statistically significant relationship between lower vitamin D levels in early pregnancy (T1) and higher depressive symptoms in late pregnancy (T2). This finding is largely consistent with prior, although limited, findings (Brandenbarg et al., 2012; Cassidy-Bushrow et al., 2012; Murphy et al., 2010; M. Robinson et al., 2014). Vitamin D is likely not the only factor involved in the

development of depression in pregnancy, however it may represent an important marker for identifying pregnant women at risk for depression. For the most part, this relationship, although not statistically significant for the total sample (N=125) in early pregnancy, held at all other time points. The hypothesis, examined by hierarchical regression, that low levels of vitamin D would "account for" elevated depressive symptoms in later pregnancy was not supported by study findings (p=.102). Although 125 women enrolled in the study, complete data was only available for 96 women. The smaller sample size used in the regression analysis testing this hypothesis may have impacted study findings.

Additionally, the study design did not control for pre-existing depression or vitamin D deficiency making it difficult to assess the temporal precedence of the association. Thus, the theoretical framework suggesting an inverse association between vitamin D and depressive symptoms was validated, however the study was not able to indicate whether low vitamin D proceeds increased depressive symptoms. The study was also unable to test for mediating effects of inflammation/neuropathology, but positive associations between vitamin D deficiency and BMI were noted in this sample. There is substantial body of evidence linking chronic inflammation with metabolic dysfunction (Lumeng & Saltiel, 2011; Sonnett et al., 2010; Wellen & Hotamisligil, 2005). Depression has been linked to metabolic disease and inflammation (Raedler, 2011; Shelton & Miller, 2010), and vitamin D is known to have a critical role in normal metabolism and to have strong anti-inflammatory properties (Arora, C. 2011; Arora et al., 2011; Guillot et al., 2010; Hoeck & Pall, 2010; Lumeng & Saltiel, 2011). Therefore, the positive association between vitamin D and BMI in this sample may indicate that underlying metabolically

mediated inflammation may have a role in the noted association between vitamin D deficiency and elevated depressive symptoms.

Several potentially confounding variables in the relationship between Vitamin D levels and depression have been noted in the literature: age, BMI, employment status, (Brandenbarg et al., 2012), educational level, ethnicity (Brandenbarg et al., 2012; Murphy et al., 2010), parity (Brandenbarg et al., 2012; Nielsen et al., 2013), socio-economic status (Brandenbarg et al., 2012; Murphy et al., 2010; Nielsen et al., 2013) pregnancy intention, smoking (Brandenbarg et al., 2012; Nielsen et al., 2013; Robinson et al., 2014), social support (Nielsen et al., 2013), and marital status (Brandenbarg et al., 2012; Murphy et al., 2010). Many of these confounding variables (e.g., BMI) are shared risk factors for both vitamin D and depression and may account for the association between them.

Confounding variables in this study were selected based on prior literature and on variables found to have significant correlations with vitamin D or depressive symptoms in this sample. Potential confounding variables on the association between vitamin D and depressive symptoms were evaluated at Time 1 (e.g., BMI, years of education, ethnicity, and pregnancy intention). Although, none of the covariates analyzed made a statistically significant unique contribution to EPDS scores in this sample, the combination of these covariates overwhelmed the influence of $25OHD_3$ on EPDS scores. BMI and years of education accounted for most of the confounding influence on the association between vitamin D levels and depressive symptoms. Higher BMIs may lead to underlying metabolic inflammation leading to both decreases in vitamin D levels and increases in depressive symptoms, thus acting as a possible mediator on the relationship. Higher

education may lead women to eat healthier diets and exercise contributing to higher vitamin D levels and lower depressive symptoms possibly explaining education's influence on the relationship.

Some unexpected but potentially significant findings occurred. Women in this study who experienced hemorrhage at delivery had lower levels of vitamin D. No studies were found on my review of the current literature investigating intrapartum/postpartum hemorrhage and vitamin D. Literature noting increased risk of stroke (Brondum-Jacobsen, Nordestgaard, Schnohr, & Benn, 2013), bleeding during periodontal surgery (Bashutski et al., 2011), and need for primary cesarean section (Merewood et al., 2009) with vitamin D deficiency have been noted and suggest there may be a link between bleeding, uterine muscle dysfunction, and vitamin D deficiency. Further research is needed to investigate the association between hemorrhage and vitamin D deficiency.

Study Limitations

The prospective single cohort design used in this study was appropriate for assessing associations between depression and vitamin D in a sample of pregnant women. This design allowed for the assessment of depression using a standardized depressive screening tool (i.e., EPDS), something that is not routinely done in the antenatal period and often not available in existing data sets. Measurement of levels of depressive symptoms and vitamin D at multiple time points allowed the study to demonstrate stability of depressive symptoms across time and to identify changes in vitamin D levels across the prenatal period, minimizing maturation threats. This prospective design

allowed for the measurement and consideration of covariates of interest that may not have been available in a retrospective design or secondary analysis.

Several limitations should be considered for this study. The relatively small convenience sample of women may limit the ability to detect differences and the generalizability of findings. Due to restrictions on enrollment methods, study staff was not able to collect data on women who declined to participate in the study. Thus, it was not possible to determine if there were significant differences between women who volunteered for the study and those who did not volunteer. Women in my study tended to be older (33 vs. 25.4 yrs) (Martin et al., 2010 National Vital Statistics) than the average maternal age for first birth in the United States, and have higher incomes (103k vs. 61.6k) (U.S. Census Bureau for California, 2010) as compared to other family household incomes in California, further limiting generalizability.

An urban setting in a developed country (e.g., United States) is an ideal location for examining the link between vitamin D deficiency and depression in pregnancy, since rates of both conditions are higher in these particular areas. However, having only one site in an urban location limits the generalizability of study findings and makes application to rural and underdeveloped regions and populations difficult. The study sought to examine levels of depressive symptoms and vitamin D in early and late pregnancy and therefore only enrolled women less than 25 weeks gestational age. As a result, this study does not include women who were late to enter or who had no prenatal care, introducing a bias towards women who seek early care and preventing the study from examining those with little no or care despite their increased risk for depression (Cypryk et al., 2008).

In addition this study suffered from approximately a 24% attrition rate and some missing data. There are a wide variety of reasons why retention of pregnant women in research is so difficult (Macklin, 2010; Wisner, Appelbaum, Uhl, & Goldkind, 2009; Merkatz, 1998). Partly the explanation lies with pregnant women themselves as the pregnancy can be lost (miscarriage) or terminated and viable pregnancies take a significant amount of time to gestate, during which women may experience life events that make it difficult for them to continue study participation (e.g. relocation). The 29 women with incomplete data for both time points, including those with miscarriage/stillbirth, were more likely to be single and of an ethnic minority as compared to rest of the sample. This may have introduced some sampling bias into the final sample.

Also, limiting this study is the use of self-report on depression screens, which is not a substitute for professional evaluation and diagnosis of depression. Ideally, women who screened positive would be examined by a mental health professional. The study design is observational so causality is unable to be established in the way that a more rigorous design may offer. However, multiple time points of data collection provided some evidence for a direction of the relationships. This design was vulnerable to testing threats as the women were screened for depression at more than one time point. This repeated screening may have heightened their awareness or influenced their responses. In an effort to minimize this threat, data assessing levels of depressive symptoms was collected at least 3 weeks apart. A history threat to internal validity existed for this study as a result of the growing popularity of vitamin D supplementation and growing awareness of depression. The design used helped to minimize a history threat, because

the entire cohort had similar exposures to historical threats, as it was a single site study, same city, and relatively similar social class. The cohort was vulnerable to maturation threats as it examined pregnant women who were moving through stages of change during the course of their pregnancy. However, the entire cohort was on the same maturation trajectory and the repeated measures throughout the study helped to identify patterns regarding maturation.

This study also did not evaluate for social support, anxiety, or life stressors, which may be underlying causes of elevated depressive symptoms. The study also did not account for dietary intake of vitamin D (e.g., fatty fish, fortified milk, etc.) and thus is unable to detect the role diet may have played in low vitamin D levels in this sample. Depression by nature can lead to poor nutrition and sedentary lifestyle, which could result in less vitamin D intake and synthesis, perhaps leading to lower vitamin D levels. It is therefore possible that high depressive symptoms may lead to low vitamin D, however this pattern was not universally observed in this study. The study also did not measure levels of vitamin D and depressive symptoms at exactly the same time at each time point. However, stability of depressive symptoms over the course of the pregnancy helps to ameliorate some of the potential bias this may have introduced. The design did not control for all threats to validity, but provides exploratory level data on the relationship between vitamin D and depression in pregnancy.

Implications

My findings support a significant association between low levels of vitamin D and elevated depressive symptoms in pregnancy. This study affirms previous studies that indicate depression and vitamin D deficiency are common in pregnancy. This study provides support for existing clinical guidelines that pregnant women at risk for vitamin D deficiency (such as those with parathyroid disease) and depression (history of depression) should be screened. The study also suggests that physicians and nurses may want to consider screening women diagnosed with depression for vitamin D deficiency and vice versa, although further research is needed before routine screening of depressed women for vitamin D deficiency can be recommended.

As more evidence becomes available, vitamin D may come to be a meaningful clinical marker for depression risk in pregnancy enabling obstetricians, midwives, and obstetric nurses to identify women at risk for depression prior to the onset of severe depression. Supplementation with vitamin D may represent a safe and important adjunct therapy for pregnant and postpartum women diagnosed with depression. As a result of the caring partnership between nurses and patients, nurses and midwives are well situated to raise awareness and decrease stigma-surrounding depression in pregnancy, and to educate women about emerging research on vitamin D and depression.

Recommendations for Future Research

This study adds to the growing but still very limited body of knowledge about levels of depressive symptoms and vitamin D during pregnancy. Both of these components are critical to the health of women and their offspring as serious

consequences can occur with the independent presence of either (i.e., depression or low levels of vitamin D). However, the examination of the relationship between the two is a relatively new area of research with very few studies. To this author's knowledge this study is the first that includes multiple time points in the pregnancy period. The identification of vitamin D deficiency as a risk factor for depression in pregnancy is an important step towards early identification and prevention of depression among women in the perinatal period and beyond. Future studies should include longitudinal assessment across pregnancy and postpartum periods with multiple time points for data collection, investigation of mechanisms underlying the association (i.e., inflammation), and randomized trials examining the effect of vitamin D supplementation on depressive symptoms and other important clinical outcomes (e.g., hemorrhage) in pregnant and postpartum women.

