ASSOCIATION OF MATERNAL SERUM VITAMIN D LEVELS AND FETAL GROWTH AND NEONATE BODY COMPOSITION

BY

Emily Zans

Submitted to the graduate degree program in Dietetics and Nutrition and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Science

Chairperson Holly R. Hull, PhD

Susan Carlson, PhD

Debra Sullivan, PhD, RD

Date Defended: November 11, 2013

The thesis committee for Emily Zans certifies that this is the approved version of the following thesis:

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Chairperson Holly R. Hull, PhD

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ABSTRACT

Background: Maternal serum 25-hydroxyvitamin D (25(OH)D) deficiency in pregnancy has been associated with decreased infant birth weight, although research has not been consistent. No research is available investigating the effects of serum 25(OH)D on estimated fetal weight (EFW). Only one study has been published relating infant body composition to maternal serum vitamin D status.

Purpose: The purpose of this study was to further investigate the relationship between maternal serum 25(OH)D and fetal growth and neonate body composition.

Methods: Sixty-three pregnant women had serum 25(OH)D analyzed late in pregnancy. Percent fat (%fat), fat mass (FM), and fat free mass (FFM) of the offspring were analyzed using air displacement plethysmography within 72 hours of life. Multiple linear regression was used to assess the relationship between maternal 25(OH)D and infant body composition. Covariates considered included pre-pregnancy body mass index (BMI), total gestational weight gain (GWG), infant gender, and infant age at test. Fifty-six and 31 participants had data estimating fetal weight in early and late gestation, respectively. The relationship between maternal serum 25(OH)D and EFW was assessed using multiple linear regression. Covariates considered in this analysis were pre-pregnancy BMI, GWG up to sonogram measurement, gestational age (GA) at measurement, and infant gender.

Results: The mean serum 25(OH)D of the sample was 52.6 nmol/L, with 50.7% below 50nmol/L, which is defined as deficient by the Endocrine Society. Across classification groups

those classified as serum 25(OH)D deficient had significantly higher pre-pregnancy BMIs, than those that were classified as having adequate or insufficient serum 25(OH)D. Gestational age at birth was the only predictor of infant birth weight (β= 171.050, p= 0.005). Infant %fat and FM were both predicted by age at test alone (β= 1.61, p = 0.037; β= 87.45, p= 0.004). FFM was predicted by infant age at test (β= 158.24, p= 0.001), gender (β= - 197.34, p= 0.004), and GA at birth (β= 194.37, p<0.001). EFW early in pregnancy was predicted by GWG (β= -3.84, p= 0.006) and GA at measurement (β= 65.65, p< 0.001). Only GA predicted EFW late in pregnancy (β= 208.83, p< 0.001). Maternal 25(OH)D did not remain significant in any of the variables.

Conclusion: Maternal serum 25(OH)D was not a predictor of birth weight, infant %fat, FM, FFM, or EFW in early or late pregnancy.

ACKNOWLEDGEMENTS

I would like to thank my mentor Holly Hull, PhD for her assistance in developing and writing this thesis. I would also like to express my appreciation for the numerous opportunities that she has given me throughout my graduate study. Dr. Hull, thank you for helping me to develop my skills as a writer, researcher and nutrition professional. Furthermore, I would like to thank Susan Carlson, PhD and Debra Sullivan, RD, PhD for their participation on my thesis committee. It truly has been an honor to work with you both.

Special thanks go to the members of Dr. Hull's laboratory. I'd like to thank Shengqi Li, MS, PhD candidate and Cheng Li, MS for their work on the Epic Study and assistance in preparing this thesis. Thank you to Megan Brinker, RD, Emily Newbold, RD, Brianna Helfrich and Mira Dewi for your work with the Thrasher Study.

Finally, I would like to thank my family and friends for being my cheerleaders and support throughout my education. Without your help from my first days of school, I definitely would not be here. Thank You!

