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The Relationship Between A Series Of Vitamin D Levels And A Blood Glucose Level In Pregnant Women In The Upper Midwest

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THE RELATIONSHIP BETWEEN A SERIES OF VITAMIN D LEVELS AND A BLOOD GLUCOSE LEVEL IN PREGNANT WOMEN IN THE UPPER MIDWEST

by

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

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PERMISSION

Title	The Relationship Between a Series of Vitamin D Levels and a Blood Glucose Level in Pregnant Women in the Upper Midwest
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Jeanine L. Melstad Senti
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who inspired me with her lifelong love of learning.

ABSTRACT

Hyperglycemia during pregnancy is associated with adverse outcomes for both mother and offspring. In order to facilitate glucose delivery to the fetus, insulin resistance increases as gestation progresses which results in hyperglycemia if the mother's body is unable to compensate by producing adequate insulin in response to these changes in glucose metabolism. Evidence suggests that calcidiol (vitamin D) influences insulin sensitivity and glucose metabolism in the non-pregnant population yet there are inconsistent findings on the effects of calcidiol on maternal glucose levels during pregnancy. A secondary analysis was completed on a de-identified data set. The original prospective study included a convenience sample of 52 nulliparous women from a community in the upper Midwestern United States. Serum vitamin D levels were obtained through laboratory measures at three predetermined points during pregnancy. Medical record abstraction provided blood glucose levels in mid-pregnancy and height and weight to calculate body mass index (BMI) at the beginning of pregnancy. Eighty-one percent of the participants had hypovitaminosis-D at some point during pregnancy and 67% had hypovitaminosis-D at all three points during pregnancy. Multiple regression analysis was completed to examine the relationship between calcidiol levels and blood glucose levels. A *t*-test was performed to look for a difference between two groups, those with adequate calcidiol levels and those with inadequate serum calcidiol levels. There was no evidence of a statistically significant relationship between calcidiol

levels at any of the three points and the blood glucose levels when controlling for the known confounding variables of BMI and age (R square .084, .071, and .075 respectively). The high percentage of hypovitaminosis-D was believed to result in inadequate variance in this sample to show a difference, if one does exist. There was a statistically significant correlation among the three calcidiol levels ($p < .05$). There was a statistically significant inverse relationship between BMI and calcidiol levels in early pregnancy ($p < .05$) and mid-pregnancy ($p < .05$). These findings suggest that many women have hypovitaminosis-D in pregnancy, these levels are not improving during pregnancy, and that women with a higher BMI may need additional vitamin D during pregnancy.

CHAPTER I

INTRODUCTION

Hyperglycemia during pregnancy is associated with adverse outcomes for both mothers and offspring (Pridjian & Benjamin, 2010) creating the need for interventions which can reduce the harm from this condition. Research evidence suggests that vitamin D influences glucose metabolism in the non-pregnant population (Forouhi, Luan, Cooper, Boucher & Wareham, 2008; Hirani, 2011) and possibly in the pregnant population (Wei, Qi, Luo & Fraser, 2013). More specifically, hypovitaminosis-D has been associated with impaired glucose metabolism in conditions such as in type 2 diabetes (Alfonso, Liao, Busta & Poretsky, 2009; Mathieu, Gysemans, Giulietti & Bouillon, 2005; Pittas et al., 2006; Tohidi et al., 2013). There is a need to determine whether vitamin D similarly influences glucose metabolism in pregnancy (Alzaim & Wood, 2013; McLeod et al., 2011).

This chapter begins with a brief overview of the adaptations of glucose metabolism in pregnancy and maternal hyperglycemia in pregnancy. It will be followed by information on vitamin D metabolism, hypovitaminosis-D, and vitamin D in pregnant women. Last, information specific to this study will be presented including the purpose statement, hypothesis, problem statement, research questions, theoretical framework, significance, and assumptions.

Adaptations of Glucose Metabolism in Pregnancy

Significant alterations occur in maternal physiology during pregnancy (Torgerson & Curran, 2006). The placental hormones reprogram the maternal physiology to meet the needs of the fetus and this includes a progressive increase in insulin resistance (Barbour et al., 2007). The insulin resistance subsequently triggers the β-cells of the pancreas to increase insulin production in order to maintain glucose homeostasis (Evensen, 2012; Jelsma et al., 2013). In some cases, the pancreatic β-cells are not able to adequately increase production which results in impaired glucose metabolism and hyperglycemia (Gabbe et al., 2012, p. 892). Since the fetus obtains glucose from the mother through the placenta, the adverse outcomes of maternal hyperglycemia during pregnancy involve both the woman and the baby (Evensen, 2012; Lehnen, Zechner & Haaf, 2013).

During pregnancy, insulin resistance progressively develops in the woman to ensure the fetus is supplied with adequate glucose (Evenson, 2012). As the pregnancy progresses, there is a gradual increase in post-prandial blood glucose levels (Gabbe et al., 2012, p. 888) and the pancreatic β-cells need to respond by producing more insulin to prevent hyperglycemia (McLeod et al., 2012). However, for some women, the β-cells of the pancreas do not adequately increase the production of insulin to compensate (Jelsma et al., 2013) which results in impaired glucose tolerance (Senti, Thiele & Anderson, 2012). These changes can be sufficiently diabetogenic for some of these women to result in gestational diabetes (Senti et al., 2012).

Maternal Hyperglycemia in Pregnancy

Normally, pregnant women develop some insulin resistance during the second and the third trimesters (McLeod et al., 2012) which can lead to hyperglycemia. Even mildly increased maternal blood glucose levels can result in higher glucose delivery that can affect the fetus (Fetita et al., 2006). The resulting hyperglycemia can have serious health consequences for the mother and her children (Flack, Ross, Ho, & McElduff, 2010; Santamari, Cignini, Trapanese, & Bonalumi, 2011). Women who develop hyperglycemia during pregnancy are at increased risk for early, induced labor, difficult labor, and operative delivery (vacuum, forceps, or cesarean). Infants of mothers with hyperglycemia are at increased risk for macrosomia, birth injury such as shoulder dystocia or fractured clavicle, and medical complications after birth including hypoglycemia, polycythemia, hypocalcemia, respiratory distress syndrome, and hyperbilirubinemia (Santamari, Cignin, Trapanese, & Bonalumi, 2011). These conditions may require more medical interventions including costly care in a neonatal intensive care unit (Riskin-Mashiah, Younes, Damti & Auslender, 2009) and separation of infants from their mothers in the critical first hours of life. Long-term effects exist beyond the newborn period and may contribute to the increasing rates of obesity and diabetes (Ryan, 2013).

The most commonly known condition which causes hyperglycemia in pregnancy is gestational diabetes mellitus which affects 2-13% of all pregnancies, depending upon the population group studied (Poel et al., 2012). Gestational diabetes mellitus is one of the most common complications in pregnancy (Macones, 2012; Poel et al., 2012) and the prevalence is rising worldwide (Lehnen, Zechner & Haaf, 2013). In addition, gestational

diabetes markedly increases the risk of a woman developing type 2 diabetes in later life (Makgoba et al., 2011).

Gestational diabetes mellitus has been extensively researched in the professional literature for many years. Less has been written about blood glucose levels in the upper limit of normal but not over the threshold for a diagnosis of GDM. Many healthy women fit into this category during pregnancy. Since there is a paucity of scientific literature on hyperglycemia during pregnancy for healthy women, some of the research on GDM will be considered as proxy to hyperglycemia in this study to gain insight into the effects of hyperglycemia in pregnancy.

One major study to fill the knowledge gap on the link between mild, maternal hyperglycemia and pregnancy outcomes was the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study which involved 15 centers in 9 countries and over 25,000 pregnant women (Coustan, Lowe, Metzger, & Dyer, 2010; Leary, Pettitt & Javanovic, 2010). The HAPO study revealed that maternal hyperglycemia at levels less than required for a diagnosis of GDM do pose a risk for adverse outcomes for the offspring (Coustan et al., 2010; The HAPO Study Cooperative Research Group, 2008). The HAPO study was powered to detect the primary outcomes of neonatal hypoglycemia, hyperinsulinemia and macrosomia in the infant, and primary cesarean birth in the mothers (Coustan et al., 2010). The secondary outcomes of the HAPO study included birth at <37 weeks gestation, birth injury (such as shoulder dystocia), hyperbilirubinemia, and need for neonatal intensive care (Coustan et al., 2010; HAPO Study Cooperative Research Group, 2002). These findings demonstrate the harm which can result from maternal hyperglycemia during pregnancy supporting the need for prevention strategies.

Brief Overview of Vitamin D Metabolism

Vitamin D comes from two sources which are identified as vitamin D2 (ergocalciferol) or vitamin D3 (cholecaliciferol). Vitamin D is unique in that it is considered both a vitamin and a pro-hormone with a vitamin D receptor found in nearly every cell in the human body (Holick, 2005; Lips, 2006; McCullough, 2007; Pittas & Dawson-Hughes, 2010). Vitamin D is synthesized from direct sunlight on exposed skin or obtained from supplements, fortified foods, or one of the few foods which naturally contain vitamin D (Thorne-Lyman & Fawzi, 2012; Specker, 2004). Synthesis of vitamin D in the skin can be affected by latitude, season of the year, cloud cover, pollution, age, body mass index (BMI), the amount of melanin in the skin, ethnicity, genetics, renal function and liver function (Collins-Fulea, Klima & Wegienka, 2012; Holmes, Barnes, Alexander, McFaul, & Wallace, 2009; Prentice, 2008; Wicherts et al., 2011).

Hypovitaminosis-D

Vitamin D deficiency is a problem worldwide (Ginde, Sullivan, Mansbach & Camargo, 2010; Hanley & Davison, 2005). Hypovitaminosis-D affects nearly 41.6% of the adults in the United States according to the National Health and Nutrition Examination Survey (NHANES) report (Forrest & Stuhledreher, 2011). Hypovitaminosis-D is often a problem for women, especially those who live in Northern latitudes, have darkly pigmented skin, or those whose clothing prohibits direct sunlight on exposed skin (Holick & Chen, 2008). Maghbooli, Hossein-nezhad, Karimi, Shafaei and Larijani (2008) reported that hypovitaminosis-D was found in 70.6% of the pregnant women in their research in Iran. In Oakland, California, 54.4% of pregnant women were found to have hypovitaminosis-D (Dror, King, Durand, & Allen, 2011). In the United

States, 69% of pregnant women were found to have hypovitaminosis-D from a secondary analysis of the National Health and Nutrition Examination Survey (NHANES) from 2001 – 2006. The NHANES study collected data on women of childbearing age which ranged from 13-44 years so this data set included younger pregnant women (Ginde, Sullivan, Mansbach & Camargo, 2010). Ginde et al. (2010) reported that younger pregnant women were more frequently nonwhite (57%), lower socioeconomic status, and not using any vitamin D supplements and all of these factors increased their risk of hypovitaminosis-D in pregnancy. They further reported the novel finding of similar rates of hypovitaminosis-D in Hispanic and non-Hispanic blacks which they concluded was likely to decreased synthesis of vitamin D in the skin due to the levels of melanin. Wagner et al. (2015) found the mean serum calcidiol level in early pregnancy was 58 nmol/L (5 – 185 nmol/L) in a sample of over 400 women.

Calcidiol in Pregnant Women

Calcidiol levels are unlikely to change in pregnancy unless there is an increase in synthesis (cholecalciferol from the sun; D3) or intake from plants and absorbed through the gut (ergocalciferol, D2), or intake from supplements (either D2 or D3) (Gabbe et al., 2012, p. 59). The active metabolite of vitamin D, calcitriol, progressively increases during pregnancy (Barrett & McElduff, 2010; Hollis, Johnson, Hulsey, Ebeling, & Wagner, 2011; Holmes et al., 2009). Holmes et al. (2009) suggest that there is an increased demand for vitamin D during pregnancy to support these higher levels of calcitriol. Hollis et al. (2011) found that women with higher intake of oral vitamin D supplements had higher calcitriol levels as pregnancy progressed when compared with women who had lower intake of vitamin D supplements.

Calcidiol and Glucose Metabolism

Insufficient vitamin D may be associated with insulin resistance with the resulting hyperglycemia. Serum calcidiol is the usual measure for vitamin D and low levels may be a risk factor for impaired glucose metabolism leading to hyperglycemia (Forouhi et al., 2008; Wang et al., 2012). Several researcher have reported an association between calcidiol levels and glucose metabolism (Chiu, Chu, Go & Saad, 2004; Forouhi et al., 2012; Moreira & Hamadeh, 2010; Holick, 2005; Pittas & Dawson-Hughes, 2010; Palomer, Gonzalez-Clemente, Blanco-Vaca & Mauricio, 2008; Song & Manson, 2010). McLeod et al. (2011) found an association between serum calcidiol levels and pancreatic β-cell function linking vitamin D with an effect on insulin production and glucose metabolism. Kayaniyil et al. (2011) reported higher calcidiol levels independently predict improved pancreatic β-cell function. These studies illustrate the emerging evidence on the positive influence of vitamin D on glucose metabolism.

Serum Calcidiol and Glucose Metabolism in Pregnancy

Vitamin D may have the potential to positively influence glucose metabolism during pregnancy. A limited number of researchers have studied the relationship between serum calcidiol levels in the first trimester and impaired glucose metabolism in pregnancy (Baker et al., 2012; Fernandez-Alfonso et al., 2012; Makgoba et al., 2011; Tomedi et al., 2013). Others have examined the relationship between serum vitamin D levels in the second trimester and the blood glucose levels in mid-pregnancy (Burris et al., 2012; Clifton–Bligh et al., 2008; Maghbooli et al., 2008; Parlea et al., 2012; Soheilykhah et al., 2010; Zhang et al., 2008). Two studies were found which looked at

serum vitamin D level in the third trimester and glucose metabolism in pregnancy (Farrant et al., 2009; Lau et al., 2011).

More specifically, some researchers have found an inverse relationship between serum calcidiol levels and maternal blood glucose levels in pregnancy. Burris et al. (2012) examined second trimester calcidiol and blood sugar levels when using a one-hour glucose screening test. Their results indicated that hypovitaminosis-D may increase the risk of impaired glucose metabolism. A few observational studies indicated a significant difference in serum calcidiol in women with impaired glucose metabolism versus normal glucose tolerance (Clifton-Bligh, 2008; Maghbooli, 2007; Soheilykhah, 2010; Zhang, 2008) and reported a correlation between maternal vitamin D status and impaired glucose metabolism. Hypovitaminosis-D has been associated with impaired glucose metabolism in both pregnant and non-pregnant humans (Alzaim & Wood, 2013). Pregnant women with hypovitaminosis-D may be at increased risk for hyperglycemia (Shin, Choi, Longtine & Nelson, 2010) but it still is not clear if this is a causal relationship (Poel et al., 2012). Therefore, a need exists to further explore the association between vitamin D and glucose metabolism.

Purpose Statement

The purpose of this study was to determine the relationship between calcidiol levels collected in early, mid, and late pregnancy and blood glucose levels in mid pregnancy in a sample of women in an upper Midwestern region of the United States. A quantitative, correlational design was used in a secondary analysis to ask new questions of an existing data set. According to Pridjian and Benjamin, (2010), hyperglycemia during pregnancy is associated with adverse outcomes for both the mother and baby. It is

known that calcidiol influences glucose metabolism in the non-pregnant population (Alfonso et al., 2009; Mathieu et al., 2005; Pittas et al., 2006; Tohidi et al., 2013) and this study investigated the influence of calcidiol on glucose metabolism in the pregnant population. The significance of this relationship is that it may inform an understanding of the effect of calcidiol on glucose metabolism in pregnancy. By identifying the correlation between calcidiol and glucose levels, there is the potential to mitigate hyperglycemia in pregnancy and improve the health of infants and mothers.

Research Hypothesis

The working hypothesis for this study was that there is an inverse relationship between calcidiol during pregnancy and mid pregnancy blood glucose levels.

Research Questions

1. Is there a correlation between the calcidiol level at 10-14 weeks gestation and the blood glucose (BG) level at 24-28 weeks gestation when controlling for the known confounding variables of age and pre-pregnancy body mass index (BMI)?

2. Is there a correlation between calcidiol level at 22-26 weeks gestation and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI?

3. Is there a correlation between the calcidiol level at 32-36 weeks gestation and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI?

Problem Statement

Although studies are emerging which support an association between vitamin D levels and impaired glucose metabolism (Alzaim & Wood, 2013), there are inconsistent

findings on the effects of vitamin D and maternal glucose levels during pregnancy. It is known that many women have hypovitaminosis-D during pregnancy (Aslam, Masood, Sattar, & Qudsia, 2012) and that some women develop impaired glucose metabolism in pregnancy (Davenport, Campbell, & Mottola, 2010; Evenson, 2012; Hunt & Schuller, 2007; Veeraswamy, Vijayam, Gupta, & Kapur, 2012). A need exists to learn more about the effect of vitamin D on maternal glucose levels in pregnant women (Joergensen, Lamont & Torloni, 2014; Senti, Thiele, & Anderson, 2013). Addressing this gap in knowledge may contribute to strategies which could improve glucose metabolism in pregnancy.

Theoretical Framework

The Neuman Systems Model (NSM) provides a unifying focus for a wide range of health concerns by using open systems-based approach (Neuman, 2011). The Neuman model is concerned with the patient's reaction and response to environmental stressors, both external and internal (Gigliotti, 2003). According to the NSM, clients who need nursing care are people dealing with stressors or anticipating stressors (such as physiologic variables) in their environment. The model defines universal, basic survival factors which include genetic factors and the innate strengths and weaknesses within the client system (Meleis, 2012). According to Neuman and Reed (2007), the NSM is comprised of several key components that provide the mechanism for attaining optimal wellness. These components include five variables within the client's system (physiological, psychological, developmental, sociocultural, and spiritual), the interaction with the environment (internal, external, or created), and three prevention levels. The

model also includes lines of resistance and lines of defense (flexible and normal) as part of the interaction (Gigliotti, 2003).

The normal line of defense is the inner boundary of the client system, defines its integrity and stability, and is used to measure variance from wellness (Neuman, 2005). The flexible line of defense is the outer boundary of the client system and functions as a buffer by preventing stressor invasion of the client's system (Neuman, 2005). The protective internal and external mechanisms which work to stabilize the client system and maintain wellness are the lines of resistance and support the basic structure of the client, such as the immune system (Neuman, 2005). The metabolic demands of pregnancy are an internal, physiological stressor which can cause imbalance from insulin resistance and hyperglycemia which results in impaired glucose metabolism. In Neuman's model, the goal of nursing is to prevent or correct instability (Meleis, 2010). Using the NSM, interventions by the nurse include helping the individual patient identify barriers to change and helping her discover ways to improve her diet and activity (external environment) to balance maintain normal blood glucose levels (internal environment). This will reduce the physiological stressors in her internal environment, restore health, and improve the outcome of the pregnancy for both her and her baby.

The diagnosis of impaired glucose metabolism creates a psychological stressor. The woman may feel overwhelmed and fearful for herself and her baby. Persson, Winkvist and Mogren (2010) found that women with this condition expressed a change in self-image and a loss of normalcy. Using the NSM, the nurse can enter into a bonding partnership by providing individualized attention for the client, compassionate care,

specific education, and encouragement (Neuman & Reed, 2007). Such a partnership can reduce the psychological stressors to restore well-being.

Significance of Study

Since hyperglycemia during pregnancy results in long-term health consequences for both mother and baby (Pridjian & Benjamin, 2010), there is a need for interventions which can improve glucose metabolism during pregnancy. Hypovitaminosis-D has been associated with impaired glucose metabolism in the non-pregnant population (Parlea et al., 2011). Since hypovitaminosis-D occurs in 70.6% of pregnancies (Maghbooli et al., 2008), a correlation between hypovitaminosis-D in pregnancy and maternal hyperglycemia would be noteworthy and may support vitamin D supplementation as an intervention to improve glucose metabolism in pregnancy (Tomedi, Simhan, & Bodnar, 2013). The goal of this research was to contribute to the science by identifying if there is an inverse relationship between maternal calcidiol status and blood glucose levels at mid-pregnancy. If such a relationship exists, the next step would be to determine therapeutic levels for maternal serum vitamin D for optimal glucose homeostasis. Appropriate supplementation could be a cost effective strategy for reducing hyperglycemia in pregnancy, thus enhancing the health of mothers and their children.

Assumptions

There were several assumptions in this study. It was assumed that the participants all volunteered for the study, met the selection criteria, were truthful in their responses, and followed instructions correctly. It was also assumed that the participants were relatively healthy when the testing was done and were not taking any medications or experiencing other conditions which could affect results. The sample was considered to

be an accurate reflection of the population studied (pregnant, nulliparous women in the upper Midwest). The last assumption was that all laboratory testing techniques were done properly with reliable and accurate results.

Summary of Key Points

1. Vitamin D deficiency is a problem worldwide and affects over 40% of the adults in the United States alone.
2. In the second half of pregnancy, women have increased insulin resistance. Insulin resistance is part of the complex process from placental hormones which reprograms the maternal physiology to meet the needs of the fetus.
3. Impaired glucose metabolism is a complication of pregnancy with significant short-term and long-term consequences for both women and their offspring.
4. Impaired glucose metabolism in pregnancy includes insulin resistance and impaired pancreatic β-cell function which affects insulin production.
5. Vitamin D has been shown to influence glucose metabolism in the non-pregnant population.
6. Both hypovitaminosis-D and impaired glucose metabolism are common conditions, especially for women in the Northern Plains of the United States.
7. Hypovitaminosis-D occurs during pregnancy for certain groups at risk.
8. Hypovitaminosis-D may be associated with elevated blood glucose levels in pregnancy.
9. Vitamin D supplementation during pregnancy may improve glucose metabolism.

10. A gap in knowledge exists regarding the relationship between the serum vitamin D levels in pregnancy and blood glucose levels at 24-28 weeks of gestation.
11. Finding a cost effective, easily implemented intervention to improved glucose metabolism in pregnancy would contribute to both science and clinical practice.

CHAPTER II

REVIEW OF LITERATURE

Introduction

This chapter contains a review of the literature related to the relationship between vitamin D levels at different times in pregnancy and the blood glucose level in mid-pregnancy. In the non-pregnant population, there is growing evidence that vitamin D is an important regulator of blood sugar metabolism and influences increased insulin secretion (Forouhi et al., 2008; Hirani, 2011) which has generated interest on the influence of maternal vitamin D status on blood glucose metabolism in pregnancy. The topics covered in this review include metabolic changes in pregnancy related to glucose metabolism, the effects of hyperglycemia during pregnancy on both the woman and her offspring, an overview of vitamin D including the effect on glucose metabolism, and the influence of vitamin D on glucose metabolism in pregnancy. The review of literature provided the foundation for the research questions for this study.

Definitions

The following definitions of terms used in the current study are written below to provide clarity:

Active vitamin D: $1,25(OH)_2D$; the hormonal form of vitamin D; also known as calcitriol. *In this paper, the term* ***calciTRiol*** *will be used.*

Blood glucose level (BG): amount of glucose in the blood measured in mg/dl. In this study, the BG was obtained at 24-28 weeks gestation after ingestion of a 50 gram glucose beverage at a GDM screening. Sometimes commonly called blood sugar.

Elevated BG at GDM screening: ≥140 mg/dl one hour after ingestion of 50 gram glucose beverage at 24-28 weeks of gestation (Hartling et al., 2012; Hollander, Paarlberg & Huisjes, 2007)

Gestational diabetes mellitus (GDM): carbohydrate intolerance of variable severity with onset or first recognition during pregnancy. A blood glucose level ≥ 140 mg/dl at the GDM screening test or a diagnosis of GDM in the medical record abstraction.

Glucose intolerance: hyperglycemia secondary to insulin resistance.

Hypovitaminosis-D: insufficient or deficient vitamin D level indicated by a blood serum calcidiol which is less than adequate for this population. In this paper, vitamin D is being studied for a non-skeletal influence and therefore level has been set at < 75 nmol/L. See the review of the literature for further details.

Hyperglycemia: an abnormally high concentration of glucose in the blood. For the purposes of this paper, this means a blood glucose level ≥ 140 mg/dl at the GDM screening test

Insulin resistance: physiologic condition in which insulin is produced by the pancreas but the cells fail to respond adequately which results in the need for the pancreas to produce higher amounts of insulin to maintain glucose homeostasis

IU: International Unit

Serum vitamin D: 25(OH)D level in the blood serum, also known as calcidiol or 25-hydroxyvitamin D. It is the stored form of vitamin D that circulates in the body. Serum calcidiol is used to measure vitamin D levels. See methods for data collection information. *In this paper, the term calciDiol will be used.*

Vitamin D: generic descriptor for all steroids exhibiting the biologic activity of ergocalciferol or cholecalciferol. They promote the proper use of calcium and phosphorus, thereby favoring proper bone and tooth formation. The physiological processes which begins with vitamin D intake from synthesis or consumption and becomes the active, hormonal form is very complex and is discussed in the review of literature.

Vitamin D_2: ergocalciferol; the activated ergosterol, the vitamin D of plant origin; sterol occurring naturally in fungi and some fish oils or synthesized from ergosterol; it arises from ultraviolet irradiation of ergosterol, and similar to cholecalciferol in activity and metabolism. It is added to foods such as milk as a dietary source of vitamin D or used as a supplement.

Vitamin D_3: cholecalciferol; vitamin D of animal origin found in the skin of animals exposed to sunlight; can also be manufactured as a supplement

VitD-early: calcidiol level drawn at 10-14 weeks gestation; also VitD.early

VitD-mid: calcidiol level drawn at 22-26 weeks gestation; also VitD.mid

VitD-late: calcidiol level drawn at 32-26 weeks gestation; also VitD.early

Weeks of gestation, weeks gestation: designation of time frame in pregnancy measured in completed weeks

Adaptations to Glucose Metabolism in Pregnancy

Pregnancy is a time of significant alterations and increasing metabolic demands for the woman (Torgersen & Curran, 2006) including changes in glucose metabolism (Cunningham et al., 2013; Evensen, 2012). In pregnancy, there are progressive changes in glucose metabolism which result in a decrease in fasting blood glucose (Gabbe et al., 2012, p. 889), sustained postprandial elevation, and hyperinsulinemia (Cunningham et al., 2013). In the first trimester, this is likely due to the increase in plasma volume (Gabbe et al., 2012, p. 889) which begins at six weeks and continues through the second trimester (Torgersen & Curran, 2006) while in later pregnancy, this is most likely as a result of the glucose needs of the fetus and placenta.

The placenta obtains glucose primarily through facilitated diffusion and the main glucose transporter in the placenta is GLUT1 (Brett, Ferraro, Holick & Adamo, 2015; Brett, Ferraro, Yockell-Lelievre, Gruslin, and Adamo, 2014; Day, Cleal, Lofthouse, Hanson & Lewis, 2013). This results in non-insulin mediated glucose utilization by both the fetus and the placenta which gradually increases with advancing gestation, which further contributes to the changing glucose metabolism in pregnancy. There is an increased potential for the transport of glucose to the fetus correlated to maternal sugar intake (Brett et al., 2015). Simultaneously as the gestation advances, women develop decreasing peripheral insulin sensitivity, a gradual decrease in fasting blood glucose levels, and a progressive increase in the postprandial blood sugar levels (Gabbe et al., 2012, p. 888-890). The availability of nutrients in the maternal circulation influences nutrient transport to the fetus by the placenta (Brett et al., 2015). The placenta is

considered to be a dynamic, responsive organ in the transport of glucose and other nutrients to the fetus.