APPENDIX A

Edinburgh Postnatal Depression Scale[1] (EPDS)

Study ID: ___ ___ ___ ___ Date:

Interviewer:

As you are pregnant or have recently had a baby, we would like to know how you are feeling. Please check the answer that comes closest to how you have felt **IN THE PAST 7 DAYS**, not just how you feel today.

Here is an example, already completed.

I have felt happy:
- □ Yes, all the time
- ☒ Yes, most of the time
- □ No, not very often
- □ No, not at all

This would mean: "I have felt happy most of the time" during the past week. Please complete the other questions in the same way.

In the past 7 days:

1. I have been able to laugh and see the funny side of things
   - □ As much as I always could
   - □ Not quite so much now
   - □ Definitely not so much now
   - □ Not at all

2. I have looked forward with enjoyment to things
   - □ As much as I ever did
   - □ Rather less than I used to
   - □ Definitely less than I used to
   - □ Hardly at all

*3. I have blamed myself unnecessarily when things Went wrong
   - □ Yes, most of the time
   - □ Yes, some of the time
   - □ Not very often
   - □ No, never

4. I have been anxious or worried for no good reason
   - □ No, not at all
   - □ Hardly ever
   - □ Yes, sometimes
   - □ Yes, very often

*5 I have felt scared or panicky for no very good reason
   - □ Yes, quite a lot
   - □ Yes, sometimes
   - □ No, not much
   - □ No, not at all

*6. Things have been getting on top of me
   - □ Yes, most of the time I haven't been able to cope at all
   - □ Yes, sometimes I haven't been coping as well as usual
   - □ No, most of the time I have coped quite well
   - □ No, I have been coping as well as ever

*7 I have been so unhappy that I have had difficulty sleeping
   - □ Yes, most of the time
   - □ Yes, sometimes
   - □ Not very often
   - □ No, not at all

*8 I have felt sad or miserable
   - □ Yes, most of the time
   - □ Yes, quite often
   - □ Not very often
   - □ No, not at all

*9 I have been so unhappy that I have been crying
   - □ Yes, most of the time
   - □ Yes, quite often
   - □ Only occasionally
   - □ No, never

*10 The thought of harming myself has occurred to me
   - □ Yes, quite often
   - □ Sometimes
   - Hardly ever
   - Never

APPENDIX B

IRB# Pro00027763

Interviewer:	__ __
Date:	__ __/__ __/__ __ __ __ (M M D D Y Y Y Y)
Study ID#:	__ __ __ __ __ __ __ __

Baseline Health History

Ethnicity: _____ Estimated Date of Delivery "Due Date": _____/_____/_____ (MM/DD/YY)

Employment:	Do you work outside of your home?	☐ No	☐ Yes **Please Specify:** How many hours do you work each week? _____
	How many minutes does it take you to travel to work? _____ minutes		
Please choose one of the following:	☐Single ☐ Partner ☐ Living with partner ☐ Married ☐ Divorced		
What is your average annual income in dollars? $_____			
How many years of education do you currently have?_____yrs			

GENERAL HEALTH: THE FOLLOWING QUESTIONS ARE REGARDING YOUR ***GENERAL HEALTH***:

Vitals	➢ How tall are you? _____ ft. _____ in. ➢ How much do you currently weigh? _____ lbs. ➢ How much did you weigh before you became pregnant? _____lbs.
Are you currently taking any medications, vitamins, herbs, or supplements?	➢ (Please List)_____ ➢ _____ ➢ _____
Exercise:	➢ Considering a 7-Day period (a week), during your leisure-time, **minutes** do you spend doing activity long enough to work up a sweat (heart beats rapidly)? _____mins
Television:	➢ How many ***hours*** a day do you watch television? _____hrs
Computer:	➢ How many ***hours*** a day do you spend on the computer? _____hrs
Sun Exposure:	➢ How many ***minutes*** of sun exposure every day? _____mins
Caffeine	➢ Do you drink caffeine? ☐ No ☐ Yes ➢ Specify: ☐Coffee ☐ Tea ☐ Cola ➢ # of Cups/Cans per day:_____ ➢ Do you skip breakfast? ☐Occasionally ☐ Frequently ☐ Never

Alcohol Consumption	➢	Do you currently drink alcohol? ☐ No ☐ Yes On average how many drinks do you have each week? _____
Tobacco Use:	➢	Do you currently smoke tobacco? ☐Daily ☐ Less than daily ☐ Not at all
Drug Use:	➢	Are you currently using recreational drugs? ☐ No ☐ Yes
Depression	➢	Do you have a family history of depression? ☐ No ☐ Yes

THE FOLLOWING QUESTIONS ARE REGARDING YOUR ***OVERALL HEALTH***

HAVE YOU HAD ANY OF THE FOLLOWING?					
Overweight	☐ **NO**	☐ **YES**	Chronic Bladder Infections	☐ **NO**	☐ **YES**
Low levels of vitamin D	☐ **NO**	☐ **YES**	Chronic Vaginal Infections:	☐ **NO**	☐ **YES**
High blood sugar (Diabetes)	☐ **NO**	☐ **YES**	HIV/AIDS	☐ **NO**	☐ **YES**
High Cholesterol	☐ **NO**	☐ **YES**	Syphilis	☐ **NO**	☐ **YES**
Blood Disorder	☐ **NO**	☐ **YES**	Herpes	☐ **NO**	☐ **YES**
Arthritis	☐ **NO**	☐ **YES**	Chlamydia	☐ **NO**	☐ **YES**
Osteoporosis/Osteopenia	☐ **NO**	☐ **YES**	HPV	☐ **NO**	☐ **YES**
Eczema	☐ **NO**	☐ **YES**	Gonorrhea	☐ **NO**	☐ **YES**
Psoriasis	☐ **NO**	☐ **YES**	ADD/ADHD	☐ **NO**	☐ **YES**
Allergies	☐ **NO**	☐ **YES**	Autism/Autism Spectrum	☐ **NO**	☐ **YES**
Hypercalcemia	☐ **NO**	☐ **YES**	Depression	☐ **NO**	☐ **YES**
Gout	☐ **NO**	☐ **YES**	Seasonal Affective Disorder (SADs)	☐ **NO**	☐ **YES**
Fibromyalgia	☐ **NO**	☐ **YES**	Anxiety	☐ **NO**	☐ **YES**
Epilepsy	☐ **NO**	☐ **YES**	Schizophrenia	☐ **NO**	☐ **YES**
Thyroid Disease	☐ **NO**	☐ **YES**	Bipolar Disorder	☐ **NO**	☐ **YES**
Metabolic Syndrome	☐ **NO**	☐ **YES**	Previous Preterm Labor (without preterm delivery)	☐ **NO**	☐ **YES**
Hepatitis B	☐ **NO**	☐ **YES**	Preeclampsia/Toxemia	☐ **NO**	☐ **YES**
Autoimmune Diseases:	☐ **NO**	☐ **YES**	High blood pressure during pregnancy	☐ **NO**	☐ **YES**
Liver Disease	☐ **NO**	☐ **YES**	Gestational Diabetes	☐ **NO**	☐ **YES**
Parathyroid Disease	☐ **NO**	☐ **YES**	Postpartum Depression	☐ **NO**	☐ **YES**
Cushing's Syndrome	☐ **NO**	☐ **YES**	Group Beta Strep (GBS)	☐ **NO**	☐ **YES**
Alzheimer's Disease	☐ **NO**	☐ **YES**	**Water breaking early**	☐ **NO**	☐ **YES**
Eating Disorder:	☐ **NO**	☐ **YES**	Rubella Infection	☐ **NO**	☐ **YES**
Bowel Disease	☐ **NO**	☐ **YES**	Vaginal Infection	☐ **NO**	☐ **YES**
Sarcoidosis	☐ **NO**	☐ **YES**	**Anemia**	☐ **NO**	☐ **YES**
Periodontal Disease	☐ **NO**	☐ **YES**	Short Cervix (Cerclage)	☐ **NO**	☐ **YES**
Heart Disease	☐ **NO**	☐ **YES**	Asthma	☐ **NO**	☐ **YES**
Cancer	☐ **NO**	☐ **YES**	High Blood Pressure	☐ **NO**	☐ **YES**
Parkinson's Disease	☐ **NO**	☐ **YES**			
Huntington's Disease	☐ **NO**	☐ **YES**			

Renal Disease	☐ **NO**	☐ **YES**
Chronic Headaches	☐ **NO**	☐ **YES**
Chronic Pain	☐ **NO**	☐ **YES**

CURRENT PREGNANCY AND HEALTH

Have you had the following ***DURING THIS PREGNANCY***?		
Anemia	☐ **NO**	☐ **YES**
Asthma	☐ **NO**	☐ **YES**
Short Cervix (Cerclage)	☐ **NO**	☐ **YES**
High Blood Pressure	☐ **NO**	☐ **YES**
Vaginal Infection	☐ **NO**	☐ **YES**
Bladder Infection	☐ **NO**	☐ **YES**
Severe Nausea/Vomiting ***Requiring Hospitalization*** (Hyperemesis Gravidarium)	☐ **NO**	☐ **YES**
Gestational Diabetes	☐ **NO**	☐ **YES**
Preeclampsia	☐ **NO**	☐ **YES**
Depression	☐ **NO**	☐ **YES**
Group Beta Strep	☐ **NO**	☐ **YES**

How many pregnancies have you had including miscarriages, abortions, stillbirths, AND including this pregnancy?
How many pregnancies resulted in live births?
How many pregnancies resulted in stillbirths (20 weeks ga)?
How many neonatal deaths occurred (<1 month of age)?
How many pregnancies resulted in your new baby being admitted to the NICU?
How many pregnancies delivered preterm? (<37 weeks ga)
How many pregnancies delivered at term?
How many pregnancies resulted in spontaneous miscarriage? (<20 weeks ga)
How many pregnancies resulted in induced abortion?
How many pregnancies resulted in multiple births?
Was the current pregnancy planned? ☐ **NO** ☐ **YES**
Are you planning to breastfeed? ☐ **NO** ☐ **YES**

APPENDIX C

Study ID : __ __ __ __ Date: ___ /___/_____

Depression and Vitamin D in Pregnancy Study Questionnaire

Visit 2

1. Current weight: _____ lbs
2. Please circle one of the choices below:
   Single Partner Living with Partner Married Divorced

3. Are you currently taking any medications and/or supplements (including vitamins)?

- Med name: _____ Dose:_____ Frequency: _____ x day
- Med name: _____ Dose:_____ Frequency: _____ x day
- Med name: _____ Dose:_____ Frequency: _____ x day
- Med name: _____ Dose:_____ Frequency: _____ x day
- Med name: _____ Dose:_____ Frequency: _____ x day
- Med name: _____ Dose:_____ Frequency: _____ x day
- Med name: _____ Dose:_____ Frequency: _____ x day
- Med name: _____ Dose:_____ Frequency: _____ x day

4. Do you have any pain currently or are you having any pain on an intermittent, ongoing basis?

□YES □ NO
(if "yes", continue to questions 5-12; if "no" stop)

5. How often do have the pain?
□ Constantly □ Daily □ Several times a week □ Once a week □ Less than once a week

6. During the past week has pain interfered with any of your daily activities? □YES □ NO

7. Rate the intensity of your pain overall:

□ No pain □ Mild □ Discomfort □ Distressing □ Horrible □ Excruciating

8. Does the pain affect your sleep? □YES □ NO

9. Does the pain have an impact on your mood? □YES □ NO

10. On a scale 1-10 with 10 being greatest intensity what is the overall intensity of you pain overall: *(please circle corresponding number below)*

1 2 3 4 5 6 7 8 9 10

11. Instructions: For each word which describes your pain, rate the intensity of that particular quality of pain for each location of pain.