Table of Contents

Chapter I

INTRODUCTION

Vitamin D has long been recognized to play a role in bone modeling, but as of late, the potential role vitamin D may play in various metabolic conditions has been presented (1). A population of particular interest in the emerging vitamin D story is pregnant women. The Institute of Medicine's (IOM) Recommended Dietary Allowance (RDA) for vitamin D in pregnancy rose from 200 IU per day to 600 IU per day in 2010 (2). Although this rise triples the previous recommendation many believe that it is still not enough (3, 4). According to the 2009 NHANES data, 28% of pregnant women had a serum 25-dihydroxy vitamin D (25(OH)D) level less than 50nmol/L. This level is classified as "at risk for vitamin D inadequacy" by the IOM. Furthermore, seven percent of pregnant women had serum levels <30nmol/L which is classified as "at risk for vitamin D deficiency" (5).

Disagreement exists as to the desired amount of vitamin D intake that is required to achieve optimal serum levels. The Endocrine Society suggests that an intake of 1500-2000 IU/d may be required to achieve optimal serum 25(OH)D in pregnancy (3). However, Hollis et al suggests that in pregnancy optimal serum 25(OH)D levels are much higher and require an intake of 4000 IU to be achieved (4).

Adequate vitamin D intake is important in pregnancy due to possible associations between maternal vitamin D status and fetal and infant outcomes. Maternal vitamin D insufficiency or deficiency has been related to increased risk of gestational diabetes (6) and pre-eclampsia (7) in the mother. In the fetus, maternal vitamin D insufficiency or deficiency has been related to growth restriction and an increase in adiposity during childhood (8). Few studies have

reported these findings and reports are contradictory (9-19). Therefore, the purpose of this thesis is to investigate the relationship between maternal serum 25(OH)D levels and fetal growth and neonate body composition (percentage body fat (%fat), fat mass (FM) and fat-free mass (FFM)).

Statement of Purpose

The purpose is to investigate the relationship between maternal serum 25(OH)D levels and fetal growth and neonate body composition.

Research Question

Is maternal serum 25(OH)D measured late in pregnancy related to fetal growth and neonate body composition at birth?

Specific aims and Hypotheses

Aim 1: Examine the relationship between maternal serum 25(OH)D levels measured late in pregnancy and fetal growth.

Hypothesis 1.1: Maternal serum 25(OH)D late in pregnancy is positively related to estimated fetal weight (EFW) by ultrasound in early and late pregnancy

Hypothesis 1.2: Maternal serum 25(OH)D late in pregnancy is positively related to infant birth weight

Aim 2: Examine the relationship between maternal serum 25(OH)D levels measured late in pregnancy and neonate body composition (%fat, FM and FFM).

Hypothesis 2.1: Maternal serum 25(OH)D is positively related to neonate %fat within 72 hours of birth

Hypothesis 2.2: Maternal serum 25(OH)D is positively related to neonate FM within 72 hours of birth

Special Note

In order to report variables in similar units, conversions from mircograms (µg) to international units (IU) and nanograms (ng) to nanomol (nmol) were completed with the follow conversion factors:

1microg= 40 IU

1ng= 2.5 nmol/L

These conversions are recognized and used by the IOM (20).

Chapter II

LITERATURE REVIEW

Vitamin D: The sunshine vitamin

Vitamin D is a unique vitamin because unlike others it can be obtained from food as well as synthesized from sunlight. It also contains hormone-like traits in its function and seco-steroid structure (1). The metabolic pathways for synthesis, transport and regulation and details of the vitamin D receptor will be discussed. Lastly, food sources of vitamin D are included. Also, for consistence all serum 25(OH)D levels are reported in nmol/L. A conversion factor of 2.5nmo/l per ng/L was used (2). Similarly, intake of vitamin D is expressed in IU with 1 microgram equaling 40IU (2).

Metabolic pathway for synthesis

Ultraviolet (UV) B rays with a length of ~285-320 nm can convert 7-dehydrocholesterol found in the skin to previtamin D_3 (1). Activation of 7-dehydrocholesterol by UV B rays varies by the amount of skin exposed (21), latitude (22), season, time of day, cloud cover (23), the use of sunscreen (24) and melanin within the skin (22). After 2 to 3 days, the unstable double bonds within previtamin D_3 are rearranged to form vitamin D_3, also known as cholecalciferol (1).