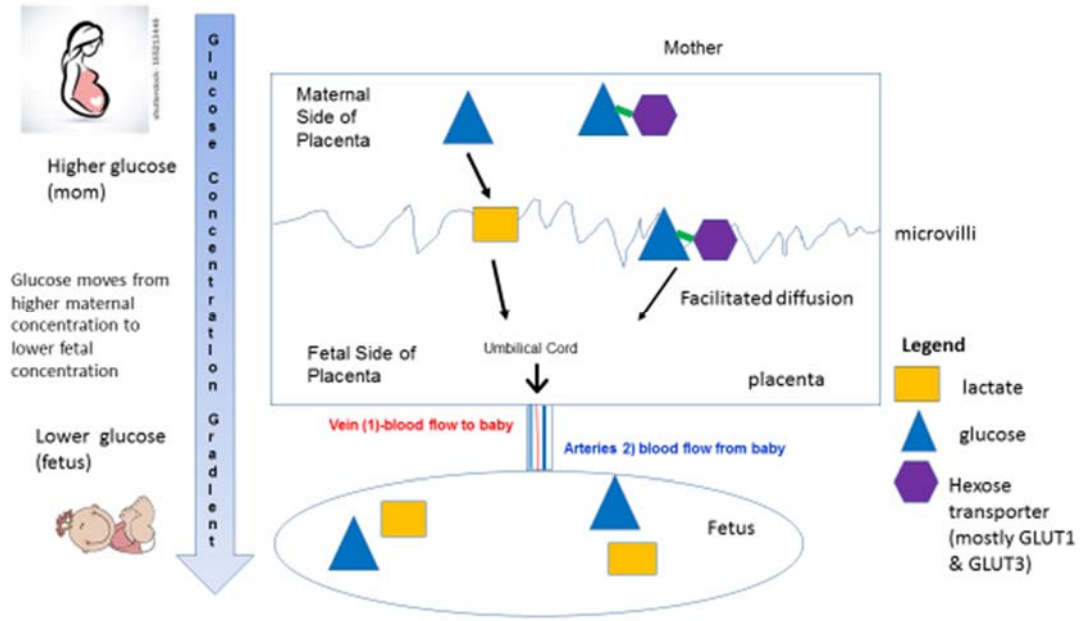


Figure 1. Glucose transport in placenta to fetus.

Changing Insulin Metabolism

Early pregnancy is characterized by alterations in maternal metabolism to provide adequate nutrition to the placenta and the fetus. These metabolic changes are attributed to pregnancy-related hormones including human placental lactogen, estrogen, progesterone, prolactin, and cortisol (Evenson, 2012; Maghbooli et al., 2008). Glucose metabolism is changed by an increase in insulin resistance and reduced uptake of glucose by maternal tissues which results in higher postprandial blood glucose levels (Maghbooli et al., 2008). Simultaneously, the placenta utilizes glucose which then results in lower fasting blood glucose levels (Gabbay-Benziv & Baschat, 2015). As the pregnancy

progresses the insulin resistance increases to facilitate glucose delivery to the fetus (Evensen, 2012).

Factors Contributing Insulin Resistance in Pregnancy

Normally, this state of increased insulin resistance in the second half of pregnancy is similar to the underlying pathophysiology of type 2 diabetes (Evensen, 2012; Jelsma et al., 2013). In both conditions, the increasing insulin resistance along with an inadequate response of the β-cells to produce the amount of insulin needed leads to hyperglycemia (Gabbe et al., 2012, p. 892). Although not yet fully understood, insulin receptors found in the placenta undergo developmental changes as the pregnancy progresses which strongly suggest a shift from maternal control to fetal control of insulin dependent functions (Desoye & Hauguel-de Mouzon, 2007). In the microvillus trees of the placenta, there is diffusion of glucose from the higher concentration in the maternal blood supply to the lower concentration on the fetal side of the placenta. The glucose supplied to the developing fetus is dependent upon the concentration gradient between the mother and baby. The main glucose transporter in this process is GLUT1 which provides a binding site at the basal membrane and faster, facilitated diffusion. The placenta also utilizes a considerable amount of glucose which buffers the glucose fluctuations to the fetus (Barta & Drugan, 2010).

Maternal Hyperglycemia in Pregnancy

Hyperglycemia occurs in pregnancy when the body is unable to respond to the physiologic changes by increasing insulin production to meet the changes in glucose metabolism (Coustan, 2013). Emerging research demonstrates that maternal hyperglycemia is clinically important, even when less severe than the level associated

with a diagnosis of gestational diabetes mellitus (The HAPO Study Cooperative Research Group, 2008). Glucose metabolism is on a continuum (Salzer, Tenenbaum-Gavish & Hod, 2015) with a linear association between maternal blood glucose levels in pregnancy and unfavorable perinatal outcomes (Tieu, McPhee, Crowther & Middleton, 2014). A fetus is more sensitive to hyperglycemia than to hypoglycemia when it comes to well-being (Evensen, 2012). Studies such as the HAPO study demonstrate that adverse outcomes for mother and baby can be a result of hyperglycemia in pregnancy (Coustan Lowe, Metzger, & Dyer, 2010). However, the threshold for maternal blood glucose levels which predicts adverse perinatal outcomes has not yet been found (Tieu, McPhee, Crowther, & Middleton, 2014).

The HAPO study examined any association between blood glucose levels and adverse pregnancy outcomes (Coustan et al., 2010). Over 25,000 women were involved in the study and various research teams analyzed the data. Fasting blood sugar (FBS) levels and/or oral glucose tolerance test (OGTT) results were used and the study was powered for the primary outcomes of macrosomia, neonatal hypoglycemia, cesarean delivery, and hyperinsulinemia (Coustan et al., 2010). The secondary outcomes in the HAPO study included shoulder dystocia, preterm birth, birth injury, neonatal intensive care admission, pre-eclampsia, and hyperbilirubinemia (Coustan et al., 2008). Both obesity and gestational diabetes mellitus pose a greater risk for adverse outcomes in pregnancy with a combination of both leading to even greater risk for these unfavorable consequences (Catalano et al., 2012).

Maternal blood glucose was considered a continuous variable for the analysis (Coustan et al., 2010). Maternal hyperglycemia at levels less severe than GDM causes

fetal hyperinsulinemia and fetal overgrowth (The HAPO Study, 2009). The adverse effects of maternal hyperglycemia on the infant can result in the need for more medical interventions at birth including costly admissions to the neonatal intensive care unit (Landon et al., 2012; Desoye & Hauguel-de Mouzon, 2007; Vambergue & Fajardy, 2011). The HAPO Study (2009) concluded that there is a need to look at treating hyperglycemia in pregnancy, considering the findings of adverse outcomes.

When the fetus is exposed to ongoing hyperglycemia in utero, there is an increased risk for the adult onset of disease (Lapillone, 2009; Lehnen et al., 2013; Tieu et al., 2014; Veeraswarmy et al., 2012). Prolonged maternal hyperglycemia during pregnancy can lead to health problems in later life for the offspring including childhood obesity, development of metabolic syndrome, and type 2 diabetes in young adulthood (Tieu et al., 2014; Veeraswamy, Vijayan, Guptz, & Kapur, 2012). Fetal overnutrition at periods critical in fetal development (Javaid et al., 2006) can lead to metabolic conditions such as obesity, glucose intolerance, or type 2 diabetes. These studies demonstrates how such conditions can originate from the effects of the intrauterine environment on the developing fetus (Hunt & Schuller, 2007; Lehnen et al., 2013; Veeraswarmy et al., 2012).

The known physiologic changes in pregnancy which can results in prolonged hyperglycemia have resulted in routine screening protocols to diagnose gestational diabetes mellitus (GDM) (Tieu et al., 2014). The protocols were implemented because GDM can occur among pregnant women traditionally considered to be low risk for complications (Holmes et al., 2009; Lehnen et al., 2013; Macones, 2012; Poel et al., 2012). Gestational diabetes mellitus (GDM) is briefly defined as carbohydrate intolerance that is first recognized during pregnancy (Asemi, Hashemi, Karamali,

Samimi, & Esmaillzadeh, 2013). Left uncontrolled, GDM can result in hyperglycemia during pregnancy with the resulting adverse effects. Infants of women with GDM have increased perinatal morbidity including macrosomia, hypoglycemia, respiratory distress syndrome, and jaundice (Coustan, 2013).

Vitamin D Metabolism

Vitamin D is recognized today as a prohormone (Institute of Medicine, 2011) and is a unique nutrient because it is obtained through cutaneous synthesis from ultraviolet rays from direct sunlight (from UVB radiation at 290-320 nm) on exposed skin (Brannon & Picciano, 2011) and can also be obtained through certain foods or dietary supplements (Specker, 2004; Tomedi, Simhan, & Bodnar, 2013). Vitamin D2 (ergocalciferol) is naturally found in a few foods or is manmade (IOM, 2011; Specker, 2004). Vitamin D3 (cholecalciferol) is produced in human skin from 7-dehydrocholesterol (7-DHC) or ingested through animal based foods (IOM, 2011). Both forms, D2 and D3, are commercially manufactured for supplements (IOM, 2011).

Both forms of vitamin D (D2 and D3) are biologically inactive and must undergo two hydroxylations before becoming biologically active (Brannon & Picciano, 2011; Holick & Chen, 2008). The first hydroxylation occurs in the liver where an enzyme, 25-hydroxylase (CYP2R1), converts vitamin D into 25-hydroxyvitamin D which is also referred to as 25(OH)D or calcidiol (Brannon & Picciano, 2011; Holick & Chen, 2008; IOM, 2011). Calcidiol is the major form of vitamin D in the circulation and is bound to a carrier protein, vitamin D binding protein (known as VDP) in the plasma (IOM, 2011). Calcidiol has a half-life of 15 days (Jones, 2008) and is the best indicator of serum vitamin D status (Holick & Chen, 2008; Olmos-Ortiz, Durand-Carbajal, & Diaz, 2015)

because it reflects the cumulative effect of sunlight, diet, and supplements in the circulation (Kovacs, 2013). Although calcidiol has a sufficient half-life to most accurately reflect the vitamin D from all sources (Brannon, 2012), it does not accurately reflect the stores within the body tissues (National Institutes of Health, n.d.).

Calcidiol is changed into calcitriol (1,25(OH)2D) through the second hydroxylation in the maternal kidneys and, in lesser amounts by the fetal kidneys, the placenta, the decidua, and diverse tissues (Barrett & McElduff, 2010; Kovacs, 2013). An enzyme in the kidneys, 1α-hydroxylase (CYP27B1), converts the calcidiol into the physiologically active form, 1,25 dihydroxyvitamin D, which is also referred to as $1,25(OH)_2D$ or calcitriol (Holick & Chen, 2008; IOM, 2011). Calcitriol synthesis in the kidney is tightly controlled with up-regulation by the parathyroid hormone and down-regulation by fibroblast-like growth factor. Some extra-renal tissues contain 1α-hydroxylase, but it is yet unknown how this contributes to calcitriol production (IOM, 2011). Calcitriol acts through the vitamin D receptors (VDR) to produce the favorable biological effects (Lapillonne, 2009). Calcitriol which has a half-life of 15 hours (Jones, 2008) yet levels of calcitriol typically do not decrease until there is severe vitamin D deficiency (NIH, n.d.). Calcitriol has a significant physiological influence on essentially every tissue and cell in the human body (Holick, 2005; Lips, 2006; McCullough, 2007; Pittas & Dawson-Hughes, 2010) and may even have a protective effect against many diseases (Lapillone, 2009).

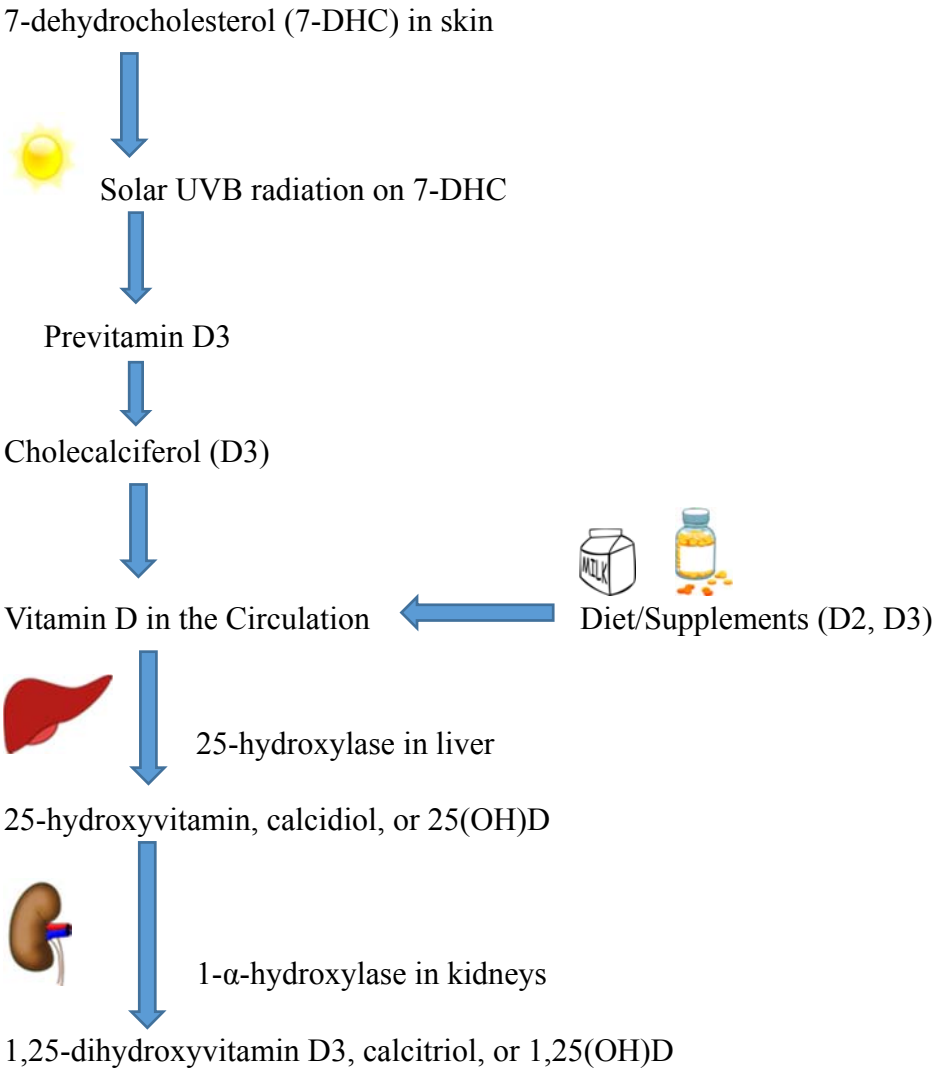


Figure 2. Vitamin D metabolism.

Hypovitaminosis-D

Concerns exist about the high prevalence of hypovitaminosis-D in pregnancy (Bodnar et al., 2007; Brannon & Picciano, 2011) which exists in almost every population studied (Dror, King, Durand, & Allen, 2011). Today, hypovitaminosis D can be attributed to lifestyle changes which result in less sunlight exposure on bare skin from the use of sunscreen, fewer hours spent outside, and clothing choices due to culture or

climate (Dawodu & Akinbi, 2013; Wagner et al., 2013; Wang et al., 2013). The reduced amount and seasonal nature of sunlight exposure, smoking, and obesity have all been associated decreased vitamin D levels (Andersen et al., 2013; Evensen, 2012). Vitamin D synthesis from direct sunlight is reduced as melanin increases in the skin or due to changes in UV radiation from sunlight when people live at higher latitudes (Holick & Chen, 2008; Schroth, Lavelle, & Moffat, 2005). The individual's metabolism of vitamin D can be influenced by factors which include renal function, liver function, ethnicity, and genetic effects (Collins-Fulea et al., 2012; Holmes et al., 2009; Prentice, 2008; Wicherts et al., 2011).

Prevalence of Hypovitaminosis D

Vitamin D deficiency is a problem worldwide and may affect as much as 42% of the adults in the United States (Forrest & Stuhledreher, 2011; Ginde, Sullivan, Mansbach, & Camargo, 2010; Hanley & Davison, 2005; Holick, 2008; Vieth, 2006). Some researchers have described hypovitaminosis D as reaching pandemic levels because it is prevalent in Asia, China, the Scandinavian countries, the United Kingdom, Canada, the northern United States, and in African Americans (Holick & Chen, 2008; Glerup et al., 2000; Hanley & Davison, 2005; Wang et al., 2012). The prevalence of hypovitaminosis-D is approximately 5-29% of pregnant women in the United States (Brannon & Picciano, 2011).

The Institute of Medicine (IOM) developed the dietary reference intakes (DRI) through a joint effort between the United States and Canadian governments (Aloia, 2011). The DRI of vitamin D was recently increased from 400 IU to 600 IU daily for adults, including pregnant women (Aloia, 2011). Many researchers believe this is still

not adequate and argue that the DRI should be 800-2000 IU (Ginde et al., 2010; Hanley & Davison, 2005; Heaney, 2005). The most effective method to achieve an increase in vitamin D is through dietary supplements (Hanley & Davison, 2005; Heaney, 2005; Thorne-Lyman & Fawzi, 2012). The IOM recommendations state that vitamin D toxicity is unlikely to occur until doses exceed 10,000 IU/day (IOM, 2011). However, vitamin D intake and serum vitamin D levels do not have a linear relationship (IOM, 2011) so the best way to determine vitamin D status is through a serum calcidiol level (Holick & Chen, 2008; Olmos-Ortiz et al., 2015).

Vitamin D in Pregnant Women

Unless there is an increase in intake of vitamin D from diet, supplements, or from synthesis in the skin, serum calcidiol levels don't change during pregnancy (Gabbe et al., 2012, p. 59). During pregnancy, calcitriol progressively increases by 50-100% in the second trimester and to twice the pre-pregnancy level in the third trimester, most likely due to production by the increasing placental mass (Cho, Hong, Oh, & Kim, 2013). This change occurs from an increase in production of calcitriol from calcidiol by the maternal kidneys as well as conversion of calcidiol into calcitriol by the placenta, the decidua and the fetal kidneys (Barrett & McElduff, 2010; Kovacs, 2013). The increase in maternal calcitriol starts in early pregnancy but the increase in free calcitriol does not occur until late pregnancy. This occurs because the maternal kidneys upregulate the expression of the enzyme, CYP27B1, which increases the production of calcitriol. The fetal kidneys, the placenta, and the maternal decidua are also sources of CYP27B1 expression but only results in a small amount of calcitriol. Their contribution is not enough to account the increase of calcitriol in maternal circulation in pregnancy (Kovacs, 2013).

Hypovitaminosis-D in Healthy Pregnant Women

Hypovitaminosis-D was detected in women of childbearing age who are considered healthy and at low risk for pregnancy complications (Holmes et al., 2009). Although vitamin D levels were significantly lower in the pregnant women in their study, Holmes et al. (2009) reported hypovitaminosis-D in the non-pregnant women from the control group in their study demonstrating that hypovitaminosis-D is prevalent in all women who are of childbearing age. Specific groups of pregnant women such as those who are veiled or have deeply pigmented skin have a high prevalence of hypovitaminosis-D (Bodnar et al., 2007; Dawodu & Akinbi, 2013; Marwaha et al., 2011; Wagner et al., 2013; Wang et al., 2013).

The Endocrine Society and the Institute of Medicine (IOM) have differing recommendations on the amount of vitamin D supplementation required in pregnancy. The IOM recommends 600 IU/day of vitamin D for all adults with no change for women during pregnancy (IOM, 2011). The Endocrine Society recommends 600 IU of vitamin D daily for pregnant women between 14-18 years of age and 1500-2000 IU daily for pregnant women ages 19-50 years of age (Holick et al., 2011). If needed, The Endocrine Society recommends supplementations of 1,500-2,000 IU of vitamin D daily to reach a target level of serum calcidiol of 75 nmol/mL (Hollis et al., 2011). However, Kovacs (2013) reports that women do not need more vitamin D during pregnancy as compared to the non-pregnant state because maternal calcitriol normally increases in pregnancy due to increased expression of CYP27B1 in the maternal kidneys. Kovacs (2013) states that there is inconsistent data for any non-skeletal benefits from vitamin D and does not

recommend additional vitamin D during pregnancy until clinical trials prove there is a need.

Hypovitaminosis-D is prevalent in many women during pregnancy (Aslam et al., 2012; Hollis et al., 2011; Maghbooli et al., 2008) underscoring the importance of investigating the effect of vitamin D on health outcomes related to this population (Barrett & McElduff, 2010; Ginde et al., 2010; Holmes et al., 2009; Mulligan, Felton, Riek, & Bernal-Mizrachi, 2010). Universal screening is not recommended at this time, but targeted screening may be indicated for pregnant women in some at-risk groups (Collins-Fulea et al., 2012). Serum levels of calcidiol increase in proportion to intake through both sunlight exposure and oral intake from supplements or food (IOM, 2011). The dose-response varies in individuals based on the degree and duration of supplementation and the baseline calcidiol levels (IOM, 2011).

Hypovitaminosis-D and Glucose Metabolism

The National Health and Nutrition Examination Survey (HANES) showed an inverse association between diabetic risk and serum calcidiol for some ethnic groups, but not for all (Scragg, Sowers, & Bell, 2004). There is evidence that vitamin D has an effect on glucose metabolism (Chiu et al., 2004; Forouhi et al., 2012; Moreira & Hamadeh, 2010; Holick, 2005; Pittas & Dawson-Hughes, 2010; Palomer et al., 2008) but the physiological reason is not fully understood. It is unknown if vitamin D affects insulin sensitivity, β-cell function in the pancreas, or a combination of these (Clifton-Bligh et al., 2008). Several researchers have shown that vitamin D affects β-cell function in the pancreas, insulin secretion and insulin resistance which subsequently affects blood glucose metabolism (Alfonso et al., 2009; Kayaniyil et al., 2011; Zhao, Ford & Li, 2010).

Others have reported an inverse association between hypovitaminosis-D and insulin resistance, plasma glucose levels, and diabetes (Chiu et al., 2004; Ford, Ajani, McGuire & Lui, 2005; Forouhi et al., 2008; George, Person & Witham, 2012; Heaney, 2005; Kayaniyil et al., 2010; Moreira & Hamadeh, 2010; Patra et al., 2012; Pittas et al., 2010). McLeod et al. (2012) reported that vitamin D may affect glucose metabolism through influence of β-cell function. In summary, there is evidence that vitamin D influences glucose metabolism through insulin production or sensitivity, though the precise details have yet to be determined.

Insulin resistance contributes to hyperglycemia and impaired glucose tolerance (IGT) which is then linked to the development of diabetes mellitus (Muscogiuri et al., 2011; Song & Mason, 2010). A study in healthy young adults with normal glucose metabolism demonstrated an association between hypovitaminosis-D and decreased insulin secretion which was normalized after vitamin D supplementation (Jorde & Figenschau, 2009). Another study demonstrated that vitamin D3 supplementation improved postprandial sensitivity in a targeted group (Nagpal, Pande, & Bharita, 2009). Some researchers have suggested that higher levels of vitamin D might have a protective effect against development of both type 2 and type 1 diabetes (Jorde & Figenschau, 2009; Harris, 2005). If such a correlation between hypovitaminosis-D and hyperglycemia exists, it creates the potential to study vitamin D supplementation as a method for improving glucose metabolism during pregnancy (Tomedi, Simhan & Bodnar, 2013).

Insulin Resistance and Hypovitaminosis-D in Pregnancy

The normal insulin resistance of pregnancy may be compounded by the metabolic consequences of hypovitaminosis-D which itself can contribute to insulin resistance and

impaired glucose metabolism. A limited number of large studies have supported the premise that maternal hypovitaminosis D during pregnancy is linked to insulin resistance, elevated fasting blood glucose levels, or GDM (Burris et al., 2013; Jensen et al., 2013; Maghbooli et al., 2008). Retrospective studies examining the relationship between a diagnosis of gestational diabetes mellitus (GDM) and serum calcidiol levels failed to find an association between hypovitaminosis-D and a diagnosis of GDM, yet the researchers reported that they did find an association between hypovitaminosis-D and elevated blood glucose levels (Farrant et al., 2009; Lau et al., 2011). These discrepancies are evidence that there continues to be a gap in knowledge on the influence of hypovitaminosis-D on glucose metabolism in pregnancy. Since pregnancy results in progressive physiological changes, the relationship between calcidiol and blood glucose in early, mid, and late pregnancy will be discussed.

Hypovitaminosis-D in Early Pregnancy

Scientists examining vitamin D levels in the first trimester (early pregnancy) did not find an association between serum vitamin D and GDM. Baker et al., 2012; Fernandez-Alonso et al., 2012; Makgoba et al., 2011; Tomedi et al., 2013; Zhang et al., 2008). Baker et al. (2012) investigated the relationship of first trimester vitamin D deficiency and the risk of GDM based on the premise that hypovitaminosis-D might be related to dysfunction of pancreatic β-cells leading to impaired glucose metabolism. The researchers used a nested case-control approach with 180 women in their sample. They used stored serum collected in the first trimester for calcidiol levels and blood glucose levels from the GDM screening at 24-28 weeks gestation. They were unable to prove their hypothesis that first trimester hypovitaminosis-D was related to higher levels of

GDM because so few of the participants in their study had hypovitaminosis-D (Baker et al., 2012).

Fernandez-Alonso et al. (2012) examined pregnancy outcomes and first trimester (early pregnancy) vitamin D levels for 466 women in a cross-sectional study in Spain. Participants had a serum blood drawn between 11-14 weeks gestation at their first prenatal visit then were followed to the end of pregnancy. Seasonal variations in vitamin D levels were found based on the first trimester being in the spring/summer compared to the fall/winter and this seasonal variation continued into the second trimester. They also found a progressive and significant decrease in vitamin D levels from the first to the third trimester which was independent of season of the year. However, perinatal outcomes did not vary based on the calcidiol levels in the first trimester (Fernandez-Alonso et al., 2012).

Makgoba et al. (2011) looked for an association between serum vitamin D in the first trimester and development of GDM in a prospective, case-controlled study of 248 women. Blood samples collected in the first trimester were used from a multiethnic group consisting of 90 women who developed GDM and a control group of 158 women. The women in the study were receiving care in inner-city London. There was a negative correlation between the calcidiol level at the initial prenatal visit in the first trimester and the glucose level obtained in mid-pregnancy at a two hour glucose test. However, there was not a statistically significant difference between the calcidiol levels in the group of women who developed GDM when compared to the group who did not develop GDM. Univariate analysis showed a positive association between calcidiol levels and maternal

age and a negative association between calcidiol levels and the first trimester BMI (Makgoba et al., 2011).

The Study of Nutrition and Pregnancy (SNAP) data was used by Tomedi et al. (2013) to investigate the association between the calcidiol levels in early-pregnancy (< 16 weeks gestation) and the blood glucose levels collected at 24-28 weeks gestation which was through a routine glucose screening test. They defined hypovitaminosis-D as a serum calcidiol level < 50 nmol/L. There were 429 women in their sample. They found that 67% of the women had hypovitaminosis-D and 11% of the women had hyperglycemia. They did not find a difference in the calcidiol and glucose levels when using the Pearson chi-squared or the Students'-test. They then looked at calcidiol as a continuous variable with logistic and multivariate linear regression models to examine the association between the maternal calcidiol levels and blood glucose levels or hyperglycemia. Smoking status was included in the data set. Among the smokers, calcidiol levels in the first trimester were lower for those with hyperglycemia at mid-pregnancy. This association was not found for the non-smokers. In their analysis, they considered that this may be due to the attributes of their cohort (young, nulliparous, with low incidence of GDM) or that smoking may concurrently reduce vitamin D levels and increase blood glucose concentrations due to oxidative stress. They reported this may have resulted in the association between hypovitaminosis-D and hyperglycemia in the participants who smoked.