	(0) None	(1) Mild	(2) Moderate	(3) Severe	Location of Pain Head (HA), Back, Leg, Abd., Pelvis, Incision, Breast, Perineum
Throbbing	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Shooting	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Stabbing	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Sharp	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Cramping	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Gnawing	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Hot-burning	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Aching	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Heavy	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Tender	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Splitting	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Tiring-exhausting	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Sickening	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Fearful	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____
Punishing-cruel	□	□	□	□	□ HA □ bk □ leg □ abd □ pel □ inc □ brst □ peri □ _____

APPENDIX D

IRB No: Pro00027763/ Ame00011714
Approval Date: 12/3/2012 **Expiration Date: 5**/31/2013

CEDARS-SINAI MEDICAL CENTER

CONSENT FORM FOR RESEARCH

RIGHTS AS A HUMAN RESEARCH PARTICIPANT

- You have the right to make a voluntary decision to participate in this research study.
- If you have questions, discuss them with the investigator before you agree to participate. You have the right to ask questions at any time and have them answered as soon as possible.
- You have the right to take time to review this research consent form carefully. You should discuss it with others, and if appropriate seek a second opinion, and make an informed decision.
- You have the right to be informed of significant new findings related to this research study which may affect your willingness to continue participating in this study.
- You have the right not to participate or to withdraw from this research study at any time without any penalty or loss of benefits to which you would be entitled outside of the study. Choosing so will not change the quality of care you receive at Cedars-Sinai Medical Center (CSMC).

Depression and Vitamin D in Pregnancy Study

1. WHO IS CONDUCTING THIS RESEARCH STUDY?

Principal Investigator:	Calvin Hobel, MD	310-423-3365
Co-Investigator:	Amy Lamb, PhD(c)	615-779-3001
Study Coordinator:	Susan Jackman, RN	310-423-4765
	Donnabeth Young, LVN	310-423-4788
After hours contact:	Amy Lamb	615-779-3001

2. WHAT IS THE PURPOSE OF THIS RESEARCH STUDY?

The purpose of the study is to understand more about mood and vitamin D levels in pregnancy. Previous research shows that there may be some relationship between vitamin D levels and mood alterations. The study will help us know more about how mood and vitamin D might work together. Lastly, the study will look at how pregnancy might influence changes in mood and vitamin D levels. There are no drugs involved with this study.

3. WHY AM I ASKED TO PARTICIPATE?

You are being asked to take part in this research study because you are a pregnant adult.

4. HOW MANY PEOPLE WILL PARTICIPATE?

About 135 people will take part in this study at CSMC.

5. HOW LONG WILL I BE IN THE STUDY?

We think you will be in the study for about 7 months, however depending on when you enroll and complete the study you may be in the study for as long as 20 months. Please see the section "What Study Procedures Are Involved?" for additional information.

6. WHAT STUDY PROCEDURES ARE INVOLVED?

This section provides a summary of procedures if you take part in this study. A flowchart of procedures is attached to the end of this consent form. The flowchart shows a timeline for research-related or standard of care procedures. Research-related procedures are those that are performed solely for the research. They would not be performed if you did not take part in the study. Standard of care procedures would occur even if you did not take part in the study.

You will be asked to participate in four study visits. Your first visit will be at the time you are enrolled into the study, the second when you are in your second or third trimester, the third when you give birth to your baby, and finally a fourth during your postpartum period (up to one year postpartum).

During the first and fourth visits some blood may be collected and you will be asked to complete two questionnaires. During the second visit you will be asked to complete two questionnaires and no blood will be collected. During the third visit, at the time you give birth, some of your blood may be collected and the baby's cord blood will be collected. Your baby will NOT have a blood draw as part of this study. At the end of the study we will review your medical record.

Blood collection:
Blood will be drawn to measure your vitamin D blood levels. Approximately 2 teaspoons of blood will be drawn 1 time every three months, for a total of 6 teaspoons. It may be possible to use the blood that your doctor is already drawing during your routine prenatal or postpartum care visits. If we are able to use the blood that is already being collected by your doctor then you may have fewer blood draws. As part of the study you will have up to 3 total blood draws. Cord blood will also be collected as part of this study. Extra cord blood will be collected during the time that routine cord blood collection takes place. The blood will be drawn from the umbilical cord after it has been separated from your baby. Less than 1 teaspoon of cord blood will be collected for research. Your baby will NOT receive a needle stick as part of this study.

Questionnaires:
You will be asked to complete two questionnaires at the first, second, and fourth study visits. We will ask you questions to evaluate how you are feeling emotionally. We will also ask you questions about yourself (e.g. age) and your health (e.g. height). We think it should take about 15 minutes to complete the questionnaires. Questionnaires will ask you to respond to questions about how you feel, if you have had thoughts of hurting yourself, or how much you weigh. If you feel uncomfortable or embarrassed answering any question, you may skip it. The questionnaire will be labeled with a unique study number that will link your identity so that only the research team can recognize you.

Medical Records:
Your medical history will be reviewed at the end of the study. It will be necessary to review your records in order to make sure the data collected about you as part of the study is correct.

7. WHAT ARE THE POSSIBLE RISKS OR DISCOMFORTS?

There might be some possible risks or discomforts associated with taking part in this study. You may experience feelings of embarrassment or a loss of confidentiality as a result of answering questions about how you feel and having your medical record reviewed. If you note having thoughts of self-harm when responding to study questionnaires you will be referred to the emergency room and your physician will be notified. At the completion of the study when the questionnaires are analyzed if you have severely elevated depressive symptoms then you and your physician will be notified.

Blood Draw
Blood drawing may cause some pain and has a small risk of bleeding, bruising, or infection at the puncture site. There is also a small risk of fainting.

Incidental Findings
It is possible that the study procedures could detect a possible medical problem that is unrelated to the purpose of this study that was previously unknown. If the research procedures uncover findings that may be important for you to know about, such as the possibility of a previously unknown medical condition, you will be informed by a member of the research team. Or, you may authorize the release and communication of the findings to your primary physician. These findings may require additional testing or treatment. The cost of any additional tests or related treatment will be your responsibility.

Because we do not yet know what different vitamin D levels mean for pregnant women and their babies, and this is not a standard test done in clinical care for pregnant women or their babies, we will not inform you or your primary physician of the findings regarding your vitamin D levels during the course of the study. However, at the completion of the study when vitamin D levels are analyzed we will inform you and your physician if you or your baby's levels are very low or high.

8. ARE THERE DIRECT BENEFITS IN TAKING PART IN THE STUDY?

You should not expect to benefit directly from taking part in this research study.

9. HOW CAN MY PARTICIPATION BENEFIT OTHERS?

While no benefit is ever guaranteed, we hope the information learned from this research study will benefit other pregnant women and babies in the future by helping us to learn more about how mood and vitamin D might influence each other in pregnancy. It may also help us know more about the role that vitamin D has in pregnancy. Finally, there is a small possibility that it may give us knowledge to help reduce the risk for developing depression in pregnancy and postpartum.

10. WHY WOULD MY PARTICIPATION BE STOPPED?

The investigator may decide to take you off this research study for any reason, even if you would like to continue. Some examples of why the investigator might take you off the study include the following: the investigator determines that it is in your best medical interest; your condition worsens; funding for the study is stopped; the whole study is stopped or modified at CSMC or at all sites for any reason; or you are unable to comply with the protocol.

You may also decide to withdraw from the research study at any time. However, the researchers may continue to analyze data previously collected from you before you withdrew from the research study.

11. ARE THERE ANY OTHER OPTIONS?

Your participation is voluntary and you can choose to not participate in this study. Your medical care will not be changed in any way as a result of this decision.

12. HOW WILL MY PRIVATE INFORMATION BE KEPT CONFIDENTIAL?

CSMC values and respects your private information. Federal and state laws protect your privacy. Every reasonable effort will be made to keep your records confidential, such as storing your private information in a secure location where only authorized individuals will have access to it. When possible, investigators will assign a unique code to your research

information so that people who see the coded data will not be able to identify you.

If information from this study is published, presented at scientific meetings, or used for teaching, your name and other personal information will not be used.

People inside and outside of CSMC may need to see your information for this study. Information collected about you during the course of this research may be subject to inspection by accrediting agencies, government and regulatory groups (e.g. Food and Drug Administration (FDA), Office for Human Research Protections (OHRP), etc.), safety monitors, and companies that sponsor the study. These agencies are responsible for the oversight of this research. You will be asked to sign a separate "Authorization Form" that outlines who your information may be shared with for the purposes of this research and under what circumstances.

13. WHAT ARE THE COSTS OF TAKING PART IN THIS STUDY?

You and your insurance company will not be charged for your participation in this research study.