Cholecalciferol within the skin diffuses into the blood bound to vitamin D binding protein. Vitamin D binding protein transports most of the cholecalciferol to the liver; however some may go to other tissues such as muscle and adipose (1). Dietary Vitamin D (ergocalciferol and cholecalciferol) is absorbed within the duodenum and distal small intestine (1). Diffusion

into the enterocyte is done via micelle formation. This requires the presence of bile acids and pancreatic lipase within the intestine. The presence of fat increases excretion of bile acids and pancreatic lipase, thus its presence in the intestine increases the efficacy of vitamin D absorption (2). Dietary vitamin D transports through the body within chylomicrons, and may be released into other tissues during hydrolysis of the chylomicron by lipoprotein lipase (2). Vitamin D from either the diet or from the skin does not remain in the circulation very long due to uptake by adipose tissue or the liver. This generally occurs within hours of absorption (25).

Once vitamin D_3 is in circulation, it must be activated by a two-step process. The first step occurs in the liver where vitamin D_3 is hydroxylated by 25-hydroxylase to form calcidiol (also known as 25-hydroxy vitamin D (25(OH)D)). In the second step, 25(OH)D reaches the kidney and is hydroxylated again by 1 α-hydroxylase forming calcitriol, also known as 1,25-dihydroxy vitamin D, the active form of vitamin D (1). Serum 25(OH)D is used to assess vitamin D adequacy. This is due to the short half-life of calcitriol of about 4 to 6 hours (25), its regulation by other hormones such as parathyroid hormone (PTH) and lack of direct association with vitamin D intake and skin synthesis (2). The half-life of serum 25(OH)D is several weeks and therefore a more stable measure of vitamin D status (25). The main storage site for $25(OH)D_3$ is thought to be the blood and muscles, while adipose and skin store vitamin D in the form of cholecalciferol (1).

Regulation of Vitamin D synthesis

Vitamin D concentrations within the body are regulated by various pathways such as formation and activation. The conversion of 7-dehydrocholesterol and previtamin D_3 to other metabolites helps to prevent toxicity when exposed to UV B rays for extended periods of time.

The formation of previtamin D_3 from7-dehydrocholesterol plateaus once 10-15% of the supply is converted (22). At this point UV rays begin to convert excess 7-dehydrocholesterol to lumisterol, and excess previtamin D_3 to tachysterol. Vitamin D binding protein has little affinity for these two compounds and they are often sloughed off with skin cells (1, 22). Absorption of vitamin D within the intestines is not regulated. This is why large doses of synthesized vitamin D can cause symptoms of toxicity (2).

Vitamin D concentration is also controlled by the 2 steps required for activation. The first hydroxylation forming 25(OH)D within the liver is not tightly regulated (1, 26). Although it has been observed that NADPH-dependent 25-hydroxylase is more efficient when circulating levels of cholecalciferol are limited (1). Hydroxylation of cholecalciferol occurs primarily in the liver, however some occurs within the intestine and kidney (26). Final hydroxylation at position 1 of 25(OH)D is more tightly regulated by 1-hydroxylase. This enzyme is stimulated by the increased presence of PTH via a cAMP/phosphatidylinositol 4,5-biphosphate (PIP_2)-medicated signal transduction mechanism (26). Also, decreased plasma calcium increases 1-hydroxylase. The activation of 1-hydroxylase by plasma PTH and calcium levels is crucial to calcium homeostasis within the body (1). Over production of 1,25 (OH) D_3 is prevented via feedback regulation where an increased concentration of the product $1,25(OH)D_3$ decreases activity of 25-hydroxylase (26). Finally, activated vitamin D is formed to increase serum phosphorus. A lower phosphorus intake stimulates serum $1,25(OH)D_3$, which then increases resorption of phosphorus from bone (1).