Zhang et al. (2008) used a subset of the Omega Study to investigate the association between serum calcidiol levels in early pregnancy and the risk for GDM. The subset consisted of 57 women with a diagnosis of GDM and 114 women without this

diagnosis as controls out of the complete data set had a total of 953 participants. The researchers used the Student's t test to examine the mean calcidiol levels between the two groups. They reported that the participants with a diagnosis of GDM had a significantly lower serum calcidiol concentration than the control group (24.2 ng/ml vs. 30.1 ng/ml, $P < 0.001$) after controlling for maternal age and BMI. They concluded that hypovitaminosis-D in early pregnancy (< 16 weeks gestation) was associated with a significantly increased risk for GDM. The researchers analyzed the characteristics of the participants and reported that, in general, the women who developed GDM were older and heavier than those who did not develop GDM. However, they report that even after controlling for these variables, the inverse association between early pregnancy calcidiol levels and GDM risk remained statistically significant (Zhang et al., 2008).

The studies described above demonstrate that science has not yet been able to determine the nature of the relationship between calcidiol levels and glucose metabolism in pregnancy. Many researchers used the diagnosis of GDM as a measure of hyperglycemia from impaired glucose metabolism. For the purposes of this study, GDM will be used as proxy to hyperglycemia in pregnancy.

Hypovitaminosis-D in Mid Pregnancy

Some researchers have reported an increased risk for GDM when women have hypovitaminosis-D in the second trimester (Burris et al., 2012; Clifton-Bligh et al., 2008; Maghbooli et al., 2008; Parlea et al., 2012; Soheilykhah et al., 2012; Yap, Gunton, Munns & McLean, 2014). Burris et al. (2012) concluded that mid pregnancy serum vitamin calcidiol levels were inversely associated with the blood glucose level obtained one hour after a 50 gram glucose load. Clifton-Bligh et al. (2008) reasoned if vitamin D

influenced insulin sensitivity or release, any effects of hypovitaminosis-D would become apparent in later pregnancy when progressive insulin resistance affects glucose metabolism. In their prospective study, they found a non-linear, inverse correlation between serum vitamin D levels and fasting glucose but this significance became borderline when they included body mass index (BMI) in the analysis (Clifton-Bligh et al., 2008).

Maghbooli et al. (2008) found higher insulin resistance and fasting blood glucose levels in a group of pregnant women with hypovitaminosis-D, even after adjusting for the confounding variables of age and BMI. However, they found hypovitaminosis-D in 71% of their sample of pregnant women and severe hypovitaminosis-D in 29% of their sample when looking at hypovitaminosis-D and insulin resistance. They reported that hyperglycemia during pregnancy was significantly higher in the group with severe hypovitaminosis-D (Maghbooli et al., 2008).

Soheilykhah et al. (2010) found hypovitaminosis-D (serum calcidiol < 50 nmol/L) in 83.3% of the participants with GDM and in 71.2% of the controls (matched in age, BMI, and gestation). They report women with increase blood glucose levels at mid pregnancy had significantly lower serum calcidiol levels compared with the control group. Furthermore, women with GDM had a 2.66 fold increased risk of having serum vitamin calcidiol levels < 37.5 nmol/L (Soheilykhah et al., 2010).

Yap et al. (2014) report there is an increased risk of gestational diabetes mellitus associated with hypovitaminosis-D in pregnancy and postulated that high dose vitamin D supplementation for women who had hypovitaminosis-D (defined as serum calcidiol < 80 IInmol/L) before 20 weeks gestation might decrease this risk. Participants with

hypovitaminosis-D were randomized to receive 5,000 IU of vitamin D daily (high dose) or 400 IU daily (low dose) and the mid-pregnancy blood glucose levels obtained from an oral glucose tolerance test were compared. There was no difference in the mean maternal glucose levels between the two groups. However, 8% of the participants in the high dose group were diagnosed with GDM compared to 13% in the low dose group, and baseline serum calcidiol showed an inverse relationship with the blood glucose levels (Yap et al., 2014).

Hypovitaminosis-D in Late Pregnancy

There are limited studies specifically examining the relationship between hypovitaminosis-D and hyperglycemia in pregnancy in late pregnancy. Farrant et al. (2009) measured serum calcidiol levels and blood glucose levels at 30 weeks of gestation in Mysore women living in India. They did not find a relationship between serum vitamin D levels at 30 weeks of gestation and gestational diabetes mellitus (GDM) but they did find a high level of hypovitaminosis-D in their sample. They reported that, in women with hypovitaminosis-D, higher vitamin D levels were associated with lower BG levels at the 30 minute interval during an oral glucose tolerance test (Farrant et al., 2009).

McLeod et al. (2012) report that insulin resistance develops in the second and third trimesters of pregnancy and that vitamin D may affect glucose metabolism through influence on the β cells of the pancreas. Lau et al. (2011) examined glycated hemoglobin (HgA1c) and serum calcidiol levels in late pregnancy. They found hypovitaminosis-D in 41% of their participants and concluded lower serum calcidiol levels was associated with impaired glucose metabolism. They reported an inverse association between the vitamin D level and glucose control in late pregnancy or the third trimester (Lau et al., 2011).

Cho et al. (2013) investigated the relationship between vitamin D levels drawn at the time of birth (late pregnancy) and the blood glucose level drawn at 24-28 weeks gestation. They found that 85% of the women diagnosed with GDM had hypovitaminosis-D (calcidiol < 20 ng/mL) and 27.5% of the women with normal pregnancy had hypovitaminosis-D. They concluded that there was an association between hypovitaminosis-D and GDM (Cho et al., 2013).

It is interesting to note that the studies in the third trimester reported an inverse association between serum vitamin D levels and blood glucose (Cho et al., 2013; Farrant et al., 2009; Lau et al., 2011) even though these studies were methodologically different. Cho et al. (2013) investigated serum calcidiol levels just before delivery and found a higher incidence of hypovitaminosis-D in the women with GDM. Farrant et al. (2009) found an association between vitamin D levels and BG levels at the 30 minute interval during an oral glucose tolerance test, but not an association with the other blood glucose levels in the test nor with a diagnosis of GDM. Lau et al. (2011) looked at serum calcidiol levels at the time of a glycated hemoglobin test in the third trimester for women with known GDM and found an inverse association between calcidiol levels and glycemic control.

Summary

Optimal health in pregnancy requires unimpaired glucose metabolism which may depend upon adequate levels of biologically active vitamin D. The GDM screening is a single value at one point in time based on a protocol and the criteria for diagnosing GDM are not consistent or have been changed periodically over the years (Coustan et al., 2010). The pregnancy related problems of GDM are a result of hyperglycemia (Aghajafari et al.,

2013; Desoye & Hauguel-de Mouzon, 2007; Landon et al., 2012; Vambergue & Fajardy, 2011). Researchers and clinicians are just a beginning to understand the impact of vitamin D during pregnancy on the health of the mother and the future health of the developing fetus (Barker, 2007; Barker & Bagby, 2010; Bergstrom, Blanck & Savendahl, 2013). Maternal hypovitaminosis-D is quite possibly a modifiable risk factor which can be targeted to improve pregnancy outcomes and even prevent hyperglycemia in pregnancy through the simple, cost effective use of vitamin D supplementation (Parlea et al., 2012; Poel et al., 2012; Senti, Thiele & Anderson, 2012). Preventing hypovitaminosis-D during pregnancy supports an optimal intrauterine environment which may impact the health of future generations (Barker, 2005, 2007; Holt, Coleman, & McCance, 2011).

CHAPTER III

METHODS

Introduction

Chapter III provides details on the study design, population and sample, and sampling procedures. Discussion includes each of the study variables which are maternal age, body mass index (BMI), calcidiol levels at three points during pregnancy, and blood glucose in mid-pregnancy. The data analysis process is discussed. The chapter ends with limitation of the study.

The purpose of this study was to determine the relationship between calcidiol levels collected in early, mid, and late pregnancy and blood glucose levels in mid pregnancy in a sample of women in an upper Midwestern region of the United States. Calcidiol influences glucose metabolism in the non-pregnant population (Alfonso et al., 2009; Mathieu et al., 2005; Pittas et al., 2006; Tohidi et al., 2013) and this study investigated the influence of calcidiol on glucose metabolism in the pregnant population. Identifying the correlation between calcidiol and glucose levels may inform the potential mechanism on the effect of calcidiol on glucose metabolism in pregnancy. There were three research questions. 1) Is there a correlation between the calcidiol level at 10-14 weeks gestation and the blood glucose (BG) level at 24-28 weeks gestation when controlling for the known confounding variables of age and pre-pregnancy body mass index (BMI)? Is there a correlation between calcidiol level at 22-26 weeks gestation and

the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI? 3) Is there a correlation between the calcidiol level at 32-36 weeks gestation and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI?

Research Design

A quantitative, correlational design was used in a secondary analysis to ask new questions of an existing data set. The level of significance was set at $p < .05$. This study was designed as a secondary analysis of data collected in a prior prospective study. An advantage of this type of study design is the ability to answer new research questions of an existing data set (Castle, 2003; Doolan & Froelicher, 2009). The primary research was a prospective study to examine differences in women that did or did not develop preeclampsia (Anderson, Ralph, Wright, Linggi, & Ohm, 2013). The primary study used a convenience sample of women recruited from a community located at latitude of 47.96 degrees north (Anderson et al., 2014). The design of the parent study determined the inclusion and exclusion criteria. Eligibility criteria included English speaking nulliparous women who were at least 18 years of age carrying a singleton pregnancy. In addition, participants needed to be enrolled between 10 and 14 weeks of gestation, received prenatal care in the community, and delivered at the local community hospital. Exclusion criteria included woman who were unable to communicate in English, were younger than 18 years of age, were not nulliparous, pregnant with multiples, or beyond 14 weeks of gestation at time of enrollment. The data set for the secondary analysis only included women who were followed through delivery.

Demographic data was collected at enrollment between 10-14 weeks gestation. Peripheral blood samples through venipuncture were drawn at 10-14 weeks gestation, 22-26 weeks gestation, and 32-26 weeks gestation. The data set acquired for the secondary analysis included laboratory results and standard measures from the medical record collected during prenatal care, including a blood glucose level at 24-28 weeks gestation. The study was presented and refined based on input from stakeholders including groups providing prenatal, labor and birth care as well as nursing staff on the inpatient unit where the participants gave birth.

Population and Sample

This study used a convenience sample of 52 nulliparous pregnant women who received prenatal care and delivered in a single healthcare system in an upper Midwestern community of the United States. All of the participants in the study needed to be at least 18 years of age, nulliparous, enrolled before 14 weeks gestation, and did not have pre-existing medical conditions. The participants volunteered by responding to recruitment measures and lived in or near the upper Midwestern community. All of the women in the study received usual prenatal care appropriate to their institution and all were followed through delivery. The study sample was compared with the population of the state of North Dakota which represents the region and with the population of the United States. This was done to determine generalizability of the study findings.

Sampling Procedures

Sampling procedures are discussed below. This begins with how participants were recruited for the study, the time points for data collection, and the process of data collection. Protection of human subjects is discussed at the end of this chapter.

Recruitment

Recruitment occurred through advertising using local print media, the internet, a local television community bulletin board, and through flyers posted in the clinics which provided ambulatory care to pregnant women in the region. One page 8x10 inch posters were created then placed on bulletin boards in the perinatal clinic areas and on public access bulletin boards in grocery stores and shopping areas. In addition, an advertisement was placed in a local "shopper-style" free paper and a similar written advertisement was run as part of scrolling announcements on a local public information television channel. Individuals who were interested in participating in the study called the phone number listed and were screened for eligibility by a research team member. Those who met inclusion criteria met with a trained research assistant at the Grand Forks Human Nutrition Research Center (GFHNRC) three times during the prenatal period.

The first meeting with the research assistant was between 10-14 weeks gestation at which time the study was reviewed with the participant, informed consent was obtained, and a blood sample was obtained through venipuncture. The participants returned two more times to the GFHNRC for subsequent blood samples through venipuncture at 22-26 weeks gestation and at 32-36 weeks gestation (Figure 3). After giving birth, medical record abstraction was performed to obtain the height, pre-pregnancy weight (or weight at first prenatal medical appointment if pre-pregnancy weight was unknown), and the blood glucose routinely obtained in mid-pregnancy between 24-28 weeks gestation.

All participants lived in or near the upper Midwestern community. All of the women in the study received usual prenatal care appropriate to their institution. All were

followed through delivery. Each woman received a $75 incentive, scaled over three data collection points, for completing the study.

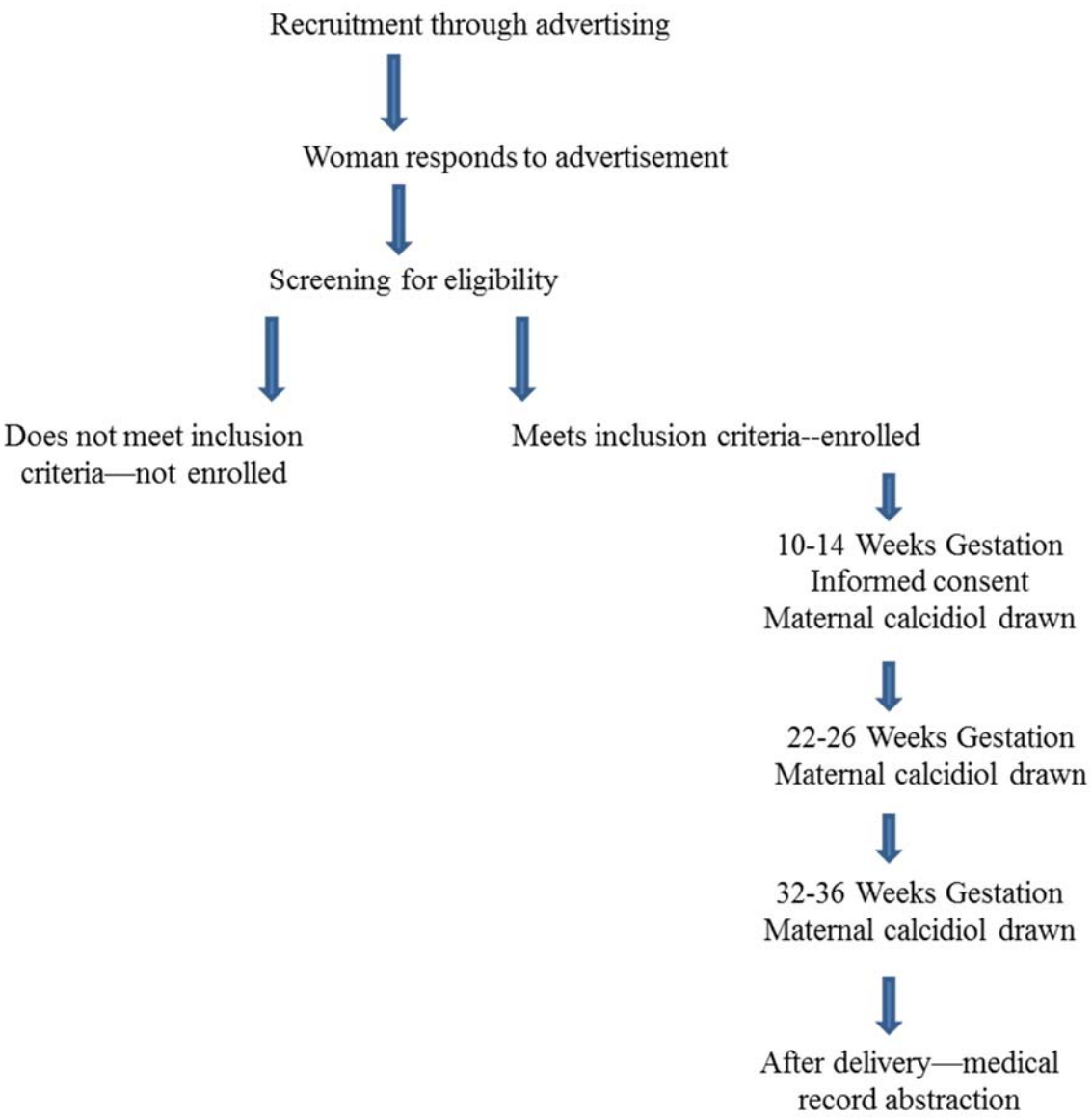


Figure 3. Recruitment and collection of data.

Instrumentation

The instrumentation used in this study is discussed in detail below. Included is the process used to obtain the serum calcidiol levels and the manner used to obtain the

other variables. Data about race was obtained and recorded at the time of study enrollment. The medical record was used to obtain pre-pregnancy or initial weight, height, and blood glucose level in mid-pregnancy. Information from the medical record was transcribed onto a tool with only the study participant number; there was no information which could identify the participants.

Methods for Calcidiol Blood Sampling

Peripheral blood samples were collected by venipuncture in early pregnancy (10-14 weeks gestation), mid-pregnancy (22-26 weeks gestation), and late pregnancy (32-36 weeks gestation). Serum was extracted from the blood samples for analysis of serum calcidiol levels. Samples were collected, processed, and stored by research staff at the Grand Forks Human Nutrition Research Center (GFHNRC) for study enrollment and for calcidiol levels. The participants reported to the GFHNRC at times scheduled for data collection at each of three time points in gestation. Using appropriate universal precautions, the research staff performed venipuncture to collect the whole blood from the participants. The blood was collected in two red top serum separator vacutainer (B-D, Franklin Lakes) blood tubes. The blood samples were kept at room temperature for one hour to allow clotting then centrifuged for 10 minutes at 1,000 rpm at 4 degrees centigrade. Serum was removed from the red top tubes, aliquoted (500 µl), and frozen at -80 degrees centigrade for storage until used for analysis of the serum vitamin D levels in the parent study.

Laboratory Analysis for Serum Calcidiol Levels

Circulating calcidiol levels were analyzed in the parent study using the Immunodiagnostic Systems LTD (IDS) 25-Hydroxy Vitamin D enzyme-immunoassay

(EIA) kit for quantitative determination of calcidiol in serum (Appendix D). Samples, controls and calibrators were diluted with biotin labelled 25-OH D. To perform the analysis, 25 µl of serum was placed in a labelled borosilicate glass or polypropylene tube then 1 ml of biotin solution (which dissociates the vitamin D from vitamin D binding protein) was added and mixed by a thorough vortex for 10 seconds. A plate with microtitre wells coated with sheep 25-OH D antibody was used and each well was filled with 200 µL of the diluted sample. The plate was sealed and incubated for 2 hours at room temperature (18-25 degrees centigrade). The plate was washed three times with a specific washing solution (WASHBUF) included in the kit. Next, an enzyme conjugate which selectively binds to the Vitamin D-biotin complex was added to each well and the covered plate was again incubated for 30 minutes at room temperature (18-25 degrees C). The wash step was repeated using the WASHBUF (as above), then a chromogenic substrate was added to the samples then the reactions was stopped by the addition of a hydrochloric acid solution. Within 30 minutes, a microtitre plate reader is used to measure the absorbance of the stopped reactions in each well with the color intensity inversely proportional to the 25-OH D concentration.

Laboratory Analysis of Blood Glucose

The blood glucose level was drawn during usual prenatal care at 24-28 weeks gestation as part of screening for gestational diabetes mellitus. Women ingested a beverage containing 50 grams of glucose, the time was noted, and a blood glucose levels was drawn in one hour per venipuncture by clinic staff. The blood glucose testing was analyzed in the clinic laboratory using standard operating procedures and the results were

entered into the medical record. The blood glucose levels used in this study were obtained from the medical record abstraction.

The blood sample is placed in either a green top or an SST tube. A label is placed lengthwise on the tube with bar codes which contain the patient identifiers and the laboratory test ordered. The tube is placed in a Megafuge 10 and spun at 32 revolutions per minute for 10 minutes to separate the plasma and the red blood cells. The rubber stopper is then removed from the tube, the tube is placed in a sample carrier, and the sample carrier is placed in the appropriated bay of the Architect ci 8200. The machine reads the bar code and automatically runs the remainder of the test then prints out results for interpretation. The lab protocols include calibration of the machine every 720 days and weekly quality control testing (personal communication, Sheila Piper, 10-6-2015).

Data Collection Procedures

The participants reported to the Grand Forks Human Nutrition and Research Center at points for data collection. The first collection point was between 10-14 weeks gestation and included an overview of the study, informed consent, and venipuncture for collection of blood for subsequent vitamin D analysis. Venipuncture was repeated at second and third collection points which were at 22-26 and 32-36 weeks gestation. Each blood sample totaled 45 milliliters (three tablespoons) placed into two red top blood collection tubes for subsequent serum extraction. Serum calcidiol levels were obtained from the serum blood draws at the GFHNRC at the three data collection points—early pregnancy, mid-pregnancy, and late pregnancy. More specifically, the data collection points were at 10-14 weeks gestation (VitD-early), 22-26 weeks gestation (VitD-mid), and 32-26 weeks gestation (VitD-late).

After birth, medical record abstraction was conducted with data recorded using a unique identification unrelated to personal participant information. The data from the medical record included maternal age at delivery, body mass index (BMI), serum blood glucose level at 24-28 weeks of gestation. BMI was calculated using the height and the pre-pregnancy weight or the first weight obtained if the pre-pregnancy weight was not available. Descriptive statistics were used to describe the study population.

Table 1. Description of Variables and Measurements.

Variable	Data Source or Measurement
Demographics	Intake information; medical record
Maternal Age	Medical record; age in years at delivery
Body Mass Index (BMI)—weight in kilograms (kg); height in meters (m)	Medical record; BMI = kg/m^2
VitD-early	Serum vitamin D level (calcidiol) in nmol/L; collected at 10-14 weeks gestation at GFHNRC
VitD-mid	Serum vitamin D level (calcidiol) in nmol/L; collected at 10-14 weeks gestation at GFHNRC
VitD-late	Serum vitamin D level (calcidiol) in nmol/L; collected at 10-14 weeks gestation at GFHNRC
Blood Glucose (BG)	Blood glucose in mg/dl; collected by clinic lab at 24-28 weeks of gestation

Data Analysis

The research questions are listed below as a reference for the discussion on data analysis. This is followed by a description of the data set and specific variables. Finally,

the statistical tests used in the analysis are described in detail. The level of significance was set at $p < .05$ and the measure of variation was standard deviation using the formula:

$$s = \sqrt{\frac{\Sigma(x - \bar{x})^2}{N - 1}}.$$

Research Questions

1. Is there a correlation between the calcidiol level at 10-14 weeks gestation and the blood glucose (BG) level at 24-28 weeks gestation when controlling for the known confounding variables of age and pre-pregnancy BMI?

2. Is there a correlation between calcidiol level at 22-26 weeks gestation and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI?

3. Is there a correlation between the calcidiol level at 32-36 weeks gestation and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI?

Data Set

Data from a total of 52 participants were analyzed to test for a correlation between serum calcidiol levels at three points in pregnancy and the blood glucose (BG) level at mid-pregnancy. The de-identified data used in this secondary analysis was obtained in a SAS file, transcribed onto an Excel spreadsheet, verified with the original data to ensure accuracy, and examined for anomalies and outliers. The data set was then loaded into a file in the Statistical Package for Social Sciences (SPSS) version 22 (IBM). These data included serum calcidiol levels in nmol/L at 10-14 weeks gestation (VitD-early), serum calcidiol levels in nmol/L at 22-26 weeks gestation (VitD-mid), serum calcidiol levels in

nmol/L at 32-36 weeks gestation (VitD-late); blood glucose (BG) at 24-28 weeks gestation; and height and weight which were used to calculate BMI and age.

The numeric value for the BMI was used as a continuous variable in the calculations. Age was a numeric, continuous variable. The numeric value for the serum calcidiol level was used as a continuous variable in the multiple regression analysis and as a categorical variable (inadequate or adequate calcidiol level) for the t-test. The numeric value for the blood glucose (BG) level was used as a continuous variable in the statistical analysis

Descriptive Data

The race was obtained for the sample, the population (North Dakota), and the nation (United States). In order to determine if the sample was representative of the region and the nation, analysis of variance (ANOVA) was performed which tested the differences between the observed and expected proportions. The results provided a basis for generalizability of findings. This is discussed in greater detail in subsequent chapters.

Multiple Regression Analysis

Statistical analysis was performed using SPSS. A multiple regression analysis was utilized to determine if there was a correlation between the independent variables of VitD-early, VitD-mid, VitD-late and the dependent variable, BG. As discussed in Chapters I and II, maternal age at delivery and BMI were known confounding variables. To control for these confounding variables, BMI and age were also included as independent variables in the multiple regression.

A regression analysis was the preferred statistical test to look for an association because all of the variables were continuous in the data set used in this analysis. Data for

each variable were checked for normality and to verify that the assumptions of the statistical procedures were met. Assumptions for linear regression are linearity and additivity, statistical independence, homoscedasticity (constant variance) and normality, and these assumptions were met for the data set. A multiple linear regression was chosen because there was more than one independent variable; a simple linear regression would be used with a single independent and a single dependent variable.

The working hypothesis for this study was that an inverse relationship would exist between serum calcidiol levels during pregnancy and the mid pregnancy blood glucose levels. This hypothesis aligned with the work of other researchers, more specifically that of Maghbooli et al. (2008), Soheilykhah, Mojibian, Rashidi, Rahimi-Saghand and Jafari (2010), and Zhang et al. (2008). A one-tailed test was chosen to test for an inverse relationship between serum calcidiol levels (VitD-early, VitD-mid, and VitD-late) and the BG levels in mid-pregnancy. When interpreting results, a perfect correlation would have been indicated by 1.00; no correlation would have been indicated by 0.00.

Independent Samples *t*-test

The data was coded into categories of inadequate serum calcidiol levels (presence of hypovitaminosis-D) and adequate serum calcidiol levels. This divided the sample into two groups (inadequate and adequate serum calcidiol levels). An independent sample t-test was used to compare means between the two groups and the BG at each of the time points of the each of the serum calcidiol levels (VitD-early, VitD-mid, and VitD-late). Cohen's *d* was included in the statistical results.

Trends in Serum Calcidiol Levels

Mean values for each of the calcidiol levels were determined and compared. Trends were examined in the calcidiol levels across the three time points and the relationship between the variables was determined. Serum calcidiol levels over the three data collection time points were described and compared.

Association Between Confounding Variables

Pearson's correlation coefficient was performed to evaluate the association between the confounding variables. This included the relationship between BG and age as well as the relationship between BG and BMI. It also included the relationship between BMI and serum calcidiol levels, and the relationship between age and the serum calcidiol level.

Protection of Human Subjects

Protection of human subjects was assured. The parent study received Institutional Review Board (IRB) approval by the University of North Dakota IRB and the Altru Health System IRB (Appendix A). Informed consent (Appendix B) was obtained upon enrollment in the parent study which included the variables used in the secondary analysis. Each participant received both written and verbal study information. All were reassured that their medical care was the same whether or not they chose to participate in the study and that there was no obligation to participate. All participants received the usual and customary care provided at their respective institutions. Since the goal of this study was to identify factors exclusive to pregnant women, enrollment of children was inappropriate.

IRB approval was submitted to perform the secondary analysis for this study to both UND and Altru Health System (Appendix A). The project was approved by both agencies and considered an Exempt Review since it was a secondary analysis of a de-identified data and the parent study had previously received IRB approval from both of these same IRBs. According to Doolan and Froelicher (2009), a secondary analysis of an existing data set has the benefit of answering new research questions without putting participants at risk for any harm. Further protection of human subjects is provided from the study design being a secondary analysis of a de-identified data set. The information from the medical record was transcribed onto a Medical Record Abstraction Form (Appendix C) which did not include any identifying information about participants. Linkage between personal identification and unique identification number was known only to the principle investigator of the parent study. The data set used in this secondary analysis was de-identified.