14. WILL I BE PAID?

You will receive a $10 gift card at the first visit and a $10 gift card at the end of the study. The total amount you will receive if you complete the whole study is $20 dollars in gift cards. If you do not complete the entire research study, you will only be paid for those visits and procedures you do complete. For any visits you make to the study office you will be reimbursed for parking. You may be required to complete a W-9 Form in order to receive payment. The W-9 Form will be maintained by our accounting department at CSMC. If payment exceeds $600 in a calendar year, a 1099 Form will be filed with the IRS in accordance with federal tax law.

If you are a CSMC employee, you should provide your employee identification number to the research team so that your payment can be appropriately processed through Payroll. For your own protection and to

comply with tax laws, your payment for participation will be reported to the IRS together with other compensation you receive from CSMC.

15. WHAT IF I BECOME ILL OR INJURED BECAUSE OF TAKING PART IN THIS STUDY?

You will not be in danger of any illness or injury from this research study. However, should you believe that you are ill or have been injured as a result of your participation, please contact the Co-Investigator listed on the first page of this consent form, under "Who Is Conducting This Research Study?"

16. WHAT IF I HAVE QUESTIONS OR PROBLEMS?

If you have questions or concerns about this research, please contact the Co-investigator or Principal Investigator listed under "Who is conducting this research study?" on the first page of this consent form.

If you have questions regarding your rights, concerns, or complaints about taking part in this study, please contact:

CSMC Institutional Review Board (IRB)
Phone: (310) 423-3783
Email: ResearchConcerns@cshs.org

The CSMC IRB has been established to review, approve, and monitor all human research at CSMC with the purpose of minimizing risks and protecting the rights and welfare of research participants.

Permission to be contacted in the future

If you agree, you will be contacted in the future to receive information on other research studies investigating vitamin D, depression, or other pregnancy complications.

☐ YES ☐ NO I agree to be contacted in the future to receive information on other research studies investigating vitamin D, depression, or other pregnancy complications.

17. CONSENT PROVISIONS

If you sign this form below, it means that:

(1) You have carefully read and understood the information presented in this informed consent form;
(2) The information concerning the research study and its involved procedures has been fully explained to you and your questions have been answered to your satisfaction;
(3) You have received all of the information you desire regarding your participation in the research study;
(4) You have considered the potential risks, anticipated benefits and alternatives (and their relative risks and benefits) of participation;
(5) You are voluntarily agreeing to participate in this research study;
(6) For research where you will receive treatment or diagnostic intervention, you agree that your right to access copies of health information created during your participation in your research will be suspended while the research study is in progress; your right to access this information will be restored upon completion of the entire study;
(7) You understand that by consenting to participate in the research, you are not giving up any of your legal rights (other than the postponement of your access to certain health information as described in this informed consent form); and
(8) You have been provided with a copy of the "Experimental Subject's Bill of Rights", if applicable to this research study, and have been provided with an opportunity to ask questions regarding the Bill of Rights.

If you have any additional questions during the course of your involvement in the research, please contact the investigator(s) and/or the IRB Office at any time.

We will give you a copy of this signed and dated consent form.

SIGNATURE BY THE SUBJECT:

Name of Subject (Print)	Signature of Subject	Date of Signature

SIGNATURE BY THE INVESTIGATOR:

I attest that all the elements of informed consent described in this form have been discussed fully in non-technical terms with the subject. I further attest that all questions asked by the subject were answered to the best of my knowledge.

Signature of the Investigator Who Obtained Consent	Date of Signature

SIGNATURE BY THE WITNESS/TRANSLATOR

(Signature of a witness is only required when a non-English speaking subject is consented with the assistance of a translator and an IRB-approved 'short form.' The signature of the witness below attests that the translator has presented the elements of consent to the subject, orally and in his/her preferred language, and that a summary of the oral presentation, in a language the subject can understand, has been given to the participant.)

Signature of Witness	*Date of Signature*

EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

In accordance with California Health and Safety Code 24172, any person who is required to consent to participate as a subject in a research study involving a medical experiment or who is requested to consent on behalf of another has the right to:

1. Be informed of the nature and purpose of the experiment.
2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.
3. Be given a description of any attendant discomforts and risks to the subject reasonably to be expected from the experiment.
4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.
5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.
6. Be informed of the avenues of medical treatment, if any, available to the subject after the experiment if complications should arise.
7. Be given an opportunity to ask any questions concerning the experiment or the procedure involved.
8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.
9. Be given a copy of any signed and dated written consent form used in relation to the experiment.
10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion, or undue influence on the subject's decision.

_____ _____

Signature of Experimental Subject **Date**

Distribution instruction for investigators:

The signed (i) Consent form, (ii) Authorization for Use and Disclosure of Identifiable Health Information and (iii) "Experimental Subject's Bill of Rights" (the latter required if the research study involves medical interventions)* should be distributed to:

1) Medical Chart
2) Subject
3) Principal Investigator's research records (original)

FLOWCHART OF PROCEDURES

Procedures	Visit #1	Visit #2	Visit #3	Visit #4
Research Related Procedures: Procedures, drugs, devices, evaluations or other services done only because of your participation in this research.				
Review of your medical records by the study team				X
Blood draw to measure Vitamin D Levels	X*		X*	X*
Completion of 2 questionnaires: Depression Scale and Demographic and Clinical Obstetric Information Questionnaire	X	X		X
Collection of Cord Blood			X*	

* **As necessary. If we are able to use the blood that is already being collected by your doctor, then you may have fewer blood draws. As part of the study you will have up to 3 total blood draws.**

REFERENCES

2004–2006 National Health Interview Survey (NHIS), National Center for Health Statistics, Centers for Disease Control and Prevention. Retrieved from http://www.cdc.gov/nchs/nhis.htm.

Adams, J. S., & Hewison, M. (2010). Update in Vitamin D. Journal of Clinical Endocrinology & Metabolism, 95(2), 471-478. doi: 10.1210/jc.2009-1773

Adams, J.S. & Hewison, M. (2006). Hypercalcemia caused by granulomaforming disorders. In: Favus MJ, ed. Primer on the metabolic bone diseases and disorders of mineral metabolism. 6th ed. Washington, DC: American Society for Bone and Mineral Research; 200–202

Agarwal, K. S., Mughal, M. Z., Upadhyay, P., Berry, J. L., Mawer, E. B., & Puliyel, J. M. (2002). The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. Archives of Disease in Childhood, 87(2), 111-113. doi: 10.1136/adc.87.2.111

Altemus, M., Neeb, C. C., Davis, A., Occhiogrosso, M., Nguyen, T., & Bleiberg, K. L. (2012). Phenotypic differences between pregnancy-onset and postpartum-onset major depressive disorder. Journal of Clinical Psychiatry, 73(12), e1485-1491. doi: 10.4088/JCP.12m07693

American College of Obstetricians and Gynecologists (2010). Screening for depression during and after pregnancy. Committee Opinion No. 453. Obstetrics & Gynecology,115,394–395.

American College of Obstetricians and Gynecologists (2011). Committee Opinion No. 495. Vitamin D: screening and supplementation during pregnancy. Obstetrics and Gynecology, 118, 197–198.

Aretaeus of Cappadocia. (1856). The Extant works of Aretaeus, the Cappadocian. Edited and Translated by Francis Adams, Publisher Sydenham Society.

Arora, C. (2011). Role of vitamin D in modulating gestational diabetes. Biopolymers and Cell, 27(2), 85-92.

Arora, P., Garcia-Bailo, B., Dastani, Z., Brenner, D., Villegas, A., Malek, S., . . . Badawi, A. (2011). Genetic polymorphisms of innate immunity-related inflammatory pathways and their association with factors related to type 2 diabetes. BMC Medical Genetics, 12(1), 95.

Baker, A. M., Haeri, S., Camargo, C. A., Jr., Espinola, J. A., & Stuebe, A. M. (2010). A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia. Journal of Clinical Endocrinology & Metabolism, 95(11), 5105-5109. doi: 10.1210/jc.2010-0996

Baker, A. M., Haeri, S., Camargo, C. A., Jr., Stuebe, A. M., & Boggess, K. A. (2012). First-trimester maternal vitamin D status and risk for gestational diabetes (GDM) a nested case-control study. Diabetes Metab Res Rev, 28(2), 164-168. doi: 10.1002/dmrr.1282

Bansil, P., Kuklina, E., Meikle, S., Posner, S., Kourtis, A., Ellington, S., & Jamieson, D. (2010). Maternal and fetal outcomes among women with depression. Journal of Women's Health, 19 (2). doi: 10.1089/jwh.2009.1387

Banti, S., Mauri, M., Oppo, A., Borri, C., Rambelli, C., Ramacciotti, D., . . . Cassano, G. B. (2011). From the third month of pregnancy to 1 year postpartum. Prevalence, incidence, recurrence, and new onset of depression. Results from the perinatal depression-research & screening unit study. Comprehensive Psychiatry, 52(4), 343-351. doi: 10.1016/j.comppsych.2010.08.003

Bashutski, J. D., Eber, R. M., Kinney, J. S., Benavides, E., Maitra, S., Braun, T. M., . . . McCauley, L. K. (2011). The impact of vitamin D status on periodontal surgery outcomes. J Dent Res, 90(8), 1007-1012. doi: 10.1177/0022034511407771

Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., & Erbaugh, J. (1961). An inventory for measuring depression. Archives of General Psychiatry, 4, 561-571.

Belderbos, M. E., Houben, M. L., Wilbrink, B., Lentjes, E., Bloemen, E. M., Kimpen, J. L., . . . Bont, L. (2011). Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. Pediatrics, 127(6), e1513-1520. doi: 10.1542/peds.2010-3054

Bener, A., Al-Hamaq, A. O., & Saleh, N. M. (2013). Association between vitamin D insufficiency and adverse pregnancy outcome: global comparisons. International Journal of Women's Health, 5, 523-531. doi: 10.2147/IJWH.S51403

Bergwitz, C. & H. Juppner (2010). Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. Annual Review of Medicine 61: 91-104.

Bodnar, L. M., Simhan, H. N., Powers, R. W., Frank, M. P., Cooperstein, E., & Roberts, J. M. (2007). High Prevalence of Vitamin D Insufficiency in Black and White Pregnant Women Residing in the Northern United States and Their Neonates. The Journal of Nutrition, 137(2), 447-452.

Borella, E., Nesher, G., Israeli, E., & Shoenfeld, Y. (2014). Vitamin D: a new anti-infective agent? Annals of New York Academy of Science. doi: 10.1111/nyas.12321

Brandenbarg, J., Vrijkotte, T. G., Goedhart, G., & van Eijsden, M. (2012). Maternal early-pregnancy vitamin D status is associated with maternal depressive symptoms in the Amsterdam Born Children and Their Development cohort. Psychosom Med, 74(7), 751-757. doi: 10.1097/PSY.0b013e3182639fdb

Brannon, P. M., & Picciano, M. F. (2011). Vitamin D in Pregnancy and Lactation in Humans. Annual Review of Nutrition, 31(1), 89-115. doi:10.1146/annurev.nutr.012809.104807

Brock, K. E., Huang, W.-Y., Fraser, D. R., Ke, L., Tseng, M., Mason, R. S., . . . Graubard, B. I. (2011). Diabetes prevalence is associated with serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in US middle-aged Caucasian men and women: a cross-sectional analysis within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. British Journal of Nutrition, 106(03), 339-344. doi: doi:10.1017/S0007114511001590

Brondum-Jacobsen, P., Nordestgaard, B. G., Schnohr, P., & Benn, M. (2013). 25-hydroxyvitamin D and symptomatic ischemic stroke: an original study and meta-analysis. Annals of Neurology, 73(1), 38-47. doi: 10.1002/ana.23738

Bunevicius, R., Kusminskas, L., Bunevicius, A., Nadisauskiene, R. J., Jureniene, K., & Pop, V. J. (2009). Psychosocial risk factors for depression during pregnancy. Acta Obstetrics and Gynecology of Scandinavia, 88(5), 599-605. doi: 10.1080/00016340902846049

Camadoo, L., Tibbott, R., & Isaza, F. (2007). Maternal vitamin D deficiency associated with neonatal hypocalcaemic convulsions. Nutrition Journal, 6, 23. doi: 10.1186/1475-2891-6-23

Cassidy-Bushrow, A. E., Peters, R. M., Johnson, D. A., Li, J., & Rao, D. S. (2012). Vitamin D nutritional status and antenatal depressive symptoms in African American women. Journal of Women's Health (Larchmt), 21(11), 1189-1195. doi: 10.1089/jwh.2012.3528

Centers for Disease Control and Prevention (2007). Preconception and interconception health status of women who recently gave birth to a live-born infant—Pregnancy Risk Assessment Monitoring System (PRAMS), United States, 26 Reporting Areas, 2004. Morbidity and Mortality Weekly Report, 56(SS10), 1–35.

Chaudron, L. H., Szilagyi, P. G., Campbell, A. T., Mounts, K. O., & McInerny, T. K. (2007). Legal and Ethical Considerations: Risks and Benefits of Postpartum Depression Screening at Well-Child Visits. Pediatrics, 119(1), 123-128. doi: 10.1542/peds.2006-2122

Chazotte, C., Comerford Freda, M., Elovitz, M., Youchah, J. (1995). *Journal of Women's Health, 4* (4), 375-380.

Cipolla, M. J. (2013). The adaptation of the cerebral circulation to pregnancy: mechanisms and consequences. Journal of Cerebral Blood Flow Metabolism, 33(4), 465-478. doi: 10.1038/jcbfm.2012.210

Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet 1982;74–76.

Cook, G. (1999). Early use of `open-air' treatment for `pulmonary phthisis' at the Dreadnought Hospital, Greenwich, 1900-1905. Postgraduate Medical Journal, 75 (884), 326–327.

Costa, D. D., Rippen, N., Dritsa, M., & Ring, A. (2003). Self-reported leisure-time physical activity during pregnancy and relationship to psychological well-being. Journal of Psychosomatic Obstetrics & Gynecology, 24(2), 111-119. doi: doi:10.3109/01674820309042808

Cox J.L., Chapman, G., Murray, D., & Jones, P. (1996). Validation of the Edinburgh postnatal depression scale (EPDS) in non-postnatal women. Journal of Affective Disorders, 39(3). doi:10.1016/0165-0327(96)00008-0

Cox, J.L., Holden, J.M., & Sagovsky, R. (1987). Detection of postnatal depression: Development of the 10-item Edinburgh Postnatal Depression Scale. British Journal of Psychiatry, 150, 782-786.

Crowther, C., Hiller, J., Moss, J., McPhee, A., Jefferies, W., & Robinson, J. (2005). Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. The New England Journal of Medicine, 352 (24), 2477-2486.

Cummings, S. R., Browner, W. S., Bauer, D., Stone, K., Ensrud, K., Jamal, S., & Ettinger, B. (1998). Endogenous Hormones and the Risk of Hip and Vertebral Fractures among Older Women. New England Journal of Medicine, 339(11), 733-738. doi: doi:10.1056/NEJM199809103391104

Dabelea, J., Snell-Bergeon, C., Hartsfield, K., Bischoff, K., Hamman, R. & McDuffie, R. (2005). Increasing prevalence of gestational diabetes mellitus (GDM) over time

and by birth cohort. Kaiser Permanente of Colorado GDM screening program. Diabetes Care, 28 (3), 579-584. doi: 10.2337/diacare.28.3.579

Das, M., Tomar, N., Sreenivas, V., Gupta, N., & Goswami, R. (2014). Effect of vitamin D supplementation on cathelicidin, IFN-gamma, IL-4 and Th1/Th2 transcription factors in young healthy females. European Journal of Clinical Nutrition, 68(3), 338-343. doi: 10.1038/ejcn.2013.268

Delatte, R., Cao, H., Meltzer-Brody, S., & Menard, M. K. (2009). Universal screening for postpartum depression: an inquiry into provider attitudes and practice. American Journal of Obstetrics and Gynecology, 200(5), e63-e64. doi: 10.1016/j.ajog.2008.12.022

Department of Health and Human Services (2009). Code of federal regulations. Title 45. Public welfare. Part 46. Protection of human subjects. Retrieved from http://www.hhs.gov/ohrp/policy/ohrpregulations.pdf on June 8, 2014.

Dietz, P.M., Williams, S.B., Callaghan, W.M., Bachman, D.J., & Hornbrook, M.C. (2007). Clinically identified maternal depression before, during, and after pregnancies ending in live births. American Journal of Psychiatry, 164, 1515-1520.

Dijkstra, S.H., van Beek, A., Janssen, J.W., de Vleeschouwer, L.H., Huysman, W.A. & van den Akker, EL. (2007). High prevalence of vitamin D deficiency in newborn infants of high-risk mothers. Archives of Disorders in Childhood, 92, 750–753

Dumville, J., Miles, J., Porthouse, J., Cockayne, S., Saxon, L., & King, C. (2006). Can vitamin D supplementation prevent winter-time blues? A randomized trial among older women. The Journal of Nutrition, Health & Aging, 10(2), 151-153.

Eskandari, F., Martinez, P. E., Torvik, S., Phillips, T. M., Sternberg, E. M., Mistry, S., . . . for the Premenopausal, O. W., Alendronate, Depression Study Group,. (2007). Low Bone Mass in Premenopausal Women With Depression. Arch Intern Med, 167(21), 2329-2336. doi: 10.1001/archinte.167.21.2329

Eyles, D. W., Feron, F., Cui, X., Kesby, J. P., Harms, L. H., Ko, P., . . . Burne, T. H. J. (2009). Developmental vitamin D deficiency causes abnormal brain development. Psychoneuroendocrinology, 34 (Supplement 1), S247-S257. doi: 10.1016/j.psyneuen.2009.04.015

Eyles, D. W., Smith, S., Kinobe, R., Hewison, M., & McGrath, J. J. (2005). Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. Journal of Chemical Neuroanatomy, 29(1), 21-30. doi: 10.1016/j.jchemneu.2004.08.006

Farrant, H. J., Krishnaveni, G. V., Hill, J. C., Boucher, B. J., Fisher, D. J., Noonan, K., . . . Fall, C. H. (2009). Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size. European Journal of Clinical Nutrition, 63(5), 646-652. doi: 10.1038/ejcn.2008.14

Flicker, L., Mead, K., MacInnis, R. J., Nowson, C., Scherer, S., Stein, M. S., . . . Wark, J. D. (2003). Serum Vitamin D and Falls in Older Women in Residential Care in Australia. Journal of the American Geriatrics Society, 51(11), 1533-1538. doi: 10.1046/j.1532-5415.2003.51510.x

Galitzer, H., Ben-Dov, I., Lavi-Moshayoff, V., Naveh-Many, T. & Silver, J. (2008). Fibroblast growth factor 23 acts on the parathyroid to decrease parathyroid hormone secretion. Current Opinion in Nephrology and Hypertension 17(4), 363-7.

Gaynes ,B.N., Gavin, N., Meltzer-Brody, S., Lohr, K.N., Swinson, T., Gartlehner, G.,… Miller, W.C. (2005). Perinatal Depression: Prevalence, Screening Accuracy, and Screening Outcomes. Evidence Report/Technology Assessment No. 119. (AHRQ Publication No. 05- E006-2). Rockville, MD: Agency for Healthcare Research and Quality.

Gerdhem, P., Ringsberg, K., Obrant, K., & Akesson, K. (2005). Association between 25-hydroxy vitamin D levels, physical activity, muscle strength and fractures in the prospective population-based OPRA Study of Elderly Women. Osteoporosis International, 16(11), 1425-1431. doi: 10.1007/s00198-005-1860-1

Gjerdingen, D. K., & Yawn, B. P. (2007). Postpartum depression screening: Importance, barriers, methods and recommendations for practice. Journal of the American Board of Family Medicine, 20, 280–288.

Glossmann, H. H. (2010). Origin of 7-dehydrocholesterol (provitamin D) in the skin. J Invest Dermatol, 130(8), 2139-2141. doi: 10.1038/jid.2010.118

Goldblatt, H. & Soames, K.N. (1923). A study of rats on a normal diet irradiated daily by the mercury vapor quartz lamp or kept in darkness. Biochemistry Journal, 17, 294-297.