CHAPTER IV

RESULTS

Introduction

The relationship between calcidiol levels at three predetermined points in pregnancy and the blood glucose level at 24-28 weeks gestation are reported. This chapter reports the descriptive statistics for the sample participants and the results of the multiple regression analysis to answer each of the research questions. A comparison of the calcidiol levels at each of the three collection points is reported. The relationship between the variables used in the analysis was determined and reported in this chapter. Finally, the sample findings are compared with the regions, the state, and the nation.

Descriptive Statistics of the Sample Participants

This section describes the characteristics of the sample participants as it relates to the study variables. Age and body mass index (BMI) are known confounding variables so are both presented. Included is a normal distribution curve to illustrate how the sample aligns with a normal distribution. Then the race of the sample participants is discussed and compared with that of the region (the state of North Dakota) and the nation (United States) as these demographics influence the generalizability of the findings from this sample.

Age

The age range in years for the 52 participants in this study was between 18.8 and 35.8 years with a mean of 25.9 (± 4.18). The normal childbearing age is between 18 and 35 years and are within the expected range and normal distribution (Figure 4).

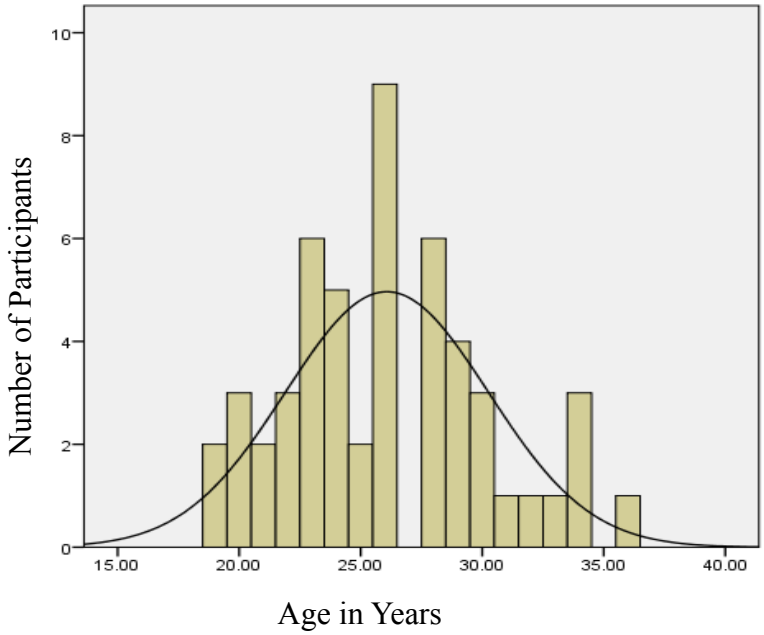


Figure 4. Distribution of age in sample.

Table 2. Age and Body Mass Index.

	Minimum	Maximum	Mean	SD
Age (years)	18.8	35.8	25.9	4.18
BMI (kg/m^2)	19.3	39.4	27.2	5.08

Body Mass Index (BMI)

The BMI was calculated for each of the participants. As a group, the sample was in the overweight category which was determined because the mean BMI was 27.2 kg/m^2

(± 5.08). BMI for the sample ranged from 19.3 to 39.40 kg/m^2 which indicated the sample had women of normal BMI to women in the obese category for BMI. There were no participants with a BMI in the underweight category. A histogram was created to examine the frequency and distribution of the BMI values for the sample and the normal distribution Bell curve was place over the histogram (Figure 5).

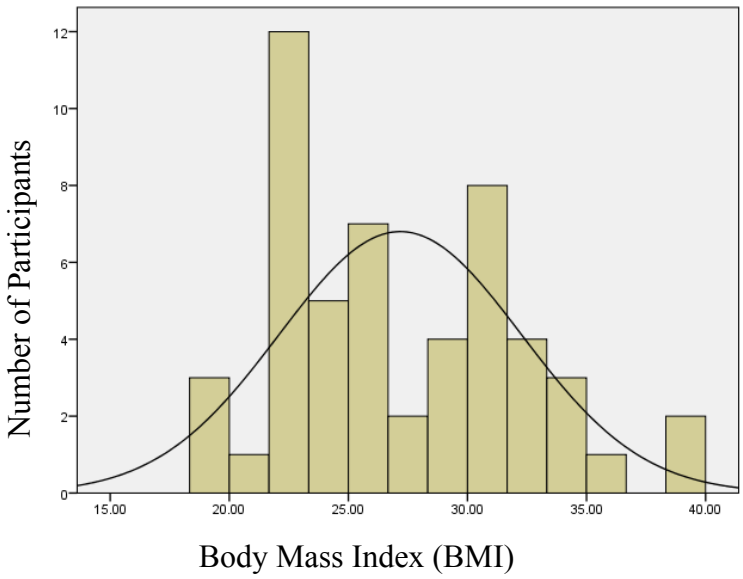


Figure 5. Distribution of BMI in sample.

Race of Study Participants

The participants were asked to self-report their race with the following results: 92.3% (n=48) Caucasian, 1.9% (n=1) Hispanic, and 5.8% (n=3) "mixed race" (Figure 6). The sample is closely representative of the population in North Dakota which has the following statistics on race: 89.6% Caucasian, 2.9 % Latino or Hispanic, 2 % black or African American, 5.4 % American Indian and Alaska Native, 1.2 % Asian, and 1.9 % "mixed race" indicating two or more races (United States Census Bureau, 2013). There were no Native American or African-American participants. This is important because

there is a higher prevalence of impaired glucose metabolism during pregnancy in Native American, African-American, and Hispanic women in the United States than in non-Hispanic white women (Alzaim & Wood, 2013). Barriers for minority participation in research studies include access to care, false perceptions, mistrust (Fisher & Kalbaugh, 2011) and personal circumstances such as job flexibility or lack of awareness about the study (Wendler et al., 2006).

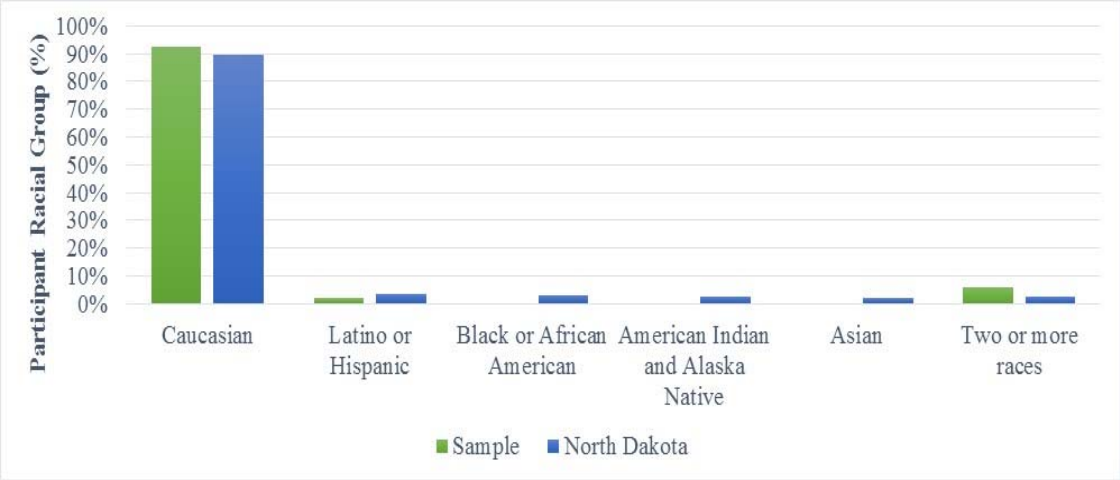


Figure 6. Race of sample and of North Dakota population.

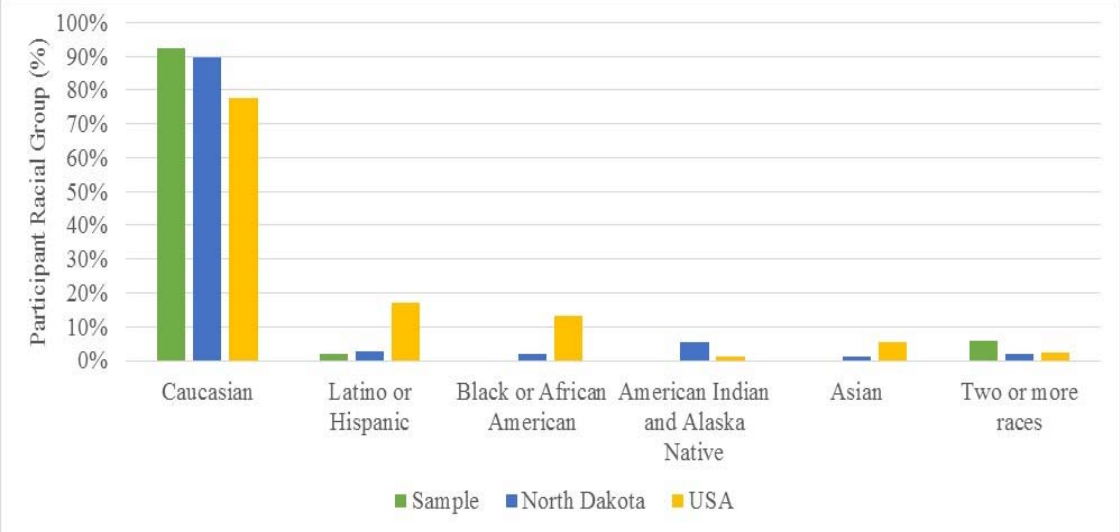


Figure 7. Race of sample, North Dakota population, and of the United States.

Table 3. Race of Sample and Population.

Self-reported race	Sample	North Dakota	United States
Total people	52	723,857	316,497,531
White alone	92.3% (n=48)	89.6%	77.7%
Latino or Hispanic	1.9% (n=1)	2.9%	17.1%
Black/African American alone	0%	1.8%	13.2%
American Indian/Alaska Native	0%	5.4%	1.2%
Asian alone	0%	1.2%	5.3%
Two or more races	5.8% (n=3)	1.9%	2.4%

N=52 in study sample. North Dakota and United States per United States Census Bureau, 2013

The ethnicity was obtained for the sample, the population (North Dakota), and the nation (United States) and is reported above (Figure 7 and Table 11) (United States Census Bureau, 2013). ANOVA was performed to ascertain if the study sample was representative of the population and the nation by testing for the difference between three groups (sample, North Dakota, and United States) the observed and expected proportions. There was not a statistically significant difference between groups at the $p < .05$ level, $F(2, 15) = 01$, $p = .99$.

Blood Glucose Levels

Women are at risk for hyperglycemia during pregnancy due to the physiological changes which occur. Blood glucose levels are obtained at mid-pregnancy in the normal course of prenatal care to screen for hyperglycemia. For the purposes of this study, the blood glucose (BG) levels in mg/dl acquired at the routine mid-pregnancy test were collected through medical record abstraction. The results are summarized below.

In this sample, 5.8 % of the participants had a BG >140 mg/dl. The mean for BG for the sample was 93.8 (± 20.41 standard deviation). As illustrated in Figure 8, this histogram of BG results is plotted with an overlay of a normal distribution Bell curve. This demonstrates that the scores generally follow a normal distribution. There is a negative skew showing that the sample had a higher frequency of BG results which were less than 100 mg/dl. It is visually apparent that the actual frequency of BG values was highest between 75 mg/dl and 100 mg/dl. The significance of this finding will be discussed in Chapter V.

Table 4. Summary of Study Sample Findings for Blood Glucose.

	Minimum	Maximum	Mean	SD
BG (mg/dl)	59.0	157.0	93.8	20.42

Serum Calcidiol Levels

Blood was collected through venipuncture at three pre-determined points in this study to obtain serum calcidiol levels. As discussed previously, serum calcidiol is the

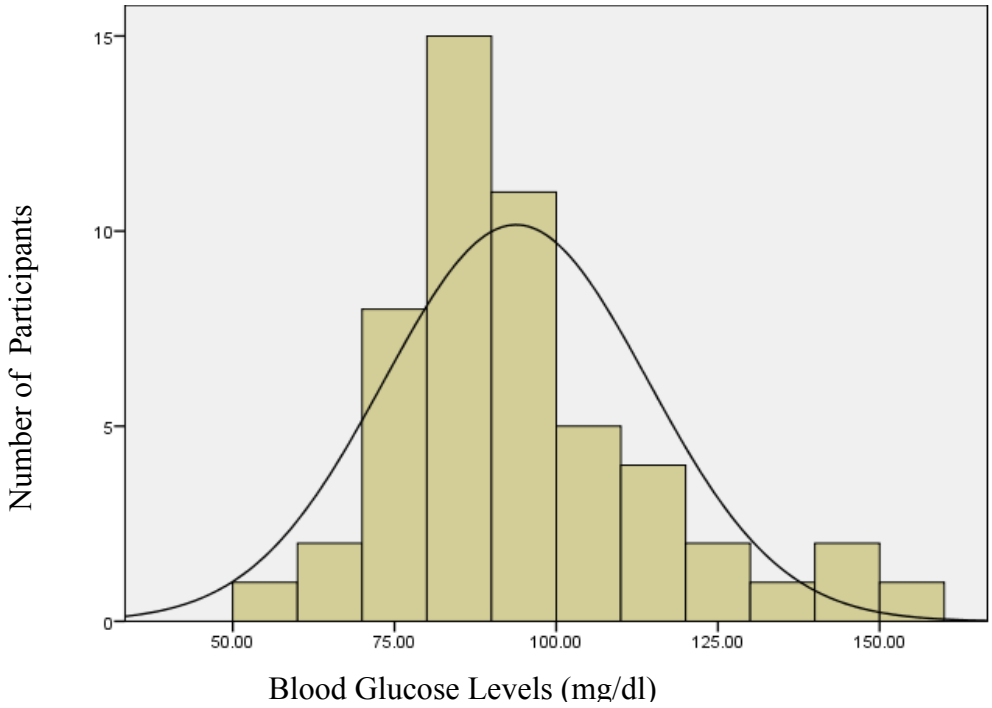


Figure 8. Distribution of blood glucose at 24-28 weeks of gestation in sample.

usual laboratory test to determine vitamin D status. For the purposes of this study, hypovitaminosis-D was defined as a serum calcidiol level of < 75 nmol/L.

Prevalence of Hypovitaminosis-D in Sample

Calcidiol levels for the sample for each of the collection points were examined. Only fifteen percent of the study participants had adequate calcidiol levels at all three of the points measured. Hypovitaminosis-D was present in 81% of the participants at one or more points during the pregnancy and 67.3% with hypovitaminosis-D at all three measurement points. In early pregnancy (VitD-early), 41 out of 52 women (78.9%) had hypovitaminosis-D. In mid pregnancy (VitD-mid), 40 out of the 52 women (77%) had hypovitaminosis-D and in late pregnancy (VitD-late), 37 out of the 52 (71%) of the participants in the sample had hypovitaminosis-D (Figure 9).

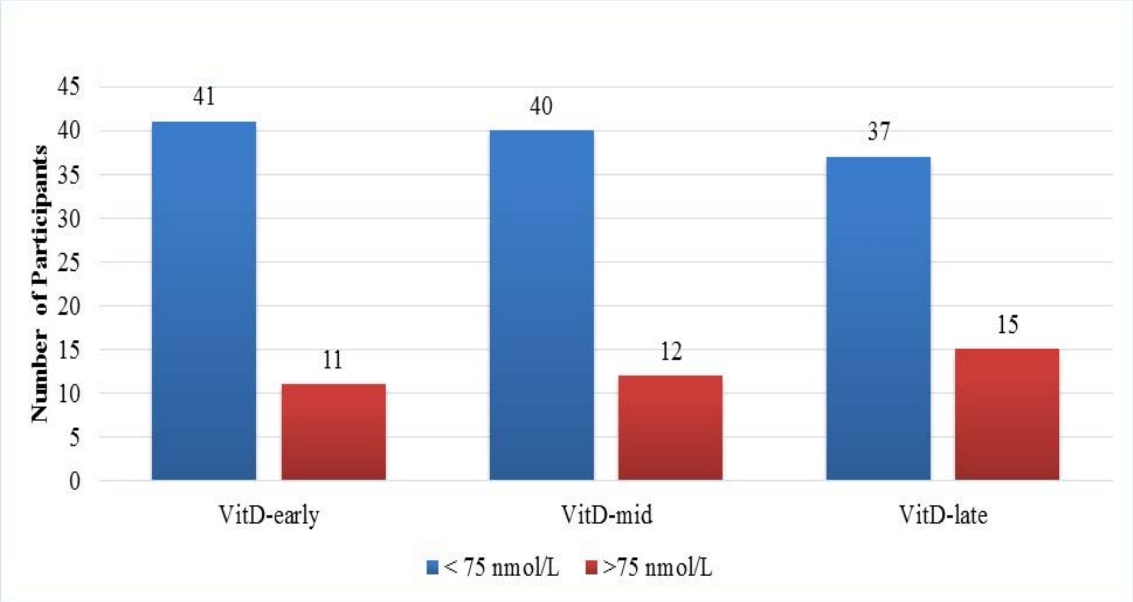


Figure 9. Number of participants by calcidiol level greater or less than 75 nmol/L.

Range of Calcidiol Levels in Sample

The range in the calcidiol levels demonstrates that some participants had levels much higher than 75 nmol/L. VitD-early ranged 41.6-123.5 nmol/L, VitD-mid ranged 36.5-154.3 nmol/L, and VitD-late ranged from 25.3-182.8 nmol/L. The mean at each of these points for the sample was < 75 nmol/L which indicates that there were inadequate calcidiol levels at all points in pregnancy for this sample, as a whole. The mean calcidiol level was 63.3 nmol/L for VitD-early, 66.3 nmol/L for VitD-mid, and 66.4 nmol/L for VitD-late (Table 5).

Scatterplot of Participant and Target Calcidiol Levels

Figure 10 visually demonstrates the calcidiol levels in nmol/L for each participant at each of the three collection points (VitD-early, VitD-mid, and VitD-late) compared to target level of greater than or equal to 75 nmol/L. It is evident that the majority of serum calcidiol levels were less than the target level. A few participants had at calcidiol levels

Table 5. Summary of Study Sample Findings of Calcidiol.

	Minimum	Maximum	Mean	SD
Vit D-early (nmol/L)	41.6	123.5	63.3	19.5
Vit D-mid (nmol/L)	36.5	154.3	66.3	20.6
Vit D-late (nmol/L)	25.3	182.8	66.4	24.5

much lower than the target and a few had a calcidiol level much higher than the target level. The clustering of calcidiol levels between 40 and 75 nmol/L emerged as one of the limitations of the study.

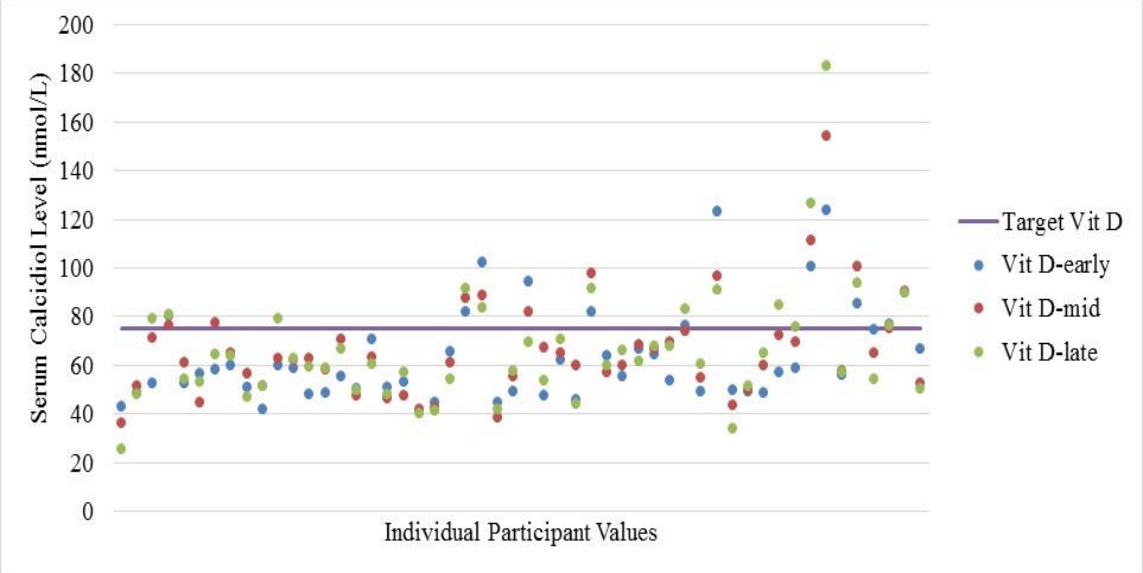


Figure 10. Serum calcidiol levels for each participant at each time point.

Serum Calcidiol Levels Over Time

The mean of the calcidiol levels at each of the three collection points illustrates that the calcidiol level for the sample slightly increased over time (Figure 11). The mean of the serum calcidiol level at 10-14 weeks gestation (VitD-early) was 63.3 nmol/L (±

2.7). The mean serum calcidiol level at 22-26 weeks gestation (VitD-mid) was 65.7 nmol/L (± 2.8). The mean calcidiol level at 32-36 weeks of gestation (VitD-late) was 65.9 nmol/L (± 3.4).

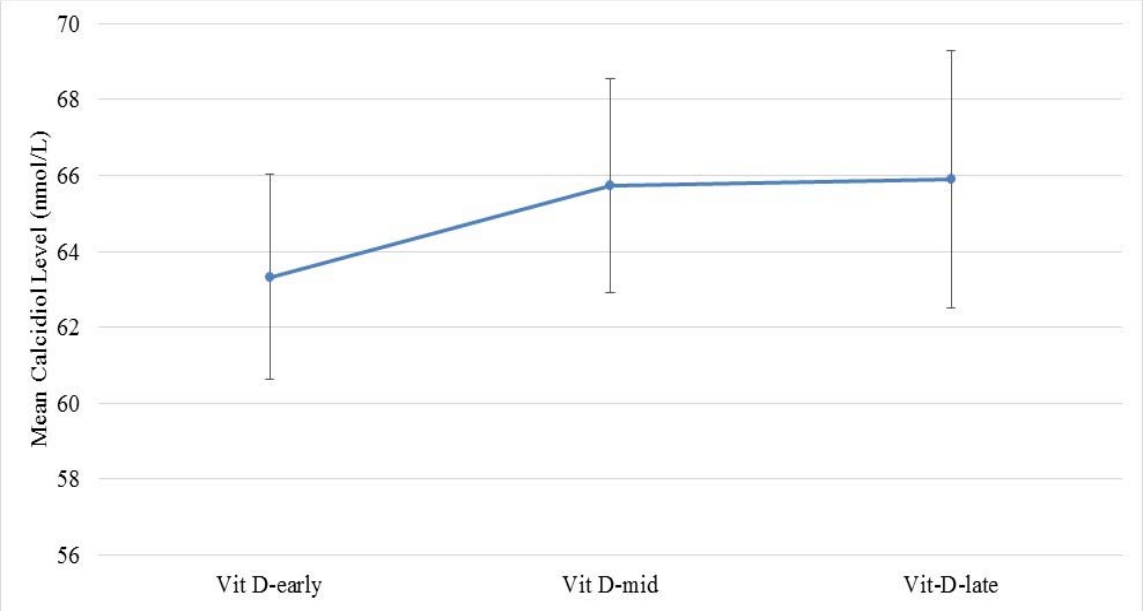


Figure 11. Mean of serum calcidiol levels at each time point (VitD-early, VitD-mid, and VitD-late). Error bars represent standard deviation.

Frequency Distribution of Calcidiol Levels Over Three Collection Points

Although the mean calcidiol levels at the three collection points have a mathematical difference of 2.6, the frequency distribution changes. This distribution moves from being skewed to the left at VitD-early to a more normal distribution at VitD-mid and VitD-late (see Figures 12, 13, and 14). When all of the serum calcidiol levels are analyzed for frequency of occurrence, the distribution has a negative skew (Figure 15) with the majority of the levels lower than the recommended level of 75 nmol/L.

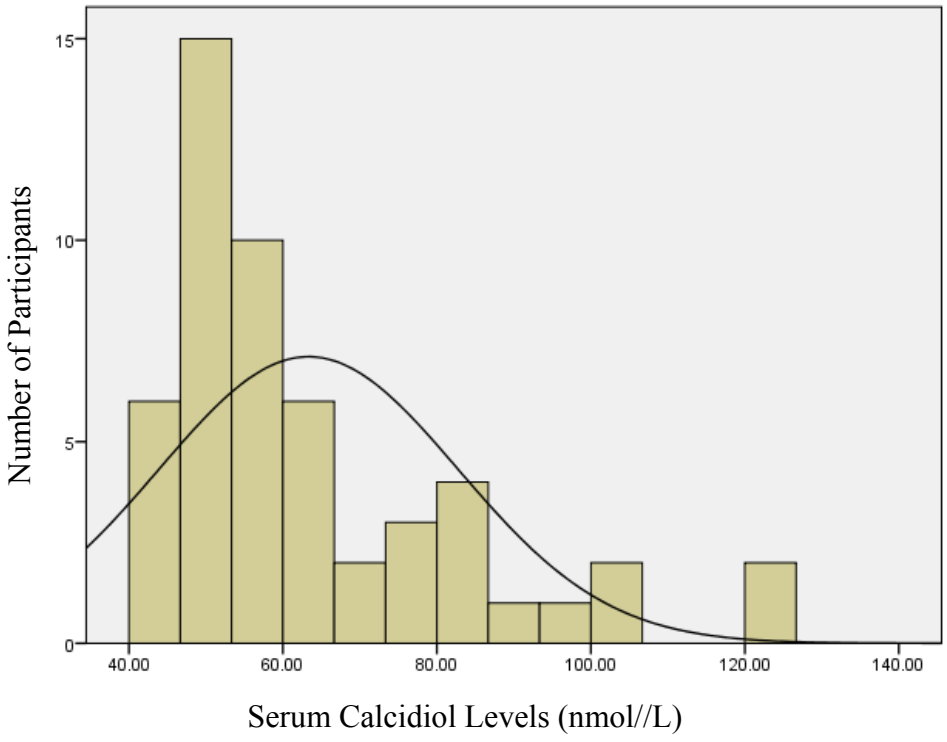


Figure 12. Distribution of serum calcidiol levels at 10-14 weeks gestation (VitD-early).

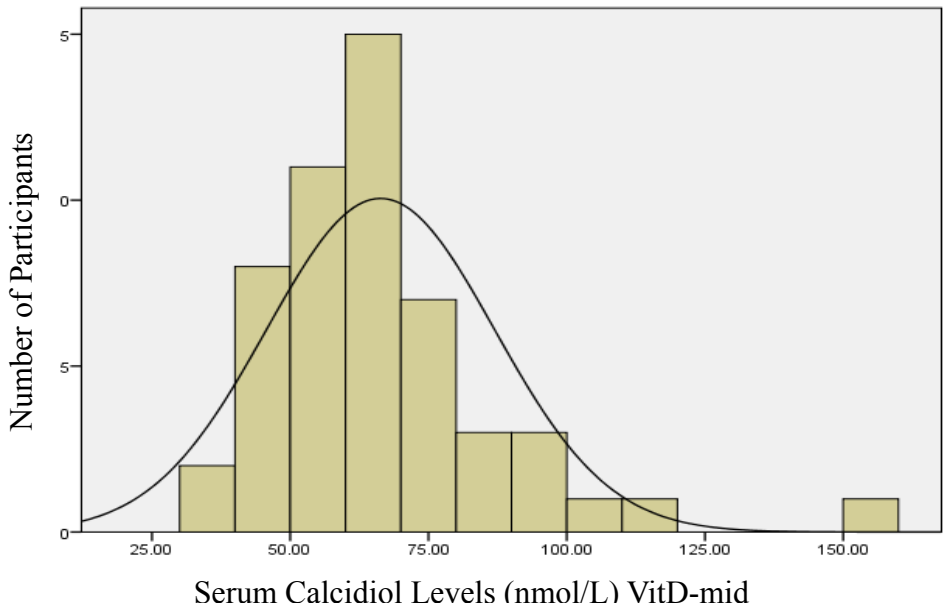


Figure 13. Distribution of serum calcidiol levels at 22-26 weeks gestation (VitD-mid).

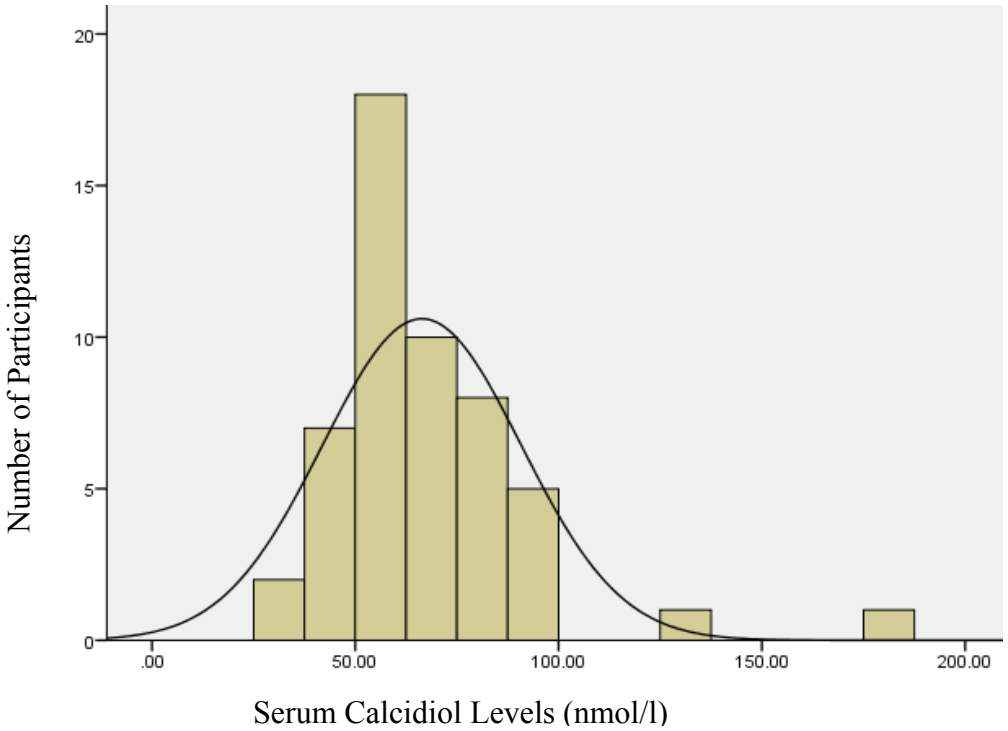


Figure 14. Distribution of calcidiol levels at 32-36 weeks gestation (VitD-late)

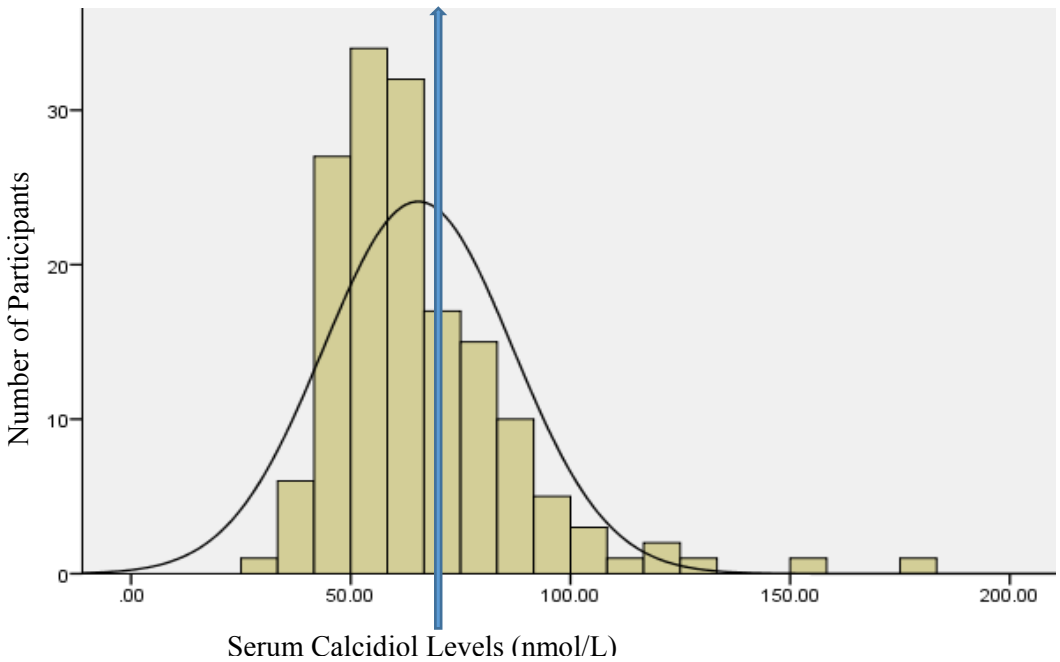


Figure 15. Distribution of All Calcidiol Levels in the Sample and Normal Curve. Arrow is placed at Level of Adequate Calcidiol Level of 75 nmol/L

Correlation in Calcidiol Levels

The trend in the calcidiol levels was analyzed to look for changes over the course of pregnancy. Data were interval and normally distributed so the Pearson correlation was utilized. There was a statistically significant correlation ($p < 0.05$) between the calcidiol levels at each point when compared to the other calcidiol levels for the participants in this sample. However, there was not a statistically significant change in the calcidiol levels over the three collection points (table 6).

Table 6. Pearson Correlation Showing the Trend in Calcidiol Levels.

	VitD-early	VitD-mid	VitD-late
VitD-early	---		
VitD-mid	.87*	---	
VitD-late	.83*	.95*	---

* Significant at $p < .05$

Research Questions and Analysis

This section will focus on the three specific research questions for the study. Multiple regression analysis was utilized to look for a correlation between the calcidiol levels at each of the three predetermined points in time and the blood glucose levels at mid-pregnancy while controlling for the known confounding variables of age and BMI. The findings from analysis will also be presented.

Correlation of Early Calcidiol Level With Mid Pregnancy Glucose Level

R-squared is a statistical value which reflects how closely the data fit the regression line reported between zero (0% correlation) and one (100% correlation). The

amount of variation explained by the regression analysis was 8.4% as evidenced by the R Square of 0.084 (Table 7). There was not a statistically significant correlation between the calcidiol level at VitD-early and BG when controlling for the known confounding variables of age and BMI. The null hypothesis was that there is no relationship between calcidiol levels at VitD-early and BG at 24-28 weeks gestation when controlling for age and BMI. These results failed to reject the null hypothesis for this research question.

Correlation of Mid Pregnancy Calcidiol Level With Mid Pregnancy Glucose Level

The amount of variation explained by the regression analysis was 7.1% as evidenced by the R Square of 0.071 (Table 7). There was not a statistically significant correlation between the calcidiol level at VitD-mid and BG when controlling for the known confounding variables of age and BMI. The null hypothesis was that there is no relationship between calcidiol levels at VitD-mid and BG at 24-28 weeks gestation when controlling for age and BMI. These results failed to reject the null hypothesis for this research question.

Correlation of Late Pregnancy Calcidiol Level With Mid Pregnancy Glucose Level

The amount of variation explained by the regression analysis was 7.5% as evidenced by the R Square of 0.075 (Table 7). There was not a statistically significant correlation between the calcidiol level at VitD-late and BG when controlling for the known confounding variables of age and BMI. The null hypothesis was that there is no relationship between calcidiol levels at VitD-late and BG at 24-28 weeks gestation when controlling for age and BMI. These results failed to reject the null hypothesis for this research question.

Table 7. R Square Analysis of VitD-early, VitD-mid, VitD-late With BG When Controlling for Age and BMI.

	R	R Square	Std. Error
VitD-early and BG	.29	.08	20.1
VitD-mid and BG	.27	.07	20.2
VitD-late and BG	.27	.08	20.2

Constants: Age, BMI

Comparison of BG With Two Groups

The data was divided into two groups, inadequate serum calcidiol levels and adequate serum calcidiol levels. An inadequate serum calcidiol level was defined as being less than 75 nmol/L or the level of hypovitaminosis-D in this study. The two groups were compared to see if there was a difference in the mean blood glucose levels between inadequate and adequate serum calcidiol levels. This comparison was done for each of the time points (VitD-early, VitD-mid, and VitD-late).

Comparison of BG With Inadequate or Adequate Calcidiol in Early Pregnancy

An independent samples t-test was conducted to compare BG in conditions of inadequate and adequate calcidiol levels at VitD-early (using the customary $p < .05$). The independent sample t test showed that the difference in the BG levels between the group with inadequate calcidiol levels (n = 41, M = 95.78, SD = 22.05) and the group with adequate calcidiol levels (N = 11, M = 86.36, SD = 10.20) was not statistically significant, $t(50) = 1.37$, $p = .18$. These results suggest that there was not a statistically significant difference in the BG between the two groups, the group with inadequate

calcidiol levels and the group with adequate calcidiol levels at VitD-early (table 8). Cohen's effect size (d = .47) suggested a moderate practical significance.

An ANOVA was employed with similar results. The difference between the means was not statistically significant at the .05 level (F= .71, df = 9, 40). The critical value of F for the .05 level was 2.82. Since the observed value of F was less than the critical value, the null hypothesis was not rejected.

Table 8. Mean Blood Glucose (BG) in mg/dl for Two Groups at 10-14 Weeks Gestation.

	N	Mean BG level	Standard Deviation
Inadequate calcidiol	41	95.78	22.05
Adequate calcidiol	11	86.36	10.20

Comparison of BG With Inadequate or Adequate Calcidiol in Mid Pregnancy

An independent samples t-test was conducted to compare BG at 24-28 weeks of gestation in conditions of inadequate and adequate calcidiol levels at 22-26 weeks of gestation (VitD-mid). An independent sample t test showed that the difference in the BG levels between the group with inadequate calcidiol levels (N = 39, M = 95.67, SD = 22.30) and the group with adequate calcidiol levels (N = 13, M = 88.15, SD = 12.29) was not statistically significant, $t(50) = 1.15$, $p = .25$. These results suggest that there was not a statistically significant difference in the BG between the two groups, the group with inadequate calcidiol levels and the group with adequate calcidiol levels at 22-26 weeks of gestation (table 9). Cohen's effect size (d = .37) suggested a small to moderate practical significance.

An ANOVA was employed with similar results. The difference between the means was not statistically significant at the .05 level (F = .28, df = 9, 40). The critical value of F for the .05 level was 2.82. Since the observed value of F was less than the critical value, the null hypothesis was not rejected

Table 9. Mean Blood Glucose (BG) for Two Groups at 22-26 Weeks Gestation.

	N	Mean BG level	Standard Deviation
Inadequate calcidiol	39	95.67	22.30
Adequate calcidiol	13	88.15	12.29

Comparison of BG With Inadequate or Adequate Calcidiol in Late Pregnancy

An independent samples t-test was conducted to compare BG at 24-28 weeks of gestation in conditions of inadequate and adequate calcidiol levels at 32-36 weeks of gestation (VitD-late). An independent sample t test showed that the difference in the BG levels between the group with inadequate calcidiol levels (N = 37, M = 95.51, SD = 21.19) and the group with adequate calcidiol levels (N = 15, M = 89.53, SD = 18.33) was not statistically significant, $t(50) = 0.96$, $p = .34$. These results suggest that there was not a statistically significant difference in the BG between the two groups, the group with an inadequate calcidiol level and the group with adequate calcidiol levels at 32-36 weeks of gestation (Table 10). Cohen's effect size value (d = .29) suggested a small practical significance.

An ANOVA was employed with similar results. The difference between the means was not statistically significant at the .05 level (F= .22, df = 9, 40). The critical

value of *F* for the .05 level was 2.82. Since the observed value of F was less than the critical value, the null hypothesis was not rejected.

Table 10. Mean BG for Two Groups at 32 – 36 Weeks Gestation.

	N	Mean BG level	Standard Deviation
Inadequate calcidiol	37	95.51	21.19
Adequate calcidiol	15	89.53	18.33

Specifically, these results suggest that there was not a statistically significant difference at any of the three points in pregnancy (VitD-early, VitD-mid, and VitD-late) in the BG level at 24-28 weeks gestation between the two groups based on inadequate calcidiol levels and adequate calcidiol levels.

Confounding Variables

Two confounding variables were often used in the literature review in the analysis of the relationship between serum calcidiol levels and blood glucose levels during pregnancy. Those two confounding variables were maternal age and BMI. Based on the literature review, both age and BMI were considered confounding variables in this study. These confounding variables were investigated separately below. First, the relationship between BG and the confounding variables of age and BMI were investigated. Then the relationship between the confounding variables of age and BMI and the serum calcidiol levels were examined. The Pearson's r is a measure of effect size in this correlation. Effect size is small when r is 0.2, medium when r = 0.5, and large when r $\geq$ 0.8 (Sullivan & Feinn, 2012). Pearson's r varies from -1 to 1 with the strength of the linear relationship getting stronger as the value is closer to 1 and weaker as the value

approaches zero. The relationship can be either positive or negative which is represented by the positive or negative value of the integer.

Relationship Between BG and the Variables of BMI and Age

Since age and BMI are known confounders of BG metabolism, each of these factors (age and BG; BMI and BG) were examined independently. Data were interval and normally distributed. To determine if there was a relationship between BG and BMI, Pearson's correlation was computed (Table 10). There was not a statistically significant correlation between the BG and BMI, r = 0.25, n = 52, p = .07. To determine if there was a relationship between BG and age, Pearson's correlation was computed (table 11). There was not a statistically significant correlation between BG and age, r = 0.07, n = 52, p = .60. In this sample, neither of the confounding variables of BMI and age had a statistically significant correlation with BG.

Correlation Between BMI and Calcidiol Levels in Early, Mid, and Late Pregnancy

First, a Pearson correlation coefficient was computed to assess the relationship between BMI and VitD-early. There was a statistically significant negative correlation between the two variables of BMI and VitD-early, r = -0.43, n = 52, p = .001. Second, a Pearson correlation coefficient was computed to assess the relationship between BMI and VitD-mid. There was a statistically significant negative correlation between the two variables, BMI and VitD-mid, r = -0.30, n = 52, p = .03. Finally, a Pearson correlation coefficient was computed to assess the relationship between BMI and VitD-late. There was not a statistically significant relationship between the two variables of BMI and VitD-late, r = -0.20, n = 52, p = .16. Overall, there was a negative correlation found between BMI and the calcidiol level at

VitD-early and VitD-mid in pregnancy, but not found at VitD-late in pregnancy. In other words, a higher BMI at the beginning of pregnancy was associated with a lower serum calcidiol level in early and mid-pregnancy (Table 11).

Correlation Between Age and Serum Calcidiol Level

A Pearson correlation coefficient was computed to assess the relationship between age and calcidiol levels at each of the three points. There was not a statistically significant relationship at any of the three points (Table 11). In summary, age did not have a statistically significant correlation with the calcidiol levels.

Table 11. Correlations All Factors.

	Age	VitD-early	VitD-mid	VitD-late	BG	BMI
Age	--					
VitD-early	- .22	--				
VitD-mid	- .23	.86*	--			
VitD-late	- .22	.80*	.95*	--		
BG	- .07	- .16	- .12	- .12	--	
BMI	.12	- .43*	- .30*	- .20	.25	--

* Correlation is significant at the 0.05 level.

Summary

The relationship between calcidiol levels in early, mid, and late gestation and blood glucose levels at 24-28 weeks gestation was investigated in a sample of pregnant women in an upper Midwestern community. The known confounding variables of maternal age and body mass index (BMI) were included in the analysis. Hypovitaminosis-D during pregnancy was found in 81% of the women and there was not

a statistically significant change in serum calcidiol levels for the participants over time as measured by the three collection points. Serum calcidiol levels were drawn at three predetermined points in pregnancy and described as VitD-early, VitD-mid, and VitD-late for the purposes of analysis. The mean of the calcidiol level at VitD-early was 63.3 nmol/L, the mean of the calcidiol level at VitD-mid was 65.7 nmol/L, and the mean calcidiol level at VitD-late was 65.9 nmol/L. The mean calcidiol at each of these time points was below the recommended level of 75 nmol/L. There was no evidence of a statistically significant relationship between the blood glucose level at 24-28 weeks of gestation and the calcidiol level at any of the predetermined points during pregnancy (VitD-early, VitD-mid, and VitD-late) when controlling for the confounding variables of age and BMI. There was not a statistically significant difference in blood glucose levels between the group with inadequate calcidiol levels and the group with adequate calcidiol levels at any of the three points.

The confounding variables were examined in relation to serum calcidiol levels and blood glucose levels. There was a statistically significant inverse relationship between BMI and VitD-early ($p < .05$), and between BMI and VitD-mid ($p < .05$). There was not a statistically significant between BMI and calcidiol levels in late pregnancy. Age did not have a statistically significant relationship with calcidiol at any point or with BMI. A statistically significant relationship did exist between the calcidiol levels at VitD-early, VitD-mid, and VitD-late as well as between BMI and calcidiol levels. The study results will be discussed further in Chapter V.

CHAPTER V

DISCUSSION

Introduction

Chapter V briefly reviews the current study, presents the findings of this research, and integrates the results with the current literature. The discussion includes the rationale which influenced the research based decisions, the characteristics of the sample, and additional findings beyond the specific research questions. This chapter concludes with limitations of the study, recommendations for further research, and implications for nursing.

Summary of the Study

This chapter begins with a brief overview of the problem. The harmful effect of hyperglycemia in pregnancy is highlighted. The potential mitigating influence of calcidiol on hyperglycemia is introduced.

Overview of the Problem

Hyperglycemia during pregnancy can result in adverse outcomes for the mother and the infant with both short term and long term consequences (Pridjian & Benjamin, 2010). The fetus obtains glucose through the placenta by facilitated diffusion (Day, Cleal, Lofthouse, Hanson, & Lewis, 2013) and is therefore dependent upon maternal blood glucose levels (Barta & Drugan, 2010). Even though fetal BG levels are 15 mg/dl lower than the maternal BG levels (Gabbe et al., 2012, p. 542), increased maternal BG

levels have been associated with fetal effects such as overgrowth (The HAPO Study, 2009). Intrauterine hyperglycemia not only results in this abnormal fetal growth but also appears to predispose the offspring for abnormal glucose tolerance later in life (Clausen et al., 2008; Luo et al., 2010). The persistent effects of hyperglycemia in pregnancy may even have generational effects (Vambergue & Fajardy, 2011).

Interventions which can reduce the harm of hyperglycemia are needed. There is growing evidence that vitamin D influences glucose metabolism. Hypovitaminosis-D has been associated with impaired glucose metabolism in non-pregnant and pregnant subjects which is of interest since hypovitaminosis-D can be treated through use of supplements (Alzaim & Wood, 2013). However, the findings on the effects of vitamin D on maternal glucose levels during pregnancy are inconsistent leading to the need for further research.

Purpose Statement

The purpose of this study was to determine the relationship between calcidiol levels collected in early, mid, and late pregnancy and blood glucose levels in mid pregnancy in a sample of women in an upper Midwestern region of the United States. A quantitative, correlational design was used in a secondary analysis to ask new questions of an existing data set. Calcidiol influences glucose metabolism in the non-pregnant population (Alfonso et al., 2009; Mathieu et al., 2005; Pittas et al., 2006; Tohidi et al., 2013) and this study investigated the influence of calcidiol on glucose metabolism in the pregnant population. The significance of this relationship is that it may inform an understanding of the effect of calcidiol on glucose metabolism in pregnancy. By identifying the correlation between calcidiol and glucose levels, there is the potential to mitigate hyperglycemia in pregnancy and improve the health of infants and mothers. The

working hypothesis for this study was that there would be an inverse relationship between calcidiol during pregnancy and mid pregnancy blood glucose levels.

In the professional literature, both maternal age and body mass index (BMI) were considered to be confounding variables in the analysis of calcidiol and BG (Clifton-Bligh et al., 2008; Maghbooli et al., 2008). Consistent with the literature, both BMI and maternal age were considered confounding variables in the current study. This research was designed to answer the following questions on the relationship between calcidiol and BG in the sample group:

Research Questions

This section begins with the research questions answered by this study. The methodology is then reviewed and the major findings are presented. The research questions were:

1. Is there a correlation between the calcidiol level at 10-14 weeks gestation and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and pre-pregnancy body mass index (BMI)?

2. Is there a correlation between calcidiol level at 22-26 weeks gestation and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI?

3. Is there a correlation between the calcidiol level at 32-36 weeks gestation and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI?

Review of the Methodology

This exploratory study was done to examine the relationship between serum calcidiol levels at early, mid, and late pregnancy and the BG levels at 24-28 weeks gestation. A secondary analysis was completed on a data set from a prospective study which used a convenience sample of 52 pregnant women. All participants were nulliparous women enrolled at less than 14 weeks gestation to allow the early pregnancy calcidiol level collected at 10-14 weeks gestation. The sample population was drawn from a community in the upper Midwestern United States at a latitude of 47.93° N. People living at this latitude are at risk for hypovitaminosis-D because the cutaneous production of vitamin D is limited due to the UV spectrum (Holick & Chen, 2008; Schroth et al., 2005). The population of the state has a 31% obesity rate (Levi, Segai, Laurent & Raybum, 2014) which is significant because obesity is associated with hypovitaminosis-D (Hemmings, 2013; Schwanlfenberg, 2007).

In the professional literature, both maternal age and body mass index (BMI) were considered to be confounding variables when investigating calcidiol and BG levels (Clifton-Bligh et al., 2008; Maghbooli et al., 2008). Lower calcidiol levels can be a result of increased BMI and increasing age can result in lower calcidiol levels (Collins-Fulea et al., 2012; Holmes et al., 2009; Prentice, 2008; Wicherts et al., 2011). Concurrently, both maternal age and BMI can influence maternal glucose levels. Maternal age greater than 30 years and maternal BMI in the obese category (> 30) are risk factors for hyperglycemia in pregnancy (Soheilykhah et al., 2010; Zhang et al., 2008). Consistent with the literature, both BMI and maternal age were considered confounding variables in this study.

Major Findings

There was no evidence of a statistically significant relationship between calcidiol levels at any of the three points and the blood glucose levels when controlling for the known confounding variables of BMI and age (R square .084, .071, and .075 respectively). However, the high percentage of hypovitaminosis-D resulted in inadequate variance in this sample to show a difference, if one does exist. Eighty-one percent of the participants had hypovitaminosis-D at some point during pregnancy and 67% had hypovitaminosis-D at all three points during pregnancy.

There was a statistically significant correlation among the calcidiol levels at the three collections points ($p < 0.01$). In other words, maternal calcidiol levels did not statistically change over the course of pregnancy in this sample. The women who had hypovitaminosis-D in early pregnancy continued to have hypovitaminosis-D in mid and late pregnancy. These findings suggest that women who have hypovitaminosis-D in pregnancy may not experience improvement in vitamin D levels over the course of pregnancy.

There was a statistically significant inverse relationship between BMI and the first ($p < 0.01$) and second ($p < 0.05$) calcidiol levels. These results suggest that women with a higher BMI may need additional vitamin D during pregnancy. Cumulatively, these findings suggest that women in the upper Midwest may not be getting adequate vitamin D during pregnancy, especially those with a higher BMI.

Findings Related to the Literature

Each of the three research questions are discussed below and integrated into the literature. First, the confounding variables of maternal age and body mass index are

briefly discussed. This is followed by a more extensive discussion on the more complex topics of blood glucose and calcidiol.

Maternal Age

Maternal age is a known confounding variable in the relationship between calcidiol levels and BG levels. Increasing maternal age can result in lower calcidiol levels (Collins-Fulea et al., 2012; Holmes et al., 2009; Prentice, 2008; Wicherts et al., 2011). Maternal age greater than 30 years is a risk factors for hyperglycemia in pregnancy (Soheilykhah et al., 2010; Zhang et al., 2008). The age range in this study was 18.82 years to 35.80 years with a mean age of 25.87 (+/- 5.13) years. There was not a statistically significant correlation between maternal age and blood glucose (p = .31) nor was there a statistically significant relationship between maternal age and the calcidiol level at 10-14 weeks gestation (p = .12), maternal age, maternal age and the calcidiol level at 24-28 weeks gestation (p = .10), or maternal age and the calcidiol level at 32-28 weeks gestation (p = .12).

Body Mass Index

Excess weight in early pregnancy is associated with adverse complications and places the woman in the high risk category for perinatal management (Bautista-Castano, Henriquez-Sanchez, Garcı´a-Herna´ndez, & Serra-Majem, 2013). A higher BMI is associated with an increased risk of insulin resistance and impaired glucose metabolism during pregnancy (Alberico et al., 2014; Power & Thomas, 2011; Rao, Disraeli & McGregor, 2004). A body mass index (BMI) below 18.5 is considered underweight, 18.5 - 24.9 is categorized as normal, 25.0 – 29.9 is in the obese category, and 30.0 and above is obese (CDC, n.d.).

Since there is a predictable and expected weight gain during pregnancy, the participant's height and either pre-pregnancy weight or first documented weight in early pregnancy were used to calculate the pre-pregnancy BMI using the formula: weight (kg) / [height (m)]^2 (CDC, n.d.). In this study, the mean BMI was 27.21 which is in the overweight category. Two of the three women in the study sample with hyperglycemia had a BMI which put them in the overweight category and the third had a BMI in the obese category.

BMI is considered a confounding variable for serum calcidiol levels because vitamin D is fat soluble and stored in adipose tissue (Achkar et al., 2015; Aloia, 2011; Hemmings, 2013; Holick, 2004). As the amount of body fat increases, there is progressive decrease in serum calcidiol levels (Arunabh, Pollack, Yeh & Aloia, 2003) from irreversible sequestration of vitamin D in the adipose tissue which leads to hypovitaminosis-D, especially if the BMI is > 30 (Hemmings, 2013; Schwalfenberg, 2007). The vitamin D insufficiency associated with obesity may occur because the increased amount of subcutaneous adipose tissue may prevent release of stored vitamin D (Wortsman, Matsuoka, Chen, Lu & Holick, 2000). A statistically significant inverse relationship was found between the calcidiol level at the first and second points during pregnancy and the BMI. Pearson's correlation between VitD-early (the calcidiol level at 10-14 weeks gestation) and BMI was -0.43 (p = .001). Pearson's correlation between VitD-mid (the calcidiol level at 24-28 weeks gestation) and BMI was -0.30 (p = .03). Pearson's correlation between VitD-late (the calcidiol level at 32-36 weeks gestation) and BMI was -0.20 (p = .16). These findings are is consistent with the findings

of other researchers who have found an inverse association between calcidiol levels and BMI (Burris et al., 2012; Ding et al., 2014; Zhang et al., 2008).