Grant, W. B. (2011). An estimate of the global reduction in mortality rates through doubling vitamin D levels. European Journal of Clinical Nutrition, 65(9), 1016-1026. doi: 10.1038/ejcn.2011.68

Grant, W. B., Garland, C. F., & Holick, M. F. (2005). Comparisons of estimated economic burdens due to insufficient solar ultraviolet irradiance and vitamin D

and excess solar UV irradiance for the United States. Photochemistry and Photobiology, 81(6), 1276-1286. doi: 10.1562/2005-01-24-RA-424

Grant, W. B., Schwalfenberg, G. K., Genuis, S. J., & Whiting, S. J. (2010). An estimate of the economic burden and premature deaths due to vitamin D deficiency in Canadaian Molecular and Nutrition Food Research, 54(8), 1172-1181. doi: 10.1002/mnfr.200900420

Grey A, Lucas J, Horne A, Gamble G, Davidson JS, Reid IR 2005 Vitamin D repletion in patients with primary hyper- parathyroidism and coexistent vitamin D insufficiency. Journal of Clinical Endocrinology & Metabolism, 90, 2122–2126

Grigoriadis, S., Vonderporten, E. H., Mamisashvili, L., Tomlinson, G., Dennis, C. L., Koren, G., . . . Ross, L. E. (2014). Prenatal exposure to antidepressants and persistent pulmonary hypertension of the newborn: systematic review and meta-analysis. BMJ, 348, f6932. doi: 10.1136/bmj.f6932

Grote, N. K., & Bledsoe, S. E. Predicting Postpartum Depressive Symptoms in New Mothers: The Role of Optimism and Stress Frequency during Pregnancy. Health and Social Work, 32(2), 107-118.

Guillot, X., Semerano, L., Saidenberg-Kermanac'h, N., Falgarone, G., & Boissier, M.-C. (2010). Vitamin D and inflammation. Joint Bone Spine, 77(6), 552-557. doi: 10.1016/j.jbspin.2010.09.018

Hamilton, M. (1960). A rating scale for depression. Journal of Neurology, Neurosurgery, and Psychiatry, 23, 56-62.

Harms, L. R., Burne, T. H. J., Eyles, D. W., & McGrath, J. J. (2011). Vitamin D and the brain. Best Practice & Research Clinical Endocrinology & Metabolism, 25(4), 657-669. doi: 10.1016/j.beem.2011.05.009

Harris, S., & Dawson-Hughes, B. (1993). Seasonal mood changes in 250 normal women. Psychiatry Research, 49(1), 77-87. doi: 10.1016/0165-1781(93)90031-b

Hatton, D. C., Harrison-Hohner, J., Matarazzo, J., Edwards, P., Lewy, A., & Davis, L. (2007). Missed antenatal depression among high risk women: a secondary analysis. Archives of Women's Mental Health, 10(3), 121-123. doi: 10.1007/s00737-007-0180-1

Haugen, M., Brantsaeter, A. L., Trogstad, L., Alexander, J., Roth, C., Magnus, P., & Meltzer, H. M. (2009). Vitamin D supplementation and reduced risk of preeclampsia in nulliparous women. Epidemiology, 20(5), 720-726. doi: 10.1097/EDE.0b013e3181a70f08

Hayes, C.E., Nashold, F.E., Spach, K.M., & Pedersen, L.B. (2003). The immunological functions of the vitamin D endocrine system. Cellular and Molecular Biology, 49(2).

Hess ,A.F. (1929). Rickets Including Osteomalacia and Tetany. Philadelphia, PA: Lea & Febiger; 1929.

Hewitt, C., Gilbody, S., Brealey, S., Paulden, M., Palmer, S., Mann, R., . . . & , Richards, D. (2009). Methods to identify postnatal depression in primary care: an integrated evidence synthesis and value of information analysis. International Journal of Technology Assessment in Healthcare, 13(36), 1-145, 147-230.

Hidaka, B. H. (2012). Depression as a disease of modernity: explanations for increasing prevalence. Journal of Affective Disorders, 140(3), 205-214. doi: 10.1016/j.jad.2011.12.036

Hoang, M. T., DeFina, L. F., Willis, B. L., Leonard, D. S., Weiner, M. F., & Brown, E. S. (2011). Association Between Low Serum 25-Hydroxyvitamin D and Depression in a Large Sample of Healthy Adults: The Cooper Center Longitudinal Study. Mayo Clinic Proceedings, 86(11), 1050-1055. doi: 10.4065/mcp.2011.0208

Hoeck, A. D., & Pall, M. L. (2011). Will vitamin D supplementation ameliorate diseases characterized by chronic inflammation and fatigue? Medical Hypotheses, 76(2), 208-213. doi: 10.1016/j.mehy.2010.09.032

Holick, M. & Chen, T. (2008). Vitamin D deficiency: a worldwide problem with health consequences. American Journal of Clinical Nutrition 87 (4), 1080S-1086S.

Holick, M. F. (2007). Vitamin D Deficiency. New England Journal of Medicine, 357(3), 266-281. doi: doi:10.1056/NEJMra070553

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., . . . Endocrine, Society. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. Journal of Clinical Endocrinology & Metabolism, 96(7), 1911-1930. doi: 10.1210/jc.2011-0385

Hollis, B. W., Johnson, D., Hulsey, T. C., Ebeling, M., & Wagner, C. L. (2011). Vitamin D supplementation during pregnancy: Double-blind, randomized clinical trial of safety and effectiveness. Journal of Bone and Mineral Research, 26(10), 2341-2357. doi: 10.1002/jbmr.463

Holmes, V., Barnes, M., Alexander, H., McFaul, P., & Wallace, J. (2009). Vitamin D deficiency and insufficiency in pregnant women: A longitudinal study. British Journal of Nutrition, 102, 876–881.

Hoogendijk, W., Lips, P., Dik, M., Deeg, D., Beekman, A., & Penninx, B. (2008). Depression is associated with decreased 25-hydroxyvitamin D and increased parathyroid hormone levels in older adults. Archives of General Psychiatry, 65(5), 508-512.

Hossain, N., Kanani, F. H., Ramzan, S., Kausar, R., Ayaz, S., Khanani, R., & Pal, L. (2014). Obstetric and neonatal outcomes of maternal vitamin D supplementation: Results of an open label randomized controlled trial of antenatal vitamin D supplementation in Pakistani women. Journal of Clinical Endocrinology & Metabolism, jc20133491. doi: 10.1210/jc.2013-3491

Hosseinpanah, F., pour, S., Heibatollahi, M., Moghbel, N., Asefzade, S., & Azizi, F. (2010). The effects of air pollution on vitamin D status in healthy women: A cross sectional study. BMC Public Health, 10(1), 519. doi: 10.1186/1471-2458-10-519

Jones, G., Strugnell, S. A., & DeLuca, H. F. (1998). Current Understanding of the Molecular Actions of Vitamin D. Physiological Reviews, 78(4), 1193-1231.

Jorde, R., Sneve, M., Figenschau, Y., Svartberg, J., & Waterloo, K. (2008). Effects of vitamin D supplementation on symptoms of depression in overweight and obese subjects: randomized double blind trial. Journal of Internal Medicine, 264(6), 599-609. doi: 10.1111/j.1365-2796.2008.02008.x

Jurutka, P. W., G. K. Whitfield, J. C. Hsieh, P. D. Thompson, C. A. Haussler and M. R. Haussler. 2001. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. Reviews in Endocrinology and Metabloic Disorders 2(2): 203-16.

Kalra, P., Das, V., Agarwal, A., Kumar, M., Ramesh, V., Bhatia, E., . . . Bhatia, V. (2012). Effect of vitamin D supplementation during pregnancy on neonatal mineral homeostasis and anthropometry of the newborn and infant. British Journal of Nutrition, 108(6), 1052-1058. doi: 10.1017/S0007114511006246

Kendall, P. C., Hollon, S. D., Beck, A. T., Hammen, C. L., & Ingram, R. E. (1987). Issues and recommendations regarding the use of the Beck depression inventory. Cognitive Therapy and Research, 11, 289–299. doi:10.1007/BF01186280

Kesby, J. P., Eyles, D. W., Burne, T. H. J., & McGrath, J. J. The effects of vitamin D on brain development and adult brain function. Molecular and Cellular Endocrinology, In Press, Corrected Proof. doi: 10.1016/j.mce.2011.05.014

Kibler, C. & Watson, S. (1935). The place of the sun in treating tuberculosis. Chest Journal*, 1* (1), 18-20. doi:10.1378/chest.1.1.18

Klieger-Grossmann, C., Weitzner, B., Panchaud, A., Pistelli, A., Einarson, T., Koren, G., & Einarson, A. (2011). Pregnancy Outcomes Following Use of Escitalopram: A Prospective Comparative Cohort Study. The Journal of Clinical Pharmacology. doi: 10.1177/0091270011405524

Lancaster, C. A., Gold, K. J., Flynn, H. A., Yoo, H., Marcus, S. M., & Davis, M. M. (2010). Risk factors for depressive symptoms during pregnancy: a systematic review. American Journal of Obstetrics & Gynecology, 202(1), 5-14. doi: 10.1016/j.ajog.2009.09.007

Lansdowne, A., & Provost, S. (1997). Vitamin D3 enhances mood in healthy subjects during winter. Psychopharmacology, 135, 319-323.

Lappe, J. M., Travers-Gustafson, D., Davies, K. M., Recker, R. R., & Heaney, R. P. (2007). Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. The American Journal of Clinical Nutrition, 85(6), 1586-1591.

LaRocco-Cockburn, A., Melville, J., Bell, M., & Katon, W. (2003). Depression screening attitudes and practices among obstetrician gynecologists. Obstetrics & Gynecology, 101, 892-898.

Lau, S., Gunton, J., Athayde, N., Byth, K. & Cheung, N. (2011). Serum 25-hydroxyvitamin D and glycated haemoglobin levels in women with gestational diabetes mellitus. Medical Journal of Australia, 194(7), 334-337.

Lawrence, R. E., Rasinski, K. A., Yoon, J. D., Meador, K. G., Koenig, H. G., & Curlin, F. A. (2012). Primary care physicians' and psychiatrists' approaches to treating mild depression. Acta Psychiatry Scandinavia, 126(5), 385-392. doi: 10.1111/j.1600-0447.2012.01887.x

Lazarus, R. (1993). From psychological stress to the emotions: A history of changing outlooks. Annual Review of Psychology, 44, 1-22. doi: 10.1146/annurev.ps.44.020193.000245

Lazarus, R.S., & Folkman, S. (1984). *Stress, appraisal, and coping*. New York: Springer Publishing Company

Lee, D., Yip, A., Chiu, H., Leung, T., & Chung, T. (2001). Screening for postnatal depression: Are specific instruments mandatory [Electronic version]? Journal of Affective Disorders, 63, 233-238.