Burris et al. (2012) had similar findings in their research with a large sample size in a healthy population. They considered the possibility that hypovitaminosis-D might be part of the causal pathway between obesity and gestational diabetes mellitus. They suggest that vitamin D supplementation for obese women in pregnancy might decrease the risk of developing GDM. Although they did not study pregnant women, Ding et al. (2014) found a negative association between BMI and serum calcidiol levels (p= 0.002) in a cross-sectional study conducted in healthy Chinese adults with normal glucose metabolism. Zhang et al. (2008) completed a nested case controlled study examining factors related to the development of GDM. They found that maternal serum calcidiol levels were inversely associated with pre-pregnancy BMI (p =.01 in controls; p = .04 in those with GDM). The study methodologies, population studied, and sample sizes all differed in these studies but had similar results in finding an inverse association between serum calcidiol levels and BMI in healthy people.

North Dakota ranks 14th in the United States for adult obesity with 31% of adults in the state classified in this category (Centers for Disease Control and Prevention, 2012). Grand Forks County where the most of the participants for this study resided, has a median adult obesity rate of 7.1% (CDC, 2012). Since the people in this region already ranks high in obesity, there needs to be heightened awareness of importance of adequate vitamin D in pregnancy for women with a higher BMI.

Blood Glucose

Blood glucose is an essential element in this study because hyperglycemia during pregnancy can result in harm. The effects of hyperglycemia in pregnancy are presented first. This is followed by a discussion of blood glucose as it pertains to the current study.

Effects of Hyperglycemia in Pregnancy

There are multiple dimensions to hyperglycemia which can impact the mother and her baby including an increased risk of an adverse outcome to the pregnancy (Santamari et al., 2011) because blood glucose levels during pregnancy have a significant influence on both maternal and fetal morbidity (Flack, Ross, Ho, & McElduff, 2010) and long-term effects exist beyond the newborn period and may contribute to the increasing rates of obesity, glucose intolerance, and diabetes in the future (Lehnen et al., 2013; Luo et al., 2010; Ryan, 2012; Veeraswamy et al., 2012).

Rationale for Choice of Blood Glucose Level

Consistent with the American Diabetes Association (2015), a normal blood glucose level at 24-28 weeks of <140 mg/dl was considered to be normal and a level greater than this was considered hyperglycemia. Much of the professional literature reports on impaired glucose metabolism in pregnancy or on GDM but not specifically on hyperglycemia in pregnancy. For the purposes of this study, GDM or impaired glucose metabolism were considered to be proxy to hyperglycemia in pregnancy.

Blood Glucose in This Study

There were a total of 52 study participants. Three participants (5.8%) had a blood glucose level > 140 mg/dl which was the defining point for hyperglycemia. This level is consistent with other results in the region. In 2013, 5.1% (n=544) North Dakota residents

had a diagnosis of GDM during pregnancy according to birth certificate information from the Division of Statistics at the ND Department of Health (personal communication, Dr. S. Pickard and C. Barth, 3/26/2015). The Pregnancy Risk Assessment Monitoring System (PRAMS) survey of 2002 (the last year of participation by the state of North Dakota) reported that 6.8% of the survey respondents in North Dakota had hyperglycemia during pregnancy (DeSisto, Kim, & Sharma, 2014). DiSisto et al. (2014) reported that there was a 9.2% prevalence of hyperglycemia during pregnancy in the United States using PRAMS data and birth certificate information from the seventeen states that participated in 2010. Therefore, the study findings are closely representative of the state where the participants reside but is not representative of the nation (Figure 14).

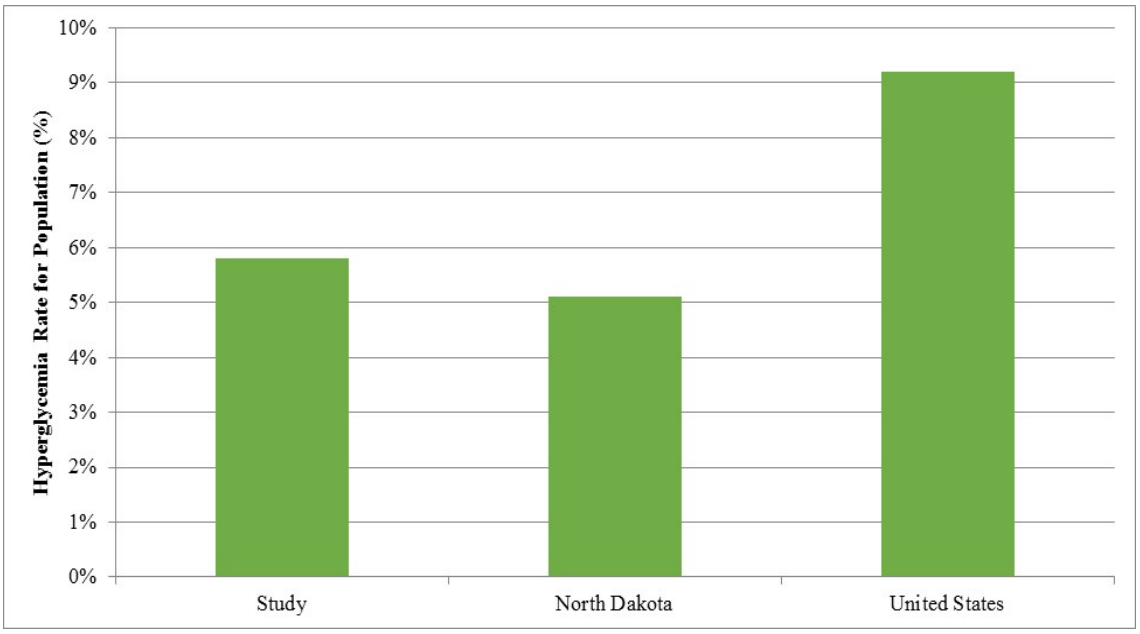


Figure 16. Rates of hyperglycemia in the study sample compared to PRAMS results for North Dakota and the United States. (DeSisto et al., 2014)

Calcidiol

The serum calcidiol level is used to assess vitamin D status (Yap et al., 2014) yet consensus has not been reached on what level of calcidiol is adequate for health (Aloia, 2011; Holick, 2007; Holick et al., 2011; Ross et al., 2011). The development of dietary reference intakes by the Institute of Medicine (IOM) was a result of a joint effort by the governments of the United States and Canada (Aloia, 2011). The appointed committee of scientists reviewed the literature and based their recommendations from the evidence of vitamin D for bone health yet determined there was insufficient evidence for causality or a dose-response relationship for any extra-skeletal vitamin D indications (Aloia, 2011; IOM, 2011). The IOM also concluded that a calcidiol level > 50 nmol/L is adequate and that vitamin D inadequacy has been overestimated in North America (IOM, 2011).

Subsequently, The Endocrine Society responded by publishing a Clinical Practice Guideline with several points of disagreement with the IOM (Hollis & Wagner, 2013; Rosen et al., 2012) which then resulted in additional professional discourse in the literature. The Endocrine Society Clinical Practice Guideline was developed by a task force with input from society members and focused on prevention of vitamin D deficiency through evaluation and treatment (Holick et al., 2011). Pramyothin and Holick (2012) bring clarity to the differences by explaining the two organizations reflect different views of the evidence and different goals. In the future, vitamin D studies will guide recommendations based on patient outcomes as a result of various calcidiol levels (Pramyothin & Holick, 2012). The Endocrine Practice Guideline recommends 600-1000 IU in pregnancy for women between 14-18 years of age and 1500-2000 IU for pregnant

women ages 19-50 years of age (Pramyothin & Holick, 2012). The Endocrine Society endorses a serum calcidiol of ≥ 75 nmol/L to be adequate (Holick et al., 2011).

Recommended Daily Allowance for Calcidiol

The IOM set the recommended daily allowance for vitamin D at 600 IU daily for persons ages 1 – 70 assuming minimal daily sunlight exposure and with no change in the recommended daily allowance in pregnancy (Ross et al., 2011). The Endocrine Society recommends pregnant women take at least 600 IU of vitamin D daily and recognize that some pregnant women may need 1500-2000 IU daily to maintain adequate calcidiol levels (Holick et al., 2011). Schwalfenberg (2007) recommends individualized dosing of vitamin D supplements to restore serum calcidiol levels to >80 nmol/L and reported that both insulin production and sensitivity improved from this intervention. However, according to Schwalfenberg (2007), between 1800-2200 IU of vitamin D is needed daily to achieve and maintain serum calcidiol levels at the target of >80 nmol/L.

Rationale for Choice of Calcidiol Level

Hollis et al. (2012) questioned if the recommendations for daily intake of vitamin D need to change during pregnancy because optimization of the active form of vitamin D, calcitriol, occurs only after the circulating calcidiol levels reach 100 nmol/L. The IOM used skeletal benefits alone to develop their guidelines (Aloia, 2011; Pramyothin & Holick 2012) while The Endocrine Society endorses a serum calcidiol level of 75 nmol/L for the non-skeletal benefits in their published Clinical Guideline (Holick et al., 2011). Since the influence of vitamin D for this research is for one of the non-skeletal benefits of vitamin D, a serum calcidiol level less than 75 nmol/L was considered to be

hypovitaminosis-D. In this study, the mean calcidiol level for VitD-early was 63.32, for VitD-mid was 65.73, and for VitD--late) was 65.89.

Table 12. Comparison of Recommended Serum Calcidiol Levels for Pregnant Women Ages 18-50 years.

Calcidiol status	Institute of Medicine	NIH Office of Dietary Supplements	United States Endocrine Society
Calcidiol deficiency	< 30 nmol/L	< 30 nmol/L	< 50 nmol/L
Inadequate/insufficient	< 40 nmol/L	30-50 nmol/L	52.5-72.5 nmol/L
Considered adequate	> 50 nmol/L	≥ 50 nmol/L	≥ 75 nmol/L
No additional benefit for bone health	> 75 nmol/L	Not applicable	Not applicable
Potential adverse effects	> 125 nmol/L	> 125 nmol/L	>150 nmol/L

Holick et al., 2011; IOM, 2011

Hypovitaminosis-D in North Dakota

Hypovitaminosis-D is of particular concern for people in North Dakota. Researchers at the Grand Forks Human Nutrition Research Center (GFHNRC) evaluated the diets of 224 women aged 40 years or more who had participated in a study at the center. Based on their results, they reported the average intake of vitamin D was 135 IU per day and concluded that most people living in the state are at risk for hypovitaminosis D (Nielsen, 2011). The people who participate in the GFHNRC studies live in communities near the GFHNRC and therefore are at risk for inadequate daily sunlight exposure. Americans living in the northern latitudes do not have adequate daily exposure

to sunlight and therefore need vitamin D supplements or fortified foods to meet the minimum daily intake of vitamin D.

Influence of the Placenta on Calcidiol

Pregnancy is a unique physiological condition because there is a placenta and a fetus. Physiological changes during pregnancy affect both vitamin D metabolism and transport resulting in an increase the circulating levels of calcitriol with little effect on the serum calcidiol levels. The mechanisms for these effects are not fully understood but both the kidney and the placenta contribute to the increased levels of calcitriol (Brannon & Picciano, 2011). The placenta has a functional endocrine system and is a known site for activation of calcidiol (Anderson et al., 2014; Barrera, Avila, Hernández, & Méndez, 2008). It contains extra-renal cells which can transform calcidiol from the maternal circulation into calcitriol. It has been proposed that the placental intervillous space could potentially be a site for admixture of maternal circulation calcidiol and the calcitriol from the fetal side of placental circulation (Adams & Hewison, 2012; Brannon & Picciano, 2011). It is yet unknown if this contributes to the calcitriol in maternal or fetal circulation (Adams & Hewison, 2012). Even though serum calcidiol levels do not increase in pregnancy, calcitriol levels gradually increase for some unknown reason, starting at about 10-12 weeks until almost double in near the end of pregnancy (Alzaim & Wood, 2013; Brannon & Picciano, 2011).

In laboratory studies, the overall data indicates that the human placenta not only has the components required for vitamin D signaling, it also has the enzymes to synthesize large amounts of calcitriol which is secreted by the placenta (Anderson et al., 2014; Avila et al., 2004; Shin et al., 2010). In addition, the kidneys increases the

synthesis of calcitriol during pregnancy and this higher concentration in maternal serum which could also facilitate transfer to the fetal circulation. When calcidiol concentration is diminished such as during vitamin D deficiency, the healthy kidney increases calcitriol production (Adams & Hewison, 2012). These factors may contribute to the fact that the maternal serum calcitriol levels increase until they are two-fold higher in the third trimester than in women who are not pregnant (Parlea et al., 2011).

Calcidiol in This Study

In this study, 81% of the participants had hypovitaminosis-D at one or more points during pregnancy and 67% had hypovitaminosis-D at all three points. It is unclear how these findings should be interpreted because only two previous studies were available reporting the vitamin D status for the region to compare with the findings in this sample. Anderson et al. (2014) studied vitamin D and pregnancy outcomes found hypovitaminosis-D in 69.5% of participants living in the same area of the Midwest. Thiele (2014) completed research in the same region and found that 61% (8 of 13) of the participants in the study had hypovitaminosis-D at enrollment. People who live at latitudes higher than 37 N are especially at risk for hypovitaminosis-D because the angle of the sun's rays prevents adequate UVB radiation for synthesis of vitamin D through the skin (Bodnar et al., 2007). Anderson et al. (2014) did not find that season of the year significantly influence serum calcidiol level or pregnancy outcomes.

Serial Calcidiol Levels During Pregnancy

Few studies were found in the literature which showed serial calcidiol levels during pregnancy which approximated the time frames in this study. Ainy, Ghazi, and Azizi (2006) noted hypovitaminosis-D of 48 Iranian women in Tehran; yet, they did find

that serum calcidiol levels increased as pregnancy progressed through the three trimesters. Marwaha et al. (2011) found a high prevalence of hypovitaminosis-D throughout the course of pregnancy for women in India. Zhao et al. (2014) found hypovitaminosis-D in all three trimesters when retrospectively testing samples obtained from 50 women during pregnancy in Northeast China where they did not take vitamin supplements. Hypovitaminosis-D was prevalent at all three points in these studies showing that the calcidiol levels did not adequately improve over time and that hypovitaminosis D was a major problem during pregnancy (Zuhur, Erol, Kuzu, & Altruntas, 2013). This supports the findings of Schroth et al. (2005) who stated intake of vitamin D fortified foods or regular multivitamins may not increase the serum calcidiol to an adequate level for maternal and fetal health. Tande et al. (2013) recommend interventions to target the nutritional status of the mother in early pregnancy because they provide an opportunity to improve pregnancy outcomes during this critical period for development of both the fetus and the placenta. Improving hypovitaminosis D is of paramount importance and readily amenable by treating with vitamin D supplements (Alzaim & Wood, 2013; Zuhur et al., 2013).

Study Findings

In this study sample the percentage of women with hyperglycemia in pregnancy was 5.8%. This was in spite of the level of hypovitaminosis-D, the lack of a statistically significant change in serum calcidiol levels, and the mean BMI of 27.21 which was in the overweight category. According to the Pregnancy Risk Assessment Monitoring System (PRAMS) report, the rate of hyperglycemia for the state (North Dakota) was 5.1% and for the nation was 9.2% (DeSisto et al., 2014). The percentage of women with

hyperglycemia during pregnancy in the study sample was similar to population of the state but not of the nation. This limits the generalizability of the study findings to the region.

Table 13. Research Findings of Calcidiol Levels at More Than One Point in Pregnancy.

	First trimester or VitD-early	Second trimester or VitD-mid	Third trimester or VitD-late
Ainy, Ghazi, Azizi, 2006	51.5 nmol/L	64.3 nmol/L	71.8 nmol/L
Marwaha et al., 2011	23.4 nmol/L	25.7 nmol/L	27.7 nmol/L
Zhao et al., 2014	28.29 nmol/L	39.23 nmol/L	40.03 nmol/L
This study	63.32 nmol/L	65.7 nmol/L	65.9 nmol/L

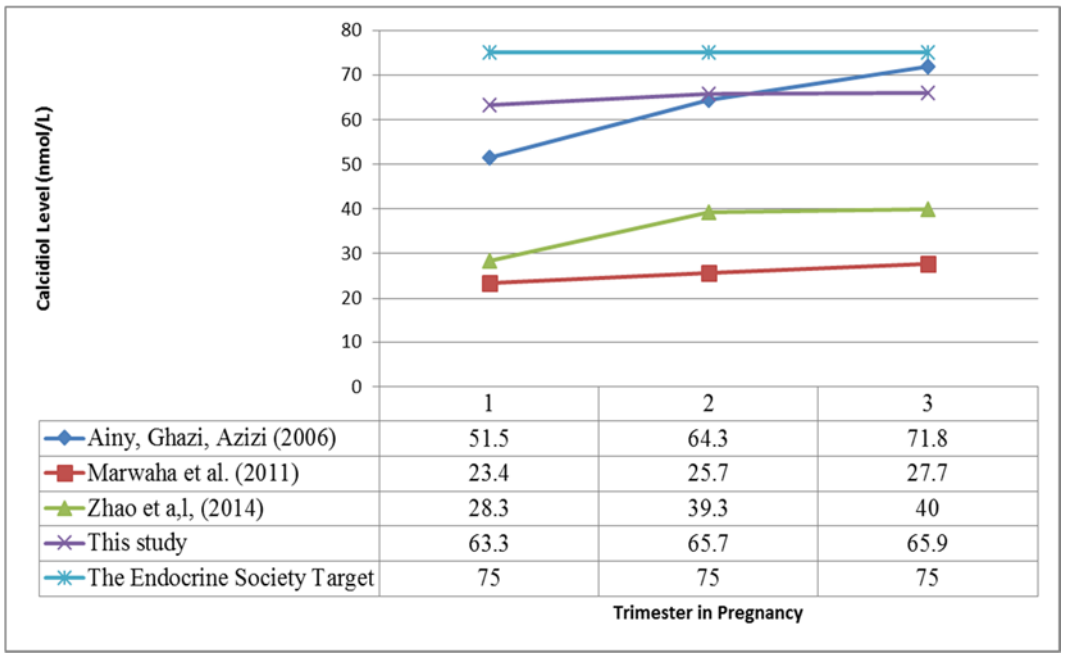


Figure 17. Calcidiol levels at three points in pregnancy.

Calcidiol and Blood Glucose in Pregnancy

There are conflicting findings on the influence of calcidiol on blood glucose metabolism in pregnancy and inconsistent methodology utilized in the research. Some investigators have reported a relationship between hypovitaminosis-D during pregnancy and impaired glucose metabolism (Alzaim & Wood, 2013; McLeod et al., 2011; Shin et al., 2010) or an increased risk for gestational diabetes mellitus (Aghajafari et al., 2013; Cho et al., 2013; Parlea et al., 2011; Zhang et al., 2008). Other researchers report they found an inverse relationship between vitamin D levels and fasting glucose in pregnant women (Burris et al., 2102; Clifton-Bligh et al., 2008; Maghbooli et al., 2008; Soheilykhah et al., 2010). In contrast, when pregnant women had mostly adequate calcidiol levels, researchers did not find a relationship between maternal calcidiol levels and hyperglycemia (Baker et al., 2012; Tomedi et al., 2013). Poel et al. (2012) also completed a systematic review and meta-analysis and found a significant inverse association between calcidiol and the incidence of impaired glucose tolerance in pregnancy but state a causal relationship has not been proven.

In pregnancy, screening for hyperglycemia is done in mid-pregnancy as part of routine prenatal care because the gestational steroid hormones cause a gradual increase in peripheral insulin resistance which intensifies after mid-pregnancy (Alzaim & Wood, 2014). This acquired insulin resistance in a normal human pregnancy is estimated to be between 40% and 70%, predominantly in the last trimester (Pridjian & Benjamin, 2010; Retnakaran et al., 2008; Yap et al., 2014). The insulin resistance results in a significant need for additional insulin secretion, therefore women with impaired pancreatic β-cell reserve pre-pregnancy are at risk for developing impaired glucose metabolism such as

occurs in gestational diabetes (Retnakaran et al., 2008; Yap et al., 2014). The combination of increasing insulin resistance, reduced pancreatic β-cell function, and insufficient secretion of insulin leads to the hyperglycemia which some women experience during pregnancy (Pridjian & Benjamin, 2010; Wang et al., 2013).

Some investigators found evidence which links hypovitaminosis-D and abnormal glucose metabolism in pregnancy (Alzaim & Wood, 2013; Cho et al., 2013; Clifton-Bligh et al., 2008; Pittas & Dawson-Hughes, 2010; Maghbooli et al., 2008). Asemi et al. (2013) found that vitamin D supplementation during pregnancy had a beneficial effect on glycemic control in women with impaired glucose metabolism. Yap et al. (2014) investigated the effects of vitamin D supplements in either 400 IU or 5000 IU daily in pregnant women with calcidiol < 80 nmol/L on the maternal glucose levels during an oral glucose tolerance test at 26-28 weeks gestation. They reported that the high dose vitamin D intervention starting at a mean of 14 weeks gestation did not improve the maternal glucose levels (Yap et al., 2014). These two examples illustrate the inconsistent findings noted by researchers on the relationship between vitamin D and hyperglycemia in pregnancy (Alzaim & Wood, 2013; Collins-Fulea et al., 2012; Dror et al., 2011). Some clarity may emerge when looking at the findings based on gestational weeks at the time of the serum calcidiol level to see how the timing of this level relates to impaired glucose metabolism.

Multiple Linear Regression Analysis

According to the literature, both maternal age and BMI are known confounders for serum calcidiol levels and for hyperglycemia in pregnancy. A multiple linear regression is a statistical model which can be used to separate the relationship of interest

from the confounding variables (Pourhoseingholi, Baghestani & Vahedi, 2012). Because of this, a multiple linear regression was employed to control for age and BMI in the analysis of the relationship between calcidiol levels and BG for each of the time points. In the analysis, calcidiol levels (VitD-early, VitD-mid, and VitD-late), BMI, and age were the independent variables and BG was the dependent variable. The results of the findings for each time point are discussed separately and integrated with the current literature.

Relationship Between Calcidiol in Early Pregnancy and Blood Glucose

Is there a correlation between the calcidiol level at 10-14 weeks gestation (VitD-early) and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and pre-pregnancy BMI? The three participants who had hyperglycemia at 24-28 weeks gestation had hypovitaminosis-D at VitD-early. R square was 0.084 which means the amount of variation explained in the regression was 8.4% indicating there does not appear to be a strong correlation VitD-early and BG at 24-28 weeks in this sample.

The current study did not find a statistically significant relationship between calcidiol levels in early pregnancy and BG levels at 24-28 weeks gestation. These findings are similar to Fernandez-Alonso et al. (2012) and Makgoba et al. (2011) who both found high levels of hypovitaminosis-D in their participants yet no association with hyperglycemia. Tomedi et al. (2013) did not find an association between calcidiol levels at < 16 weeks gestation and BG in non-smoking women who participated in their study; however, they did find an inverse association between first trimester calcidiol levels and BG at 24-28 weeks gestation in participants who were smokers. Baker et al. (2013) did

not find a relationship between calcidiol levels in early pregnancy and BG at 24-28 weeks gestation, but a limitation of that study was the low prevalence of hypovitaminosis-D in their participants.

In contrast, Lacroix et al. (2013) investigated calcidiol levels and insulin resistance, and reported a modest association between first trimester hypovitaminosis-D and insulin resistance at 24-28 weeks gestation, which suggests a higher risk for hyperglycemia. Zhang et al. (2008) concluded that hypovitaminosis-D in early pregnancy (< 16 weeks) was associated with an increased risk for hyperglycemia when controlling for maternal age and BMI. They report that the women in their study who had hyperglycemia were older and heavier than the normoglycemic women in their sample. Parlea et al. (2011) identified cases of GDM and matched them to controls, then analyzed frozen, stored serum samples which had been collected from those women at 15-18 weeks gestation. The groups below and above the fourth quartile for calcidiol levels (73.5 nmol/L) were compared. They report a significant association between calcidiol levels <73.5 nmol/L at 15-18 weeks gestation after controlling for maternal age, BMI, and race.

The high prevalence of hypovitaminosis-D in the current study resulted in limited variation in the sample which could have prevented evidence of a relationship between calcidiol and BG, if one did exist. The sample sizes, methods, and early pregnancy calcidiol levels among the research varied and these inconsistencies are a limitation when comparing these studies. The findings in the literature are not conclusive or consistent. To summarize these findings including the current study, there was no statistically significant relationship found between hypovitaminosis-D in early pregnancy and BG at

24-28 weeks gestation; however, hypovitaminosis-D possibly increases the diagnosis of GDM.

The hypothesis for the current study was that there would be an inverse relationship between calcidiol levels in early pregnancy and the BG level at 24-28 weeks gestation. The state of the science does not definitively prove nor disprove this hypothesis. Baker et al. (2012) reported that 73% of the participants in their sample had serum calcidiol levels $\geq$ 75 nmol/L which may have prevented finding the relationship between calcidiol levels in early pregnancy and BG at 24-28 weeks gestation. Their findings included a recommendation that research be done with participants who had a high prevalence of hypovitaminosis-D to determine if there was a correlation between calcidiol levels in early pregnancy and BG levels at 24-28 weeks gestation. The current study addresses that recommendation. The sample for the current study had a high prevalence of hypovitaminosis-D in early pregnancy and there was not a statistically significant correlation to hyperglycemia at 24-28 weeks. Based on the current science, there is no evidence of a statistically significant relationship between calcidiol levels in early pregnancy and BG levels at 24-28 weeks gestation.

Relationship Between Calcidiol in Mid Pregnancy and Blood Glucose

Is there a correlation between calcidiol level at 22-26 weeks gestation (mid) and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI? The three the study participants who had hyperglycemia in mid pregnancy also had hypovitaminosis-D at 22-26 weeks (VitD-mid) in addition to early pregnancy, as noted above. The relationship between VitD-mid and BG was analyzed controlling for maternal age and BMI with an R square of 0.071 which means

the amount of variation explained by the model is 7.1%. There does not appear to be a strong correlation between the calcidiol levels at VitD-mid and the BG levels at 24-28 weeks of gestation in this sample.

When compared with the early and late pregnancy, more studies have been done in mid-pregnancy investigating the relationship between calcidiol and BG levels but there were inconsistent findings. Most of these studies examined the relationship between calcidiol levels and a diagnosis of GDM, which is a condition of impaired glucose metabolism which, left untreated, leads to hyperglycemia in pregnancy. For the purposes of this study, GDM was used as a proxy to hyperglycemia in pregnancy. The time periods being studied varied, even though most were in the second trimester or mid-pregnancy. The method for the blood glucose level was inconsistent with some researchers using a fasting BG level, others using a BG level obtained after either a one-step GDM screening test or others after a two-step GDM screening test. The calcidiol levels used to define hypovitaminosis-D also varied in these studies.

McLeod et al. (2011) examined a subset of the HAPO study and looked at the calcidiol level drawn concurrently as a fasting BG level at 24 to 32 weeks of gestation. They found calcidiol levels were inversely related to the fasting BG levels which suggests that glucose metabolism might be influenced by calcidiol. Most of their participants were normoglycemic and there was an extremely low prevalence of hypovitaminosis-D in their sample because of subtropical location. Zuhur et al. (2013) obtained serum calcidiol levels when testing for impaired glucose tolerance in pregnancy and found that only the pregnant women severely deficient for calcidiol (<12.5 nmol/L) were at risk for hyperglycemia.