Leonard, B., & Maes, M. (2012). Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. Neuroscience and Biobehavior Review, 36(2), 764-785. doi: 10.1016/j.neubiorev.2011.12.005

Lissner, D., Mason, R.S., & Posen, S. (1981). Stability of vitamin D metabolites in human blood, serum, and plasma. Clinical Chemistry, 27, 773– 7744.

Locyer, V., Porcellato, L. & Gee, I. (2011). Vitamin D deficiency and supplementation: are we failing to prevent the preventable? Community Practice, 84(3), 23-26.

Logsdon, M. C., & Myers, J. A. (2010). Comparative performance of two depression screening instruments in adolescent mothers. Journal of Women's Health, 19(6), 1123-1128. doi: 10.1089/jwh.2009.1511

Logsdon, M. C., Usui, W., & Nering, M. (2009). Validation of edinburgh postnatal depression scale for adolescent mothers. Journal of Women's Mental Health, 12, 433-440.

Lumeng, C. N., & Saltiel, A. R. (2011). Inflammatory links between obesity and metabolic disease. The Journal of Clinical Investigation, 121(6), 2111-2117.

Maes, M., Ruckoanich, P., Chang, Y. S., Mahanonda, N., & Berk, M. (2011). Multiple aberrations in shared inflammatory and oxidative & nitrosative stress (IO&NS) pathways explain the co-association of depression and cardiovascular disorder (CVD), and the increased risk for CVD and due mortality in depressed patients. Program of Neuropsychopharmacology and Biological Psychiatry, 35(3), 769-783. doi: 10.1016/j.pnpbp.2010.06.008

Maiya, S., Sullivan, I., Allgrove, J., Yates, R., Malone, M., Brain, C., . . . Burch, M. (2008). Hypocalcaemia and vitamin D deficiency: an important, but preventable, cause of life-threatening infant heart failure. Heart, 94(5), 581-584. doi: 10.1136/hrt.2007.119792

Makgoba, M., Nelson, S.M., Savvidou, M., Messow, C.M., Nicolaides, K. & Sattar, N. (2011). First-trimester circulating 25-hydroxyvitamin D levels and development of gestational diabetes mellitus. Diabetes Care, 34, 1091–1093.

Martin, J., Hamilton, B.E., Sutton, P.D., Ventura, S.J., Mathews, T.J….Kirmeyer, S. (2010). Births: final data for 2007. National Vital Statistics Report, 58, 1–85.

Marwaha, R.K., Tandon, N., Chopra, S., Agarwal, N., Garg, M.K., Sharma, B., Kanwar, R.S., Bhadra, K., Singh, S., Mani, K., & Puri, S. (2011). Vitamin D status in

pregnant Indian women across trimesters and different seasons and its correlation with neonatal serum 25-hydroxyvitamin D levels. British Journal of Nutrition, 106 (9), 1383-1389. doi: 10.1017/S000711451100170X

Matsuoka, L.Y., Ide, L., Wortsman, J., MacLaughlin, J.A.& Holick, M.F. (1987). Sunscreens suppress cutaneous vitamin D3 synthesis. The Journal of Endocrinology and Metabolisim, 64, 1165–1168.

Matthey, S. (2008). Using the Edinburgh Postnatal Depression Scale to screen for anxiety disorders. Depression and Anxiety 25, 31–926.

Matthey, S., Fisher, J., & Rowe, H. (2013). Using the Edinburgh postnatal depression scale to screen for anxiety disorders: conceptual and methodological considerations. Journal of Affective Disorders, 146(2), 224-230. doi: 10.1016/j.jad.2012.09.009

McCollum, E.V., Simmonds, N., Becker, J.E. & Shipley, P.G. (1922). Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. Journal of Biological Chemistry, 53, 293-312.

McGrath, J. J., Burne, T. H., Feron, F., Mackay-Sim, A., & Eyles, D. W. (2010). Developmental vitamin D deficiency and risk of schizophrenia: a 10-year update. Schizophr Bull, 36(6), 1073-1078. doi: 10.1093/schbul/sbq101

McPherson, M., Smith-Lovin, L. & Brashears, M. (2006). Social isolation in America: Changes in core discussion networks over two decades. American Sociological Review, 71, 353.

Mellanby, E. & Cantag, M.D. (1919). Experimental investigation on rickets. Lancet 196, 407-412.

Merewood, A., Mehta, S. D., Chen, T. C., Bauchner, H., & Holick, M. F. (2009). Association between vitamin D deficiency and primary cesarean section. Journal of Clinical Endocrinology & Metabolism, 94(3), 940-945. doi: 10.1210/jc.2008-1217

Milgrom, J., Gemmill, A. W., Bilszta, J. L., Hayes, B., Barnett, B., Brooks, J., . . . Buist, A. (2008). Antenatal risk factors for postnatal depression: A large prospective study. Journal of Affective Disorders, 108(1-2), 147-157. doi: 10.1016/j.jad.2007.10.014

Mora, P. A., Bennett, I. M., Elo, I. T., Mathew, L., Coyne, J. C., & Culhane, J. F. (2009). Distinct trajectories of perinatal depressive symptomatology: evidence from

growth mixture modeling. American Journal of Epidemiology, 169(1), 24-32. doi: 10.1093/aje/kwn283

Murphy, P. K., Mueller, M., Hulsey, T. C., Ebeling, M. D., & Wagner, C. L. (2010). An Exploratory Study of Postpartum Depression and Vitamin D. Journal of the American Psychiatric Nurses Association, 16(3), 170-177. doi: 10.1177/1078390310370476

Murray, L., & Carothers, A.D. (1990). The validation of the Edinburgh Post-natal Depression Scale on a community sample. British Journal of Psychiatry, 157, 288-290.

Nakhai-Pour, H. R., Broy, P., & Bérard, A. (2010). Use of antidepressants during pregnancy and the risk of spontaneous abortion. Canadian Medical Association Journal, 182(10), 1031-1037. doi: 10.1503/cmaj.091208

National Institute of Mental Health (2008). Depression. Bethesda, MD: National Institute of Mental Health Science Writing, Press & Dissemination Branch.

National Research Council. (2003). Overview of Food Fortification in the United States and Canada. Dietary Reference Intakes: Guiding Principles for Nutrition Labeling and Fortification. Washington, DC: The National Academies Press.

National Research Council. (2011). Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: The National Academies Press.

National Sleep Foundation (2009). Sleep in America Poll: Summary of Findings. National Sleep Foundation. Retrieved from http://sleepfoundation.org/media-center/press-release/sleep-america-poll-summary-findings on June 8, 2014.

Nielsen, N. O., Strom, M., Boyd, H. A., Andersen, E. W., Wohlfahrt, J., Lundqvist, M., . . . Melbye, M. (2013). Vitamin D Status during Pregnancy and the Risk of Subsequent Postpartum Depression: A Case-Control Study. PLoS One, 8(11), e80686. doi: 10.1371/journal.pone.0080686

Nowak, D, Stezar, L., Mehlberg, L., Zajechowski, J., Luzzi, V. & Feldkamp, C. (2011). Pre-analytical Variables Affecting the DiaSorin Liaison® Vitamin D Assay. Clinical Chemistry, 57 (10), A29.

O'Hara, M.W., & Swain, A.M. (1996). Rates and risk of postpartum depression: A meta-analysis. International Review of Psychiatry, 8, 37–54.

Ota, K., Dambaeva, S., Han, A. R., Beaman, K., Gilman-Sachs, A., & Kwak-Kim, J. (2014). Vitamin D deficiency may be a risk factor for recurrent pregnancy losses

by increasing cellular immunity and autoimmunity. Human Reproduction, 29(2), 208-219. doi: 10.1093/humrep/det424

Pilowsky, D.J. (2008). Children of depressed mother 1 year after the initiation of maternal treatment: Findings from the STAR*D-Child study. American Journal of Psychiatry, 165.

Powe, C. E., Seely, E. W., Rana, S., Bhan, I., Ecker, J., Karumanchi, S. A., & Thadhani, R. (2010). First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. Hypertension, 56(4), 758-763. doi: 10.1161/HYPERTENSIONAHA.110.158238

Pratt, L.A., & Brody, D.J. (2008). National health and nutrition examination survey (data brief, no.7): Depression in the United States household population (2005–2006). Retrieved July 30, 2009 from http://www.cdc.gov/nchs/data/databriefs/db07.htm

Prie, D., & Friedlander, G. (2010). Reciprocal control of 1,25-dihydroxyvitamin D and FGF23 formation involving the FGF23/Klotho system. Clinical Journal of American Society of Nephrology, 5(9), 1717-1722. doi: 10.2215/CJN.02680310

Quick, J. A., Murphy, E. W., & United States. (1982). The fortification of foods: A review. Washington, DC: U.S. Dept. of Agriculture, Food Safety and Inspection Service, Science Program.

Radloff, L.S. (1977). The CES-D scale: A self-report depression scale for research in the general population. Applied Psychological Measurement, 1, 385-401.

Raedler, T. J. (2011). Inflammatory mechanisms in major depressive disorder. Current Opinion in Psychiatry, 24(6), 519-525 510.1097/YCO.1090b1013e32834b32839db32836.

Rai, D., Lee, B. K., Dalman, C., Golding, J., Lewis, G., & Magnusson, C. (2013). Parental depression, maternal antidepressant use during pregnancy, and risk of autism spectrum disorders: population based case-control study. BMJ, 346, f2059. doi: 10.1136/bmj.f2059

Ramos-Lopez, E., Kahles, H., Weber, S., Kukic, A., Penna-Martinez, M., Badenhoop, K., & Louwen, F. (2008). Gestational diabetes mellitus and vitamin D deficiency: genetic contribution of CYP27B1 and CYP2R1 polymorphisms. Diabetes Obesity and Metabolism, 10(8), 683-685. doi: 10.1111/j.1463-1326.2008.00879.x

Rao, K. (2009). Recent research in stress, coping and women's health. *Current Opinion Psychiatry, 22*(2), 188-193. doi: 10.1097/YCO.0b013e328320794a

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A. & Pollmacher, T. (2001). Cytokine-associated emotional and cognitive disturbances in humans. Archives of General Psychiatry, 58, 445–452.

Richards, J., Papaioannou, A., Adachi, J.D., et al. (2007). Effect of Selective Serotonin Reuptake Inhibitors on the Risk of Fracture. Archives of Internal Medicine, 167(2), 188-194. doi:10.1001/archinte.167.2.188.

Roberts, S. L., Bushnell, J. A., Collings, S. C., & Purdie, G. L. (2006). Psychological health of men with partners who have post-partum depression. Australian and New Zealand Journal of Psychiatry, 40(8), 704-711. doi: 10.1080/j.1440-1614.2006.01871.x

Robinson, C. J., Alanis, M. C., Wagner, C. L., Hollis, B. W., & Johnson, D. D. (2010). Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. American Journal of Obstetrics & Gynecology, 203(4), 366 e361-366. doi: 10.1016/j.ajog.2010.06.036

Robinson, C. J., Wagner, C. L., Hollis, B. W., Baatz, J. E., & Johnson, D. D. (2013). Association of maternal vitamin D and placenta growth factor with the diagnosis of early onset severe preeclampsia. Am J Perinatol, 30(3), 167-172. doi: 10.1055/s-0032-1322514

Robinson, M., Whitehouse, A. J., Newnham, J. P., Gorman, S., Jacoby, P., Holt, B. J., . . . Kusel, M. M. (2014). Low maternal serum vitamin D during pregnancy and the risk for postpartum depression symptoms. Archives of Womens Mental Health. doi: 10.1007/s00737-014-0422-y

Ruhrah J. (1925). Pediatrics of the Past. New York, NY: Paul B. Hoeber, Inc

Sambrook, P. N., Chen, J. S., March, L. M., Cameron, I. D., Cumming, R. G., Lord, S. R., . . . Seibel, M. J. (2004). Serum Parathyroid Hormone Predicts Time to Fall Independent of Vitamin D Status in a Frail Elderly Population. Journal of Clinical Endocrinology & Metabolism, 89(4), 1572-1576. doi: 10.1210/jc.2003-031782

Schneider, B., Weber, B., Frensch, A., Stein, J., & Fritze, J. (2000). Vitamin D in schizophrenia, major depression and alcoholism. Journal of Neural Transmission, 107(7), 839-842. doi: 10.1007/s007020070063

Shand, A. W., Nassar, N., Von Dadelszen, P., Innis, S. M., & Green, T. J. (2010). Maternal vitamin D status in pregnancy and adverse pregnancy outcomes in a group at high risk for pre-eclampsia. BJOG, 117(13), 1593-1598. doi: 10.1111/j.1471-0528.2010.02742.x

Shelton, R. C., & Miller, A. H. (2010). Eating ourselves to death (and despair): The contribution of adiposity and inflammation to depression. Progress in Neurobiology, 91(4), 275-299. doi: 10.1016/j.pneurobio.2010.04.004

Shivakumar, G., Brandon, A. R., Snell, P. G., Santiago-Muñoz, P., Johnson, N. L., Trivedi, M. H., & Freeman, M. P. (2011). Antenatal depression: a rationale for studying exercise. Depression and Anxiety, 28(3), 234-242. doi: 10.1002/da.20777

Sit, D.K., & Wisner, D.L. (2009). Identification of postpartum depression. Clinical Obsetrics and Gynecology, 52(3), 456-468.

Soheilykhah, S., Mojibian, M., Rashidi, M., Rahimi-Saghand, S., & Jafari, F. (2010). Maternal vitamin D status in gestational diabetes mellitus. Nutrition and Clinical Practice, 25(5), 524-527. doi: 10.1177/0884533610379851

Sohr-Preston, S. L., & Scaramella, L. V. (2006). Implications of Timing of Maternal Depressive Symptoms for Early Cognitive and Language Development. Clinical Child and Family Psychology Review, 9(1), 65-83. doi: 10.1007/s10567-006-0004-2

Sonnett, T. E., Levien, T. L., Gates, B. J., Robinson, J. D., & Campbell, R. K. (2010). Diabetes Mellitus, Inflammation, Obesity: Proposed Treatment Pathways for Current and Future Therapies. The Annals of Pharmacotherapy, 44(4), 701-711. doi: 10.1345/aph.1M640

Soranus & Temkin, O. (1956). Soranus' gynecology. Baltimore: Johns Hopkins University Press

Stein, A., Malmberg, L. E., Sylva, K., Barnes, J., & Leach, P. (2008). The influence of maternal depression, caregiving, and socioeconomic status in the post-natal year on children's language development. Child: Care, Health and Development, 34(5), 603-612. doi: 10.1111/j.1365-2214.2008.00837.x

Stewart, W. F., Ricci, J. A., Chee, E., Hahn, S. R., & Morganstein, D. (2003). Cost of Lost Productive Work Time Among US Workers With Depression. JAMA, 289(23), 3135-3144. doi: 10.1001/jama.289.23.3135

Stowe, Z. N., Hostetter, A. L., & Newport, D. J. (2005). The onset of postpartum depression: Implications for clinical screening in obstetrical and primary care. American Journal of Obstetrics and Gynecology, 192(2), 522-526. doi: 10.1016/j.ajog.2004.07.054

Straub, H., Adams, M., Kim, J. J., & Silver, R. K. (2012). Antenatal depressive symptoms increase the likelihood of preterm birth. American Journal of Obstetrics and Gynecology, 207(4), 329 e321-324. doi: 10.1016/j.ajog.2012.06.033

Su, K.P., Chiu, T., Huang, C., Ho, M., Lee, C., Wu, P., et al. (2007). Different cutoff points for different trimesters? The use of the Edinburgh Postnatal Depression Scale and Beck Depression Inventory to screen for depression in Taiwanese women. General Hospital Psychiatry, 29. doi:10.1016/j.genhosppsych.2007.05.005

t Jong, G. W., Einarson, T., Koren, G., & Einarson, A. (2012). Antidepressant use in pregnancy and persistent pulmonary hypertension of the newborn (PPHN): a systematic review. Reproductive Toxicology, 34(3), 293-297. doi: 10.1016/j.reprotox.2012.04.015

Thacher, T.D., Fischer, P.R., Strand, M.A. & Pettifor, J.M. (2006). Nutritional rickets around the world: causes and future directions. Ann Trop Paediatrics, 26, 1–16.

Thielen K, Nygaard E, Andersen I, Rugulies R, Heinesen E, et al. (2009) Misclassification and the use of register-based indicators for depression. Acta Psychiatrica Scandinavia, 119, 312–319.

U. S. Census Bureau (2013). State and country quickfacts, California. Median household income 2008-2012. American Community Survey, 5-Year Estimates. Retrieved from http://quickfacts.census.gov/qfd/states/06000.html on June 3, 2014.

U.S. Department of Health and Human Services. Human Resources and Service Administration (2007). Depression during and after pregnancy: A resource for women, their families, and friends. Retrieved from ftp://ftp.hrsa.gov/mchb/pregnancyandbeyond/depression.pdf

U.S. Food and Drug Administration (2011). FDA Drug Safety Communication: Selective serotonin reuptake inhibitor (SSRI) antidepressant use during pregnancy and reports of a rare heart and lung condition in newborn babies. Retrieved from http://www.fda.gov/Drugs/DrugSafety/ucm283375.htm#hcp

van Etten, E., & Mathieu, C. (2005). Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. Journal of Steroid Biochemistry and Molecular Biology, 97(1-2), 93-101. doi: 10.1016/j.jsbmb.2005.06.002

Villegas, L., McKay, K., Dennis, C. L., & Ross, L. E. (2011). Postpartum depression among rural women from developed and developing countries: a systematic

review. Journal of Rural Health, 27(3), 278-288. doi: 10.1111/j.1748-0361.2010.00339.x

Wactawski-Wende, J., Kotchen, J. M., Anderson, G. L., Assaf, A. R., Brunner, R. L., O'Sullivan, M. J., . . . Manson, J. E. (2006). Calcium plus Vitamin D Supplementation and the Risk of Colorectal Cancer. New England Journal of Medicine, 354(7), 684-696. doi: doi:10.1056/NEJMoa055222

Wang, X., Chen, C., Wang, L., Chen, D., Guang, W. & French, J. (2003). Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. Fertility and Sterility, 79(3), 577-584. doi: 10.1016/s0015-0282(02)04694-0

Western Regional Climate Center (2010). Historical climate information: California mean monthly and annual average number of clear days- Los Angeles City. Retrieved from http://www.wrcc.dri.edu/htmlfiles/westcomp.clr.html#CALIFORNIA

Wilcox, H., Field, T., Prodromidis, M., & Scafidi, F. (1998). Correlations between the BDI and CES-D in a sample of adolescent mothers. Adolescence, 33(131), 565-574.

Wilkins, C., Sheline, Y., Roe, C., Birge, S., & Morris, J. (2006). Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. American Journal of Geriatric Psychiatry, 14(12), 1032-1040. DOI:10.1097/01.JGP.0000240986.74642.7c

Wisner, K.L., Sit, D.K., Hanusa, B.H., et al. (2009). Major depression and antidepressant treatment impact on pregnancy and neonatal outcomes. American Journal of Psychiatry, 166(5), 557–566.

Wootton, A. (2005). Improving the measurement of 25-hydroxyvitamin D. The Clinical Biochemist Reviews, 26(1), 33-36.

Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF 2000 Decreased bioavailability of vitamin D in obesity. American Journal of Clinical Nutrition 72:690–693

Yonkers, K. A., Wisner, K., Stewart, D., Oberlander, T., Dell, D., Stotland, N., Ramin, S., Chaudron, L., & Lockwood, C. (2009). The management of depression during pregnancy: a report from the American Psychiatric Association and the American College of Obstetricians and Gynecologists. General Hospital Psychiatry, 31 (5), 403-413. doi:10.1016/j.genhosppsych.2009.04.003

Yonkers, K.A., Smith, M.V., Gotman, N., & Belanger, K. (2009). Typical somatic symptoms of pregnancy and their impact on a diagnosis of major depressive disorder. General Hospital Psychiatry 31. doi:10.1016/jgenhosppsych.2009.03.005

Zerwekh, J. E. (2008). Blood biomarkers of vitamin D status. The American Journal of Clinical Nutrition, 87(4), 1087S-1091S.

Zhang, C., Qiu, C., Hu, F. B., David, R. M., van Dam, R. M., Bralley, A., & Williams, M. A. (2008). Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. PLoS One, 3(11), e3753. doi: 10.1371/journal.pone.0003753

Zhou, C., Assem, M., Tay, J.C., Watkins, P.B., Blumberg, B., Schuetz, E.G. & Thummel, K.E. (2006). Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. Journal Clinical Investigation, 116, 1703–1712.