Other researchers report an inverse association between mid-pregnancy calcidiol and BG at 24-28 weeks gestation. Burris et al. (2012) found an inverse association between calcidiol levels and blood glucose levels at 26-28 weeks gestation. They reported that women with severe calcidiol deficiency (serum levels <25 nmol/L) had a significantly increased risk of GDM. Soheilykhah et al. (2010) and Zhang et al. (2008) found that hypovitaminosis-D in the second trimester was associated with a 2.66-fold increase in hyperglycemia at 24-28 weeks gestation. Clifton-Bligh et al. (2008) found an inverse correlation between calcidiol levels and fasting BG when controlling for maternal BMI. Maghbooli et al. (2008) defined hypovitaminosis-D as serum calcidiol <25 nmol/L) with hypovitaminosis-D in 70.6% of the women which they report was associated with insulin resistance.

The current study did not find evidence of a significant relationship between calcidiol drawn in mid-pregnancy (22-26 weeks gestation) and the BG level at 24-28 weeks gestation when controlling for maternal age and BMI. These findings were similar to those of Zuhur et al. (2013) who did not find a statistically significant relationship between deficient and insufficient levels of calcidiol and impaired glucose metabolism unless the hypovitaminosis-D was severe at < 12.5 nmol/L. Similarly, Tomedi et al. (2013) did not find an association between calcidiol levels at < 16 weeks and hyperglycemia in non-smoking pregnant women. As with the studies on calcidiol levels in early pregnancy, there was variation in the collection times for the calcidiol level, the definition of hypovitaminosis-D, and the method for obtaining the BG (fasting BG level versus a BG level obtained after either a one-step or a two-step GDM screening test).

The inconsistent findings across studies prevent conclusive evidence of the relationship between hypovitaminosis-D in mid pregnancy and BG levels at 24-28 weeks gestation.

Relationship Between Calcidiol in Late Pregnancy and Blood Glucose

Is there a correlation between the calcidiol level at 32-36 weeks gestation (late) and the BG level at 24-28 weeks gestation when controlling for the known confounding variables of age and BMI? Two of the three participants with hyperglycemia at the GDM screening test also had hypovitaminosis-D at 32-36 weeks gestation (VitD-late) and one had adequate serum calcidiol at this third measurement point. The relationship between calcidiol at 32-36 weeks gestation (VitD-late) and BG at 24-28 weeks gestation was analyzed, controlling for maternal age and BMI. The R square was 0.075 which means the amount of variation explained by the model is 7.5%. There does not appear to be a strong correlation between VitD-late and BG at 24-28 weeks gestation in this sample. This is consistent with the literature.

Three other researchers reported on the relationship between calcidiol levels and BG levels in late pregnancy, but none of the calcidiol levels were obtained after 32 weeks gestation. Since the current study examined the relationship between calcidiol levels at 32-36 weeks of gestation and BG, this which limits application of the study findings. For comparison, studies which examined the relationship between calcidiol levels in the third trimester of pregnancy and BG levels at mid-pregnancy were utilized. Farrant et al. (2009) reported an inverse association between calcidiol levels at 30 weeks of gestation and the blood glucose level at a 30 minute glucose screening test. Lau et al. (2011) found that lower serum calcidiol levels in the third trimester of pregnancy were independently associated with impaired glucose metabolism. As noted above, McLeod et al. (2011)

found vitamin D levels were inversely related to fasting BG levels in samples drawn at the same time from participants between 24 to 32 weeks of gestation. Based on the current science, there is no evidence of a statistically significant relationship between calcidiol levels in late pregnancy and BG levels in mid-pregnancy.

Limitations

This study examined the relationship between serum calcidiol levels at three points in pregnancy and the blood glucose level at mid-pregnancy. There are several limitations to this study. This was a correlation study and this type of research cannot prove causation by linking exposure to outcome for individuals. It can, however, link the association by showing a positive or negative relationship between two variables, but this association does not prove causation (Grimes & Schultz, 2002).

Research design was influenced by the review of the literature and use of a secondary analysis. Secondary analysis is an efficient and cost effective research design but this method does have inherent restrictions because of the inability to change the sample size (McArt & McDougal, 2007). It is possible that the size of the sample was inadequate to detect the effects of hypovitaminosis-D on glucose metabolism in pregnancy.

The participants were drawn from a geographic location that is fairly homogenous in terms of socioeconomic status, ethnicity, and racial diversity. The participants self-selected by responding to the advertising and volunteering to be in the study. The characteristics of pregnant women who volunteer for research studies compared to women who do not participate in research studies are unknown for this study. Study criteria included English speaking so it is possible that participation was limited to less

diverse ethnic groups. Participants also needed to be at least 18 years of age so a limitation was that the sample did not represent younger women of childbearing potential.

The primary data set included additional information which was beyond the scope of this study. The literature review informed the choice of variables known to influence the outcome of interest in this population. Pre-pregnancy baseline serum calcidiol levels were not known. The source of vitamin D intake (food, supplements, or exposure of unprotected bare skin to direct sunlight) was not included. Factors such as season of the year, education level, marital status, and religion were not included and are a limitation of this study. Although these factors were of interest, they are beyond the scope of the study reported here. Other limitations of this study are discussed further in the concluding remarks.

Calcidiol Testing Processes

Laboratory results inform decision making therefore the accuracy and validity of the testing process is important. Serum plasma calcidiol levels are a good biomarker for the status of vitamin D (van den Ouweland, Beijers, Demacker & van Daal, 2010); however, high variability has been reported in serum calcidiol measurements among laboratories (Binkley et al., 2004). There are two main methods for determining calcidiol levels based either on competitive immunoassay or on chromatographic separation followed by direct detection through non-immunological means (Wallace, Gibson, de la Hunty, Lamberg-Allardt, & Ashwell, 2010). Specificity is problematic for immunoassays due to the proportion of 25OHD2 when quantifying the proportion of calcidiol (Wallace et al., 2010). The methods using chromatography are more specific but

require expensive equipment, more labor intensive, and technically more difficult (Arneson & Arneson, 2013).

All of the discrepancies led to a new program being established in 2011, the Vitamin D Standardization Program (VDSP). The VDSP was formed under the combined efforts of the Center for Disease Control, National Institute of Health Office of Dietary Supplements, National Institute of Standards and Technology, the Belgian Laboratory for Analytical Chemistry, Ghent University, and the National Centre for Environmental Health (Enko, Kriefshauser, Stolba, Worf, & Halwachs-Baumann, 2015). It is anticipated that the work of the VDSP to standardize processes and emerging automated processes will also improve precision (Arneson & Arneson, 2013).

The test used for the serum calicidiol levels for this study was the IDS 25-Hydroxy Vitamin D EIA kit which uses an immunoassay method. The IDS EIA manufacturer reports the sensitivity for their test is with 5 nmol/L (Appendix D). Care was taken to ensure accuracy in testing, however, the validity and reliability of serum calcidiol testing is problematic at this point in time. The availability of consistent, specific, affordable methods is a limitation for studies involving serum calcidiol levels such as this one.

Surprises

There were two surprises which emerged from the current study. The first was the statistically significant impact of BMI on serum calcidiol levels in early pregnancy. With the increasing rate of obesity in the United States, this finding highlights the need for awareness of possible hypovitaminosis-D during pregnancy when women who present for prenatal care have a higher BMI (Wang & Beydoun, 2007). The second was

the lack of evidence of change in serum calcidiol levels when measured in early, mid, and late pregnancy in this study. This is a surprise considering the focus on quality nutrition in pregnancy including taking a daily prenatal vitamin and adequate dietary intake. Hypovitaminosis-D may contribute to a poor foundation for life-long health therefore further research is needed to determine how to ensure women have adequate vitamin D in pregnancy (Kaludjerovic & Vieth, 2010). These findings suggest that prenatal vitamins do not have adequate vitamin D. They also suggest that women with a higher BMI who live in the upper Midwest should have a serum calcidiol level checked in early pregnancy.

Conclusions

The current study examined the relationship between vitamin D measured as serum calcidiol levels and the blood glucose levels in pregnancy. This research examined the correlation between serum calcidiol and BG at three points during pregnancy. The first was early pregnancy defined as 10-14 weeks gestation (VitD-early), the second was mid-pregnancy defined as 22-26 weeks gestation (VitD-mid), and the third was late pregnancy defined as 32-26 weeks gestation (VitD-late). In each case, the confounding variables of maternal age and BMI calculated at the beginning of pregnancy were controlled for in the multiple regression analysis. This study did not find a statistically significant relationship between serum calcidiol levels and blood glucose levels when controlling for the confounding variables of age and BMI at any of the three time points in pregnancy.

Implications for Action

Application of nursing research is an essential component to improving health. The implications for action in response to the current study are presented here. This begins with suggestions for nursing policy, is followed by recommendations for nursing education, and ends with application in nursing practice. The implications for nursing research are in the section on recommendations for further research.

Implications for Nursing Policy

Nursing evaluates the client as a system to ensure optimal wellness according to the Neuman System Model (Neuman & Reed, 2007). Protective internal and external mechanisms (known as Lines of Resistance) both stabilize and support the client system (Neuman, 2005). The goal of nursing, according to the model, is to prevent or correct instability (Meleis, 2010) through nursing interventions which improve health. The physiological stressors of pregnancy can lead to impaired glucose metabolism for some women. Nursing has the potential to improve the health of pregnant women by advising them on the importance of vitamin D during pregnancy and recommending practices to attain adequate intake of vitamin D through food sources and taking a daily prenatal vitamin. The scope of practice for the registered nurse includes assessment of client needs and education to meet those needs. Giving the registered nurse the skills, knowledge and ability to prevent hypovitaminosis-D in pregnancy would be a reasonable application of the Neuman System Model and improve health of the population.

Nurses are in a key position to influence decisions which improve health. The findings of this study and similar studies demonstrate that hypovitaminosis-D may be a problem for many people in North Dakota. Nurses can educate the public on the

importance of vitamin D for health and how to get adequate amounts. Nurses can advocate for higher levels of vitamin D supplementation and calcidiol screening. Considering the high rates of hypovitaminosis-D, the population of pregnant women in this region should be considered at increased risk for deficiency and screened in early pregnancy for hypovitaminosis-D.

Implications for Nursing Education

Several implications which emerge from this research for nursing education. Theoretically, this is an example on how the environment affects the health of patients and demonstrates an application of the Neuman Systems Theory. Nurses need to be aware of the importance of vitamin D within the internal environment of human body and also be knowledgeable on sources of vitamin D from exposure to sunlight, from diet, and from supplements (Brannon & Picciano, 2011; Specker, 2004; Tomedi et al., 2013; Ross et al., 2011). Nursing curricula should include the unique nature of vitamin D as both a vitamin and prohormone along with the implications for health.

Implications for Nursing Practice

Nurses are in a unique position to influence the health of mothers and their offspring by teaching the prenatal patient about the importance of vitamin D. With knowledge about what influences vitamin D status, the nurse can recommend testing or additional supplementation for women at risk for hypovitaminosis-D. The nurse can identify expectant mothers who are at risk for hypovitaminosis-D and consult with the care team to optimize health for the women and their babies (Schroth et al., 2005). The nurse can educate patients about the importance of adequate vitamin D during pregnancy and inform the patients on how to safely obtain adequate vitamin D. Women of

childbearing age could be informed of the potential health benefits that taking a supplement with the current recommended daily allowance of 600 IU of vitamin D prior to conception could improve serum calcidiol levels (IOM, 2011). In addition, the findings of Ginde et al. (2010) demonstrate the need to recognize that pregnant adolescents (ages 13-19 years) were at increased risk for hypovitaminosis-D in pregnancy because they are more frequently are from a minority race, lower socioeconomic status, and not using any vitamin D supplements. Nurses in practice can provide education and support to improve vitamin D status in this at risk group.

Recommendations for Further Research

Nurse scientists are in an ideal position to study health related outcomes such as calcidiol in pregnancy. The importance of vitamin D beyond bone health is becoming increasingly apparent. Recent work highlights the action of vitamin D on cell proliferation and is essential for maternal-fetal health during pregnancy and birth outcomes (Shin et al., 2010; Hollis & Wagner, 2013). Unfortunately, the ethical concerns of conducting research on pregnant women limits studies in this population. Careful design must ensure no harm to either the woman or the fetus. There is a need for rigorous intervention trials to determine if vitamin D supplementation for pregnant women with hypovitaminosis-D status decreases the risk of hyperglycemia (Alzaim & Wood, 2013; Baker et al., 2012; Joergensen et al., 2014; McLeod et al., 2011; Pittas & Dawson-Hughes, 2010; Senti et al., 2013). The conflicting results on the relationship between hypovitaminosis-D and glucose metabolism in pregnancy creates an opportunity for nurse researchers to contribute to the science that can improve health.

These findings suggest that epidemiological studies are needed to better understand the regional implications of hypovitaminosis-D due to latitude, lifestyle, and culture. There is a need for public health studies on rates of hyperglycemia in pregnancy and comprehensive rates of GDM beyond the HAPO studies, especially because of the implications for the lifelong health of the offspring. Since a pattern of hypovitaminosis-D has been shown from various small studies at higher latitudes, larger studies are needed to determine the prevalence of vitamin D deficiency in such populations. Finally, there is a need to investigate the relationship between calcidiol and hyperglycemia in pregnancy using a larger population with a greater variance in calcidiol and glucose levels.

Concluding Remarks

There are several possible reasons for results in this study. First, there may not be an association (causal relationship) between hypovitaminosis-D and hyperglycemia in mid-pregnancy. The high prevalence of hypovitaminosis-D in the study participants did not provide enough variation to show a difference if one does exist. The research design was an analysis of an existing data set which precluded increasing the sample size. Participant characteristics closely represented the region where the participants resided but were not representative of the nation. This limits the generalizability of the findings.

The findings of this study are consistent with the professional literature demonstrating the hypovitaminosis-D in pregnancy is widespread and a public health concern (Alzaim & Wood, 2013; Tande et al., 2013; Zuhur et al., 2013). A limitation of this study is that dietary intake and vitamin use was not included in the outcome. Presumably, a majority of pregnant women would be taking a prenatal vitamin because this is fairly normal behavior in pregnancy. If that assumption is true, the high incidence

and severity of hypovitaminosis-D is alarming. The women in this study were aware they were pregnant in the first trimester because they were enrolled in the study prior to 14 weeks gestation and the sample was representative of the population, consistent in terms of disease burden and ethnicity in the population of interest.

There was no evidence of a statistically significant relationship between the serum calcidiol levels in early, mid, or late pregnancy and the BG level at mid-pregnancy in this investigation. This research contributes to the science by demonstrating that serum calcidiol levels collected in at three different points in pregnancy which were mostly below 75 nmol/L were not associated with hyperglycemia at 24-28 weeks gestation in pregnancy. The studies reported in the literature shows inconsistent findings on the relationship between calcidiol and BG in pregnancy. A need exists for randomized controlled trials to determine the effect of vitamin D supplementation on glucose homeostasis during pregnancy (Fanos, Vierucci & Saggese, 2013; Joergensen et al., 2014; Olmos-Ortiz et al., 2015; Senti, Thiele & Anderson, 2013). There is also a need for further research to generate a body of evidence to determine if a change in the daily recommended intake of vitamin D is needed to ensure adequate serum calcidiol levels in pregnancy. This research has the potential to benefit women and offspring both during pregnancy and for lifelong health.

APPENDICES

Appendix A
Institutional Review Board Approvals

Altru HEALTH SYSTEM

Institutional Review Board (IRB)

Copy to Jeanine 4/17/14

Research Project Action Report

Revised 5/10/11

Date: April 15, 2014 IRB # ST-132

Principal Investigator: Jeanine Senti

Department: Nursing Phone # 218-791-5192

Address to which notice of approval should be sent: 706 Central Plains Ct, Grand Forks, ND 58201

Research Coordinator: _____ Phone # _____

Project Title: The Relationship Between Early Pregnancy Vitamin D Levels and Late Pregnancy Blood Glucose Levels.

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

FULL BOARD APPROVAL w/Minor Modifications:

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

FULL BOARD APPROVAL:

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

APPROVAL GRANTED BY ONE REVIEWER:

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

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

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

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

☐ Amendment approved _____

☐ Administrative change approved _____

☐ Protocol revision approved _____

☐ Revised consent form approved _____

☒ Other **New Study.**

REMARKS: This is not human subjects research since dataset is de-identified + no interaction with subjects occurs. IRB review is not required.

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

4/15/14
Date

Appendix B
Consent Form

UNIVERSITY OF UND NORTH DAKOTA

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

Consent to Participate

You are invited by Cindy Anderson, PhD, WHNP-BC at the University of North Dakota and the Grand Forks Human Nutrition Research Center (GFHNRC) to participate in a study to identify the importance of the nutrient vitamin D in development of blood vessels in the placenta during pregnancy. The placenta is the organ that develops during pregnancy that provides oxygen and nutrients to the baby. This requires blood vessel development that allows enough blood to be delivered to the baby. For example, when the baby doesn't get enough nutrients, he or she may not grow as expected and may be born small, increasing the risk for problems after birth. Your participation in this study would require approximately 30-45 minutes of your time at each of three visits during your pregnancy. During each visit, we will ask you to complete a questionnaire about the frequency of foods you have eaten in the past three months. You will also be asked about your time in the sunshine. We will collect blood for analysis of proteins and nutrients. We will also collect your placenta after your baby is born. After your baby is born, we will collect information related to your pregnancy from your medical records.

STUDY SCHEDULE, PROCEDURES, RISKS, AND DISCOMFORT

You will be invited to participate by members of the research team familiar with this study. If you agree to participate, you will sign a copy of the informed consent statement and a form allowing us to access your medical information related to your pregnancy. You will receive a copy of both forms for your records. We will measure your height and complete a urine pregnancy test at your first visit that takes about 10 minutes. You will then complete a Sun Exposure Index and Food Frequency Questionnaire that takes 20 minutes, done at each visit. Collection of a blood sample will be done at each visit, taking about 5 minutes. Finally, we will collect your placenta after you have delivered your baby. The placenta is not usually used for any other purpose and is discarded after delivery. If the placenta is needed for a medical purpose, our sampling will not interfere. Participation in this study is not a substitute for regular prenatal care.

Procedures, Risks, and Discomfort

I. Urine Pregnancy Test

You will be asked to provide a urine sample for testing to confirm your pregnancy. The purpose is to assure your eligibility for the study.

Risks and Discomfort: There may be some embarrassment about providing a urine sample. The urine sample will be obtained in a clinical area specifically designed for this use. If the test is negative for pregnancy, you will be referred to your primary medical provider for follow-up and excused from the study.

II. Height

An instrument will measure your height.

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

III. Food Frequency Questionnaire

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

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

IV. Sun Index

You will be shown a diagram that shows body surface areas and asked to mark the areas of the body that have been exposed to sunshine/ultraviolet (UV) radiation without sunscreen in the past week. The purpose of this is to estimate the amount of vitamin D you are making from being out in the sun.

Risks and Discomfort: There may be some embarrassment about discussing your body. This will be minimized by assurance of privacy and professionalism by the research staff.

V. Blood Sampling

Blood totaling about four tablespoons will be taken for analysis needed in this study.

Risks and Discomfort: There may be discomfort when the needle enters the skin, lasting a few seconds. The discomfort due to the needle in the vein should be minimal, lasting less than one minute during collection of the blood. Genetic information will not be used in establishing medical diagnoses.

VI. Placenta Collection

After your baby is born, your placenta will be delivered as a normal part of childbirth. Samples of the placenta will be saved and used for analysis as part of this study. No additional time will be needed to complete this, since collection of the placenta is part of the usual childbirth routine. In most cases, the placenta is unused and destroyed after childbirth. Samples will be used for analyses for this study and will be destroyed after the analysis is completed.

Risks and Discomfort: There are no known risks associated with this procedure. However, you may be a little uncomfortable while waiting for the placenta to be delivered.

BENEFITS

Direct benefits to you as a participant include knowing that you have participated in a study that will provide helpful information for identifying the importance of nutrients for development of the placenta and babies during pregnancy.

ADVERSE REACTIONS
Adverse reactions are unlikely, but may include faintness during blood sampling. Research/clinical staff are on-site if adverse reactions require attention.

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

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

STATEMENT OF PRIVACY RIGHTS
You have the right to withdraw permission for the researchers to use or share your protected health information. We will not be able to withdraw all of the information that has already been used or shared with others to carry out the research or information used for oversight. If you withdraw your permission, you must do so in writing by contacting Cindy Anderson. You have the right to choose not to sign this form. If you decide not to sign, you cannot participate in the research study. Refusing to sign will not affect the care that you receive, now or in the future, and will not cause penalty or loss of benefits that you would be entitled. You also have the right to request to see your protected health information that is used or shared during this research after the study is completed. To request this information, please contact Cindy Anderson.

QUESTIONS
You are free to ask questions at any time during the study. Contact the principle investigator, Cindy Anderson PhD, WHNP-BC (women's health nurse practitioner), at 701.777.4354 for any information or if problems arise during the study. She can be reached by mail at the College of Nursing, University of North Dakota, Grand Forks, North Dakota, 58202-9025. If you have any other questions or concerns, please call the Office of Research Development and Compliance at the University of North Dakota at 701.777.4279 or the Altru Institutional Review Board at 701.780.1750.

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

CONSENT

University of North Dakota
Institutional Review Board
Approved on MAY 16 2011
Expires on MAY 15 2012

APPROVED
SEP 14 2008
ALTRU HEALTH SYSTEM
INSTITUTIONAL
REVIEW BOARD

Appendix C
Medical Record Abstraction Tool

Study 608 **ID Number:**

Reviewed by:_____ On: ___/___/_____

Instructions

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

A. Detailed Past Obstetrical History

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

Gravida Status

G: ___	P: ___	T: ___	A: ___	L: ___

Pregnancy Outcome	
1: **Date:** ___/___/___ Time: _____ *mm/dd/yy* **Outcome:** ___ **GA:** ___ / ___ *wks days* **GA code:** ___ **Gender:** ___	**Type of Delivery** ___ 1) __ / __ / ____ *lbs / oz* **OR** 2) _____ *grams*

Maternal Birth Weight		Fetal/Infant	
Description	Code	Description	Code
1)_____	1) ___	1)_____	1) ___
2)_____	2) ___	2)_____	2) ___
3)_____	3) ___	3)_____	3) ___

If LB & Child NOT Living

Date of Death: ___/___/___
mm/dd/yy

Reason for death:
_____

Code: ___

Outcome	GA (Gestational Age) code	Gender	Maternal Complications	Fetal/Infant Complications	Child NOT Living (Reason for Death)
LB=Live birth SB=Stillbirth SA=Spontaneous Abortion TA=Therapeutic Abortion EP=Ectopic Pregnancy NP=Molar Pregnancy	Enter time in weeks/days if documented. If number of weeks is not documented, enter: FT=full term (≥ 37+ weeks) MT=near term (32-36 wks) PT=early preterm (24-31 wks) ET=early termination (<20 wks) **Type of Delivery** 01=vaginal 02=op vaginal (forceps or vacuum) 03=C-section	01=male 02=female	00=None noted 01=Preeclampsia 02- Gestational Hypertension 03=Placenta previa 04=Placental abruption 05=Pre-gestational diabetes – Type I 06=Pre-gestational diabetes – Type II 07=Gestational diabetes 08=PPH requiring blood transfusion 09=PTL requiring treatment 10=PPROM 99=Other	00=None noted 01=Intra-Uterine Growth Restriction 02- Small for Gestational Age 03- Large for Gestational Age 04-Respiratory Distress Syndrome 05-Meconium Aspiration 06=Other genetic 07=Other chronic disease 08-Observe for sepsis 09-Hydrocephalus 99=Other	01=Cardiovascular 02=Congenital Defect 03=Respiratory 04=Prematurity 05-Sepsis 06-Unknown 99=Other

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

B. History of Medical Conditions

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

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

C. Surgical Conditions

1. Has the woman had any surgeries:

(circle one)

No 0

Yes..... 1

IF YES

List the surgery and the year:

	Surgery	Year
a.	_____	__ __ __ __
b.	_____	__ __ __ __
c.	_____	__ __ __ __
d.	_____	__ __ __ __
e.	_____	__ __ __ __
f.	_____	__ __ __ __
g.	_____	__ __ __ __
h.	_____	__ __ __ __

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

D. Diabetes

1. Did the woman enter pregnancy with a diagnosis of diabetes:

(circle one)

No 0

Yes 1

IF YES

a. At what age was diabetes first diagnosed: _____ years *(please specify)*

b. What type of diabetes:

(circle one)

Type 1 DM 1

Type 2 DM 2

Unspecified 3

c. Which of the following treatments were used at the time of LMP/conception:

		(circle one for each) NO	YES
1.	Diet	0	1
2.	Insulin injections	0	1
3.	Insulin pump	0	1
4.	Oral hypoglycemic agent	0	1
5.	Oral insulin sensitizing agent	0	1

d. Were any of the following complications of diabetes noted:

		(circle one for each) Present, at entry to prenatal care	Present, found during pregnancy	Absent
1.	Nephropathy/renal insufficiency/renal failure	0	1	2
2.	Retinopathy/blindness...	0	1	2
3.	Peripheral neuropathy...	0	1	2
4.	Coronary artery disease	0	1	2

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

E. Prescription Medication, Vitamins and Vaccines

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

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

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

F. Prenatal Care Visits

Instructions

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

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

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

6

G. General Prenatal Labs

1. **Mother's Blood Type:** *(circle one)*
   - A.....1
   - B.....2
   - O.....3
   - AB.....4

2. **RH Factor:** *(circle one)*
   - Positive.....1
   - Negative.....2

a. Antibody screen: ***(circle one)***
   - Positive.....1
   - Negative.....2

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

__ __ / __ __ / __ __ __ __

IF SCREEN POSITIVE

	Date Identified mm/ dd / yy	Type(s) Identified	Titer
1.	__ __ / __ __ / __ __	1. __ __ __ __ 2. __ __ __ __ 3. __ __ __ __	1. __ : __ __ __ 2. __ : __ __ __ 3. __ : __ __ __
2.	__ __ / __ __ / __ __	1. __ __ __ __ 2. __ __ __ __ 3. __ __ __ __	1. __ : __ __ __ 2. __ : __ __ __ 3. __ : __ __ __
3.	__ __ / __ __ / __ __	1. __ __ __ __ 2. __ __ __ __ 3. __ __ __ __	1. __ : __ __ __ 2. __ : __ __ __ 3. __ : __ __ __
4.	__ __ / __ __ / __ __	1. __ __ __ __ 2. __ __ __ __ 3. __ __ __ __	1. __ : __ __ __ 2. __ : __ __ __ 3. __ : __ __ __

Type of Antibody:
Enter alpha code (upper & lower case):

Type	Code
D	D
Kell	Kell
E	E
e	e
C^w	Cw
C	C
Ce	Ce
Kp^a	Kpa
Kp^b	Kpb
cE	cE
k	k
Jk	Jk
s	s
Wr^a	Wra
Fy^a	Fya
M	M
Other	Oth

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

8

3. HCT/HgB:

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

4. PAP smear: *(circle one)*

Normal.....1
Abnormal, ASCUS.....2
Abnormal, LGSIL.....3
Abnormal, HGSIL.....4
Abnormal, CIS/invasive disease.....5
Unsatisfactory/insufficient for evaluation.....6

5. Rubella: *(circle one)*

Immune.....1
Nonimmune.....2

6. GBS:

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

7. RPR/VDRL:

	Date mm/ dd / yy	Result 01=Reactive 02=Nonreactive	Titer	FTA Result 01=Positive 02=Negative
a.	__ __/ __ __/ __ __	__ __	__ : __ __ __	__ __
b.	__ __/ __ __/ __ __	__ __	__ : __ __ __	__ __

8. Urinalysis:

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

9. Urine culture:

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

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

H. Specialized Testing

Instructions

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

1. Specialized testing:

(circle one)

No..... 0

Yes..... 1

2. Hemoglobin (HgB) A1C:

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

3. Twenty-four hour urine protein:

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

3.

4. 4. Pre-Eclampsia Labs:

5.

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

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

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

7

5. Other Lab Tests:

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

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

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

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

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

12

10. Hepatitis B Surface Antigen:

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

Other hep B serology codes:

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

11. PPD: *(circle one)*

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

12. Chlamydia:

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

13. Gonorrhea:

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

14. Diabetes screen:

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

15. GTT:

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

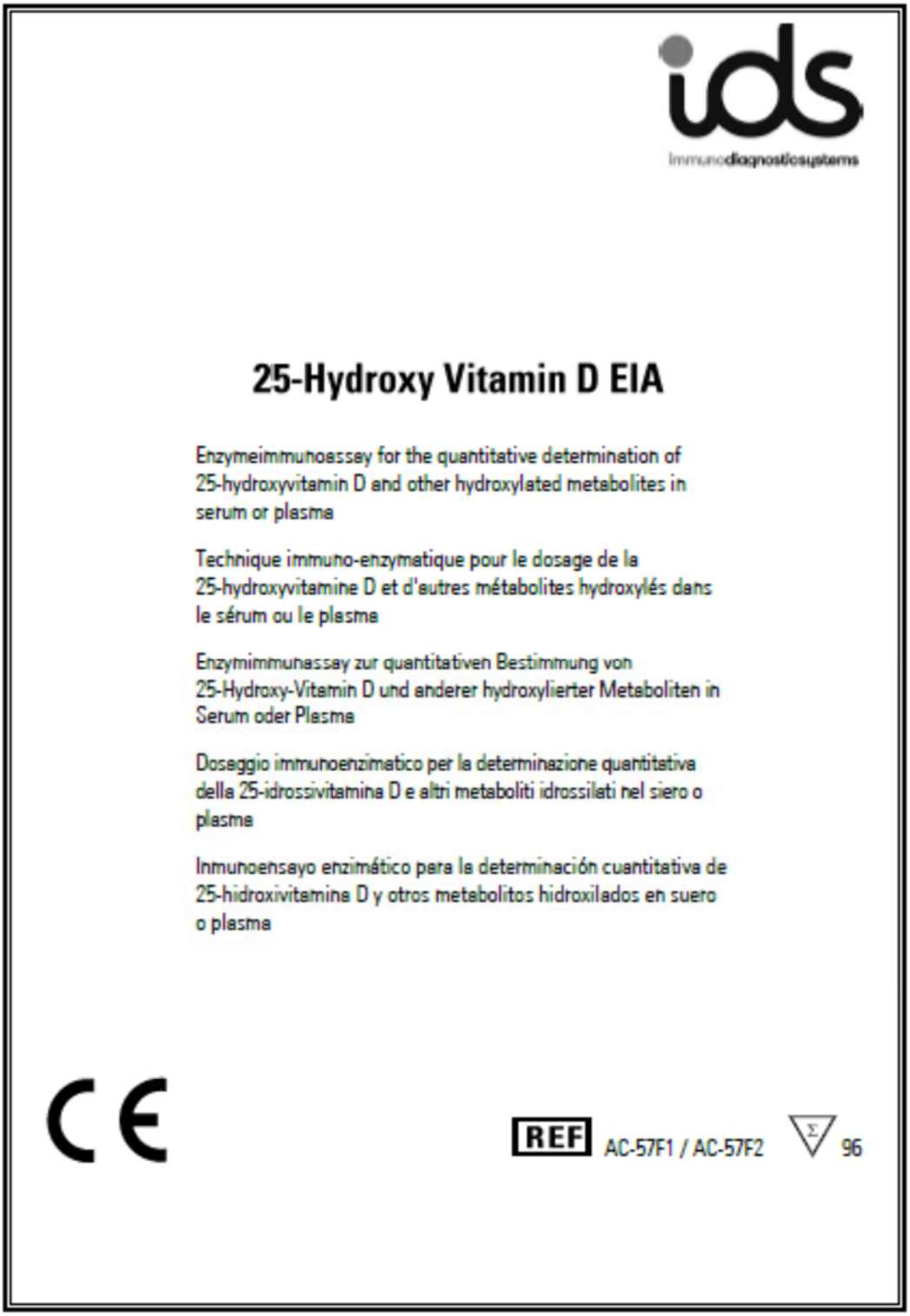
I. Prenatal Ultrasound

Instructions

Complete the Prenatal Ultrasound Diagnosis on all women enrolled prospectively into the study using medical records. Complete one row for each ultrasound the woman received. If the number of ultrasounds is more than eight, note the first 4 ultrasounds on lines #1-4 and last 4 on lines #5-8. For each ultrasound record up to three results.

	Date mm/dd/yy	Measurements CRL=Crown-rump length BPD=Biparietal diameter HC=Head circumference AC=Abdominal circumference FL=Femur length	Result* Code (see codes on page 17)	Specify	AFI (Amniotic Fluid Index) cm	*BioPhysical Profile*	
1.	__/__/__	Consistent with: 1. CRL _____ cm _____ wks gestation 2. BPD _____ cm _____ wks gestation 3. HC _____ cm _____ wks gestation 4. AC _____ cm _____ wks gestation 5. FL _____ cm _____ wks gestation _____ wks gestation	1. __ __ __ 2. __ __ __ 3. __ __ __ 4. __ __ __ 5. __ __ __	_____ _____ _____ _____ _____	__ __ __.__ ☐ Check if measurement is normal.	Fetal Tone Gross body movements Fetal breathing movements Amniotic fluid volume	____/2 ____/2 ____/2 ____/2
2.	__/__/__	Consistent with: 1. CRL _____ cm _____ wks gestation 2. BPD _____ cm _____ wks gestation 3. HC _____ cm _____ wks gestation 4. AC _____ cm _____ wks gestation 5. FL _____ cm _____ wks gestation _____ wks gestation	1. __ __ __ 2. __ __ __ 3. __ __ __ 4. __ __ __ 5. __ __ __	_____ _____ _____ _____ _____	__ __ __.__ ☐ Check if measurement is normal.	Fetal Tone Gross body movements Fetal breathing movements Amniotic fluid volume	____/2 ____/2 ____/2 ____/2
3.	__/__/__	Consistent with: 1. CRL _____ cm _____ wks gestation 2. BPD _____ cm _____ wks gestation 3. HC _____ cm _____ wks gestation 4. AC _____ cm _____ wks gestation 5. FL _____ cm _____ wks gestation _____ wks gestation	1. __ __ __ 2. __ __ __ 3. __ __ __ 4. __ __ __ 5. __ __ __	_____ _____ _____ _____ _____	__ __ __.__ ☐ Check if measurement is normal.	Fetal Tone Gross body movements Fetal breathing movements Amniotic fluid volume	____/2 ____/2 ____/2 ____/2
4.	__/__/__	Consistent with: 1. CRL _____ cm _____ wks gestation 2. BPD _____ cm _____ wks gestation 3. HC _____ cm _____ wks gestation 4. AC _____ cm _____ wks gestation 5. FL _____ cm _____ wks gestation _____ wks gestation	1. __ __ __ 2. __ __ __ 3. __ __ __ 4. __ __ __ 5. __ __ __	_____ _____ _____ _____ _____	__ __ __.__ ☐ Check if measurement is normal.	Fetal Tone Gross body movements Fetal breathing movements Amniotic fluid volume	____/2 ____/2 ____/2 ____/2

Appendix D
EIA Kit



Intended Use

For In Vitro Diagnostic Use

The IDS 25-Hydroxy Vitamin D EIA kit is an enzymeimmunoassay intended for the quantitative determination of 25-hydroxyvitamin D (25-OH D) and other hydroxylated metabolites in human serum or plasma. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of vitamin D sufficiency in adult populations.

Summary and Explanation

Vitamin D is a commonly used collective term for a family of closely related seco-steroids. Upon exposure to sunlight, 7-dehydro-cholesterol, located deep in the actively growing layers of the epidermis, undergoes photolytic cleavage of the "B" ring to yield pre-vitamin D_3 which is isomerised to vitamin D_3 (cholecalciferol). Vitamin D_3 and vitamin D_2 (ergocalciferol) may also be obtained by dietary supplementation or from a limited number of foods. Vitamin D_2 is metabolised in a similar way to vitamin D_3.

Vitamin D is stored in adipose tissue and enters the circulation bound to vitamin D binding protein (VDBP) and albumin. In the liver, vitamin D is hydroxylated to give 25-hydroxyvitamin D which also circulates as a complex with VDBP. A small proportion of the 25-OH D is further hydroxylated in the kidney, under direct regulation by parathyroid hormone and ionised calcium levels, to form the biologically-active calcitropic hormone 1,25-di-hydroxyvitamin D. Further hydroxylation and metabolism of vitamin D produces compounds that are water soluble and readily excreted.

Hepatic vitamin D 25-hydroxylase activity is not tightly regulated, and changes in cutaneous production of vitamin D_3, or ingestion of vitamin D (D_3 or D_2), will result in changes in circulating levels of 25-OH D [1].

Serum concentration of 25-OH D is considered to be the most reliable measure of overall vitamin D status and thus can be used to determine whether a patient is vitamin D sufficient[2]. Assessment of vitamin D status may be required to determine the cause of abnormal serum calcium concentrations in patients.

Method Description

The IDS 25-Hydroxy Vitamin D EIA kit is an enzymeimmunoassay for the quantitation of 25-OH D and other hydroxylated metabolites in serum or plasma. Calibrators, controls and samples are diluted with biotin labelled 25-OH D. The diluted samples are incubated in microtitre wells which are coated with a highly specific sheep 25-OH D antibody for 2 hours at room temperature before aspiration and washing. Enzyme (horseradish peroxidase) labelled avidin, is added and binds selectively to complexed biotin and, following a further wash step, colour is developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microtitre plate reader, colour intensity developed being inversely proportional to the concentration of 25-OH D.

Warnings and Precautions

The IDS 25-Hydroxy Vitamin D EIA kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IDS Limited will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Human serum: Calibrators CAL and Controls CTRL
Human material used in the preparation of this product has been tested by FDA recommended assays for the presence of antibody to Human Immunodeficiency Virus (HIV I and II), Hepatitis B surface antigen, antibody to Hepatitis C, and found negative. As no test can offer complete assurance that infectious agents are absent, the reagents should be handled in accordance at Biosafety Level 2.

Sodium azide
Some reagents in this kit contain sodium azide as a preservative, which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

0.5M hydrochloric acid
Stop Solution HCL contains 0.5M hydrochloric acid.

R36/38	Irritating to eyes and skin.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37	Wear suitable protective clothing and gloves.

Tetramethylbenzidine
TMB Substrate SUBS contains 3,3',5,5'-tetramethylbenzidine.

R21/22	Harmful by contact with skin and if swallowed.
S36/37	Wear suitable protective clothing and gloves.

Preparation of Reagents

25-D Biotin Solution 25-D BIOTIN SOLN: 25-D Biotin Concentrate 25-D BIOTIN 50x is supplied lyophilised. Add 3 mL of Buffer BUF to the bottle of lyophilised 25-D Biotin Concentrate 25-D BIOTIN 50x (blue colour). Replace the stopper and stand for 10-15 minutes at room temperature. Invert several times to ensure complete reconstitution. Add the reconstituted 25-D Biotin Concentrate 25-D BIOTIN 50x (3 mL) back into the bottle containing the remaining Buffer BUF. Mix well by inversion. The 25-D Biotin Solution (50 mL) is green in colour. Mark the bottle "25-D Biotin Solution". Store at 2-8°C in the dark.

Wash Solution WASHBUF SOLN:
Add the contents of each bottle of Wash Concentrate WASHBUF 20x to 950 mL of distilled or de-ionised water and mix. Store at room temperature.

All other reagents are supplied ready for use. Allow all reagents to come to room temperature before use.
Reagents should be mixed by repeated inversion before use in the assay.

Shelf Life and Storage of Reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Reconstituted 25-D Biotin Solution [25-D BIOTIN] [SOLN] can be stored at 2-8°C for up to 8 weeks. The 25-D Biotin solution must be stored as above in the dark immediately after use.

Unused Antibody Coated Plate [MICROPLAT] strips must be returned to the foil pouch with the desiccant sachet. Fold over the end of the foil pouch and seal in one of the plastic selfseal bags provided. Store at 2-8°C for up to 8 weeks.

Controls [CTRL] can be stored at 2-8°C for up to 8 weeks after opening.

Wash Solution [WASHBUF] [SOLN] can be stored at room temperature for up to 8 weeks.

Indications of possible deterioration of kit reagents

The presence of abnormal particulate matter in any of the reagents.

A decrease in the absorbance of the zero calibrator.

A shift in the slope of the curve from its normal position.

Specimen Collection and Storage

The assay should be performed using serum or plasma (EDTA or heparin) specimens. Specimens should be separated as soon as possible after collection. For long term storage, store at -20°C. Avoid repeated freeze/thaw of samples.

Automated Platforms

Users should be aware that this product was designed and developed to be performed manually using the protocol described below. The protocol is not necessarily applicable to automated platforms. Users who wish to perform the assay on an automated platform should therefore be aware that they may have to optimise their own protocol and that this may differ significantly from that described below. Guidance on this point can be obtained from IDS Technical Services.

Procedure

Materials Provided

1. **CAL 0 - 6 – Calibrators (REF AC-5701A - AC-5701G):**
   Buffered human serum containing 25-hydroxy-vitamin D and 0.09% sodium azide. The exact value of each Calibrator is printed on the QC Report, 1 mL per bottle, 7 bottles per kit.

2. **MICROPLAT - Antibody Coated Plate (REF AC-5702W):**
   Microplate with 25-hydroxyvitamin D sheep polyclonal antibody linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desiccant.

3. **25-D BIOTIN 50x - 25-D Biotin Concentrate (REF AC-5703):**
   Lyophilised buffer containing 25-hydroxy-vitamin D labelled with biotin, and proprietary stabilisers, 1 mL per bottle. 1 (F1) or 2 (F2) bottles per kit.

4. **BUF - Buffer (REF AC-5703B):**
   Proprietary reagent for dissociating 25-hydroxy-vitamin D from binding proteins, 50 mL per bottle. 1 (F1) or 2 (F2) bottles per kit.

5. **ENZYMCONJ - Enzyme Conjugate (REF AC-5704):**
   Phosphate buffered saline containing avidin linked to horseradish peroxidase, protein, enzyme stabilisers and preservative. 22 mL per bottle. 1 (F1) or 2 (F2) bottles per kit.

6. **CTRL 1 - 2 – Controls (REF AC-5705A - AC-5705B):**
   Human serum containing 25-hydroxy-vitamin D and 0.09% sodium azide. 1 mL per bottle, 1 bottle per control level.

7. **SUBS - TMB Substrate (REF AC-SUBS):**
   A proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide, 28 mL per bottle. 1 (F1) or 2 (F2) bottles per kit.

8. **HCL - Stop Solution (REF AC-STOP):**
   0.5M Hydrochloric Acid, 13 mL per bottle. 1 (F1) or 2 (F2) bottles per kit.

9. **WASHBUF 20x - Wash Concentrate (REF AC-WASHL):**
   Phosphate buffered saline containing Tween, 50 mL per bottle.

10. **Adhesive Plate Sealer**
    8 per kit.

11. **Documentation**
    Package Insert and QC report.

Materials Required but not Provided

1. Disposable 12 x 75 mm borosilicate glass or polypropylene tubes.
   Note: polystyrene tubes are not suitable. Do not reuse tubes.
2. Precision pipetting devices to deliver 25 µL and 200 µL.
3. Repeating pipettes to deliver 1 mL, e.g. Eppendorf Multipipette 4780, or similar.
4. Precision multi-channel pipettes to deliver 100 µL and 200 µL.
5. Vortex mixer.
6. Automatic microplate washer (optional).
7. Photometric microplate reader and data analysis equipment.

Assay Procedure

Reconstitute or prepare reagents as described in "Preparation of Reagents".

1. Prepare labelled borosilicate glass or polypropylene tubes, one for each Calibrator [CAL], Control [CTRL] and sample [SPE].
2. Add **25 µL** of each Calibrator [CAL], Control [CTRL] or sample to the appropriately labelled tubes.
3. Add **1 mL** of 25-D Biotin Solution [25-D BIOTIN] [SOLN] to all tubes. Vortex thoroughly for 10 seconds.
4. Add **200 µL** of each diluted Calibrator, Control or sample to the appropriate wells of the Antibody Coated Plate [MICROPLAT] in duplicate.
   Cover the plate with an adhesive plate sealer. Incubate at 18-25°C for 2 hours.
5. Wash all wells three times with Wash Solution [WASHBUF] [SOLN].
   a) Automatic plate wash: Set plate washer to dispense at least 300 µL of Wash Solution [WASHBUF] [SOLN] per well. Fill and aspirate for 3 cycles.
   b) Manual wash: Decant the contents of the wells by inverting sharply. Dispense 250 µL of Wash Solution [WASHBUF] [SOLN] to all wells. Decant and repeat twice.

   Tap the inverted plate firmly on absorbent tissue to remove excess Wash Solution [WASHBUF] [SOLN] before proceeding to the next step.
6. Add **200 µL** of Enzyme Conjugate [ENZYMCONJ] to all wells using a multichannel pipette.
   Cover the plate with an adhesive plate sealer. Incubate at 18-25°C for 30 minutes.
7. Repeat wash step 5.
8. Add **200 µL** of TMB Substrate [SUBS] to all wells using a multichannel pipette.
   Cover the plate with an adhesive plate sealer. Incubate at 18-25°C for 30 minutes.

   Note: TMB Substrate is easily contaminated. Only remove the required amount for the assay from the bottle. Dispose of unused TMB Substrate. Do not return to bottle.
9. Add **100 µL** of Stop Solution [HCL] to all wells using a multichannel pipette.
10. Measure the absorbance of each well at 450 nm (reference 650 nm) using a microplate reader within 30 minutes of adding the Stop Solution.

Calculation of Results

A variety of data reduction software packages are available, which may be employed to generate the calibration curve and to calculate the concentrations of unknown samples and controls. A 4 parameter logistic (4PL) curve fit, omitting Calibrator 0, is recommended. Alternatively, a smoothed spline fit can be used. Other curve fitting algorithms are not recommended.

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the ordinate against concentration of 25-hydroxyvitamin D on the abscissa. Calibrator 0 should not be included in the calibration curve.

Read the mean absorbance value of each unknown sample off the curve in nmol/L (nM).

Conversion of Units:

$$X\ \text{nmol/L} \xrightarrow{\times 0.40} Y\ \text{ng/mL}$$
$$X\ \text{nmol/L} \xleftarrow{\times 2.5} Y\ \text{ng/mL}$$

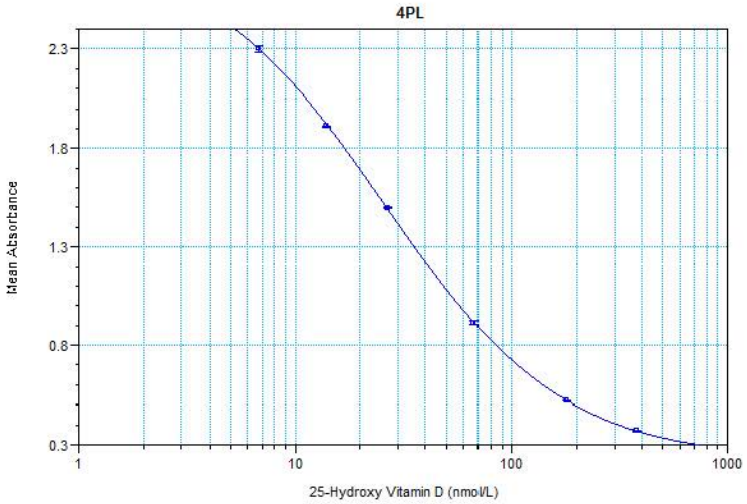
Sample Assay Data

This data is for illustration only and must not be used for the calculation of any sample result.

Well	Description	Abs.	Mean Abs.	Result (nmol/L)
A1, A2	Calibrator 0 0 nmol/L	2.476 2.530	2.503	
B1, B2	Calibrator 1 6.8 nmol/L	2.313 2.288	2.301	
C1, C2	Calibrator 2 14 nmol/L	1.912 1.908	1.910	
D1, D2	Calibrator 3 27 nmol/L	1.495 1.499	1.497	
E1, E2	Calibrator 4 67 nmol/L	0.919 0.905	0.912	
F1, F2	Calibrator 5 179 nmol/L	0.521 0.522	0.522	
G1, G2	Calibrator 6 380 nmol/L	0.372 0.368	0.370	
H1, H2	Sample 1	1.237 1.257	1.247	39
A3, A4	Sample 2	0.951 0.969	0.960	62
B3, B4	Sample 3	0.591 0.612	0.602	138

Typical Calibration Curve

This sample calibration curve is for illustration only.



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Calibration

25-OH D Calibrators are standardised using U.V. quantification.

Quality Control

Two kit controls are provided which should be tested as unknowns. These are intended to assist the user in assessing, on a day-to-day basis, the validity of results obtained with the assay. The QC report, which is included with each kit, states the **mean concentration** of each control as determined by IDS over a number of manual assays, and a **range** which is equivalent to +/- 20% of the mean concentration. IDS recommends that customers maintain graphic records of the control values that they generate, together with a calculation of the running mean, SD and % cv. This will allow them to monitor how current and historic control lots perform relative to the supplied QC data, and to identify assays which give control values significantly different from their normal range.

When interpreting control data users should note the following points:

a) This product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. **Nevertheless, it is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories**[3]. The range given in the IDS QC report may not therefore be appropriate for users who find that their own mean value for any control is significantly different from that given in the IDS QC report. In such cases users should set their own range as described in the CLSI Document C24-A3, *Internal Quality Control Testing: Principles and Definitions*. This caveat is particularly relevant for customers who run this product on an automated platform, and who by definition do not follow the Assay Procedure described above. Users should note that where the IDS QC range is used inappropriately, they have an increased risk of rejecting assays that are acceptable, and of erroneously reporting assays that are not acceptable.

When setting their own ranges, customers should be aware of the statistical relationship between multiples of standard deviation (SD) and the false rejection rate of acceptable assays. For example, if the range of each control is defined as +/- 2 SD, an assay using two control levels will have a false rejection rate of 9.8% [3].

b) Customers who are unable to set their own control ranges or who choose to use the range given in the IDS QC report should not automatically fail the assay should a control value fall outside of the acceptable range stated in the IDS QC report. In such a case they should assess the validity of the results in the context of whether the control value is significantly different from the current laboratory running mean or the historical levels obtained in earlier assays relative to the IDS QC mean value.

Limitations of Use

1. This kit is intended for use with human serum and plasma samples that have been obtained using standard methods. The kit is not suitable for human serum or plasma samples that have been further processed in any way which would alter the matrix composition of the sample.
2. Samples containing analyte concentrations in excess of the highest calibrator should be re-assayed after dilution. The preferred diluent is serum or plasma with a known low concentration of 25-Hydroxy Vitamin D (this will minimise changes to the sample matrix), but Phosphate Buffered Saline containing 9% (w/v) Bovine Serum Albumin can also be used. If a low concentration serum or plasma is used, its' concentration should be confirmed by re-assaying it in the same assay as the diluted material. A 4-fold dilution is recommended: 1 part sample plus 3 parts diluent. The actual concentration of the sample can then be calculated using the formula:

   $$A = (4M) - (3D)$$

   Where ;

   A= Actual concentration

   M = Measured concentration

   D = Diluent concentration

   For example:

   Measured concentration = 60

   Diluent concentration = 10

   Actual concentration $= (4 \times 60) - (3 \times 10)$

   $= 240 - 30$

   $= 210$
3. As in the case of any diagnostic procedure results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
4. The performance characteristics of this assay have not been established in a paediatric population.
5. In rare cases, interference due to extremely high titres of antibodies to avidin can occur.
6. The following substances have been tested and found not to interfere in the IDS 25-Hydroxy Vitamin D assay:

Haemoglobin	tested up to 1470 mg/dL
Bilirubin	tested up to 513 µmol/L
Lipid	tested up to 5.6 mmol/L triglyceride

Expected Values

Each laboratory should determine ranges for their local population.

There is no universal agreement on the optimal concentration of 25-OH D. Ranges should be based on clinical decision values

that apply to both sexes of all ages rather than population based reference ranges for 25-OH D. To that end, a large study examined the relationship of intact PTH with vitamin D levels in serum. A plateau for iPTH was seen at ~30 ng/mL[4]. Similarly, Calcium (Ca) absorption increased with increasing 25-OH D level until ~30 ng/mL 25-OH D was reached. Optimal Ca absorption requires levels of 25-OH D exceeding 30 ng/mL[5].

In the case of 25-OH D, there are also many other factors that may influence values: diet, time of day, sun exposure, season of year[6], geographic location[7], age[8], use of sunscreen and/or protective clothing[9,10] and skin pigmentation[11]. Thus, sampling a group of apparently healthy individuals is not the ideal way to establish the reference range.

The US National Osteoporosis Foundation recommends a level >30 ng/mL to protect bone health[12]. Similarly, the US National Kidney Foundation considers levels <30 ng/mL to be insufficient or deficient[13].

From a review of the available literature, the recommendations for 25-OH D levels are:

	Range	
Level	**nmol/L**	**ng/mL**
Deficient	<25	<10
Insufficient	25-74	10-29
Sufficient	75-250	30-100
Potential Intoxication	>250	>100

The following range has been determined using the IDS 25-Hydroxy Vitamin D EIA kit and is provided for guidance only. Each laboratory should determine ranges for their local population.

Normal adults 47.7 - 144 nmol/L (n = 36)

Performance Data

Accuracy

The IDS 25-Hydroxy Vitamin D EIA kit was compared against a recognised radioimmunoassay for the quantitative determination of 25-hydroxyvitamin D and other hydroxylated metabolites. A population of 180 samples, selected to represent a wide range of 25-hydroxyvitamin D [9.3 - 151.2 nmol/L], were assayed by each method. Least squares regression analysis was performed on the comparative data: IDS = 1.01(x) + 0.7; correlation coefficient (r) = 0.9

Sensitivity

The sensitivity, defined as the concentration corresponding to the mean minus 2 standard deviations of 10 replicates of the zero calibrator, is 5 nmol/L.

Precision

Intra assay mean (nmol/L)	n=10 % CV	Inter assay mean (nmol/L)	n=11 % CV
39.0	5.3	40.3	4.6
67.1	5.6	72.0	6.4
165	6.7	132	8.7

Recovery

Recovery was assessed by adding 25-OH D to samples prior to assay.

Sample	Measured (nmol/L)	Expected (nmol/L)	Recovery %
A	122	126	97
A	95.6	98.4	97
B	147	141	104
B	123	118	105
		Mean	**101**

Linearity

Linearity was assessed by diluting samples with buffer (PBS containing 9%BSA) prior to assay.

Sample	Measured (nmol/L)	Expected (nmol/L)	% M/Exp
A	83.9		
A/2	41.0	42.0	98
A/4	20.8	21.0	99
A/8	13.1	10.5	125
B	83.9		
B/2	43.5	42.0	104
B/4	23.1	21.0	110
B/8	10.7	10.5	102
C	104		
C/2	45.9	52.0	88
C/4	22.5	26.0	87
C/8	14.1	13.0	108
		Mean	**102**

Specificity

The specificity of the antiserum was assessed with the following analytes at 50% binding of the zero calibrator.

Analyte	Cross-Reactivity
25-Hydroxyvitamin D_3	100%
25-Hydroxyvitamin D_2	75%
24,25-Dihydroxyvitamin D_3	≥100%
Cholecalciferol (D_3)	<0.01%
Ergocalciferol (D_2)	<0.30%

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