Vitamin D receptor

In order for vitamin D to elicit a physiological effect, it must first gain access to the cell through the vitamin D receptor (VDR). The VDR is member of a superfamily of nuclear

receptors that includes sex and adrenal steroids (27). Superfamily receptors are compartmentalized into the amino-acid domain at the NH_2 terminus, DNA-binding domain and ligand binding domain at the COOH terminal (26-28). In the ligand domain, calcitriol binds to VDR and is phosphorylated and then binds with retinoid X or retinoic acid receptors (1, 26, 27, 29). This causes an allosteric change in the receptor making it able to bind with vitamin D response elements within target genes (1, 27). Zinc fingers, located in the DNA-binding domain (26), interact with hexonucleotide sequences in the vitamin D response element (29) causing it to enhance or inhibit transcription of genes which code for specific proteins (1, 26). The primary known proteins that results from VDR interaction with genes include osteocalcin, 24 hydroxylase, and calbindin (1).

VDR presence in multiple tissues not related to calcium homeostasis raised questions that it may play a role in other mechanisms of the body. Receptors have been found in organs such as the lung, muscle, skin, and placenta (1, 30). Furthermore, Ramagopalan et al found 229 genes that had a significant change in expression in response to vitamin D (31).

Food sources of vitamin D

Dietary vitamin D is available in two forms, D_3 and D_2. Vitamin D_3 is synthesized in human skin and found naturally in the diet from some animal sources (2). Relatively few foods are a natural source of vitamin D. Fatty fish such as swordfish, salmon, and tuna have the highest IU of vitamin D per serving ranging from 137-566 IU. This is followed by beef liver and egg yolk which have about 41 IU (32). Many foods are fortified with either a man-made form or plant derived form of vitamin D, vitamin D_2, known as ergocalciferol.

Although it is not required for milk to be fortified with vitamin D_2, in the United States, most manufacturers fortify milk with 100 IU vitamin D_2 per cup (2). Other fortified sources of vitamin D include, orange juice and ready-to-eat breakfast cereals (32). Hill *et al* in 2012 discovered that 44% of the American and Canadian intake of vitamin D comes from fortified milk products (33). Since vitamin D_3 and D_2 undergo the same two step activation process to form calcitriol and have similar abilities to cure vitamin D deficiency rickets, they are considered equivalent (26).

Vitamin D's role in health

Vitamin D is known to play a role in calcium uptake and therefore bone health in children (rickets) and adults (osteomalacia) (2). Research has also identified additional roles vitamin D may play in other conditions including inflammation (34), cardiovascular disease (29, 34-39) and type 2 diabetes (40-48). These conditions and their relationship to vitamin D are discussed below.

Bone health

Vitamin D's role in calcium homeostasis and bone modeling is well understood (1). When serum calcium decreases, the parathyroid gland releases PTH. A rise in PTH stimulates the kidneys to produce the active form of vitamin D, calcitriol. Calcitriol then increases intestinal absorption of calcium by activating VDRs in the intestinal mucosal cells. Receptor activation in these cells stimulates the production of calbindin, a calcium transport protein, which increases enterocyte absorption of calcium from intestine (1). Calcitriol and PTH stimulate the maturation

of osteoclasts in the bone, a cell which catabolizes the bone matrix releasing calcium and phosphorus in the blood stream, thus increasing serum calcium levels. Furthermore, calcitriol aids in the remodeling of the bone matrix. As serum calcium and calcitriol increase, there is a decrease in PTH, thus decreasing maturation of osteoclast cells which break down bone (2).

Inflammation

The discovery of vitamin D receptors in various tissues has sparked the investigation of vitamin D's role in other aspects of health. Calcitriol has been studied for its ability to decrease inflammation via various pathways, one being the inhibition of (cyclooxygenase) COX-2 (34). Tumor growth is facilitated by the COX-2 enzyme production of prostaglandins. In human prostate cancer cells, Moreno *et al* discovered that calcitriol has the ability to decrease expression of COX-2 enzymes and decrease the ability of prostaglandins to facilitate tumor growth (34).

Cardiovascular disease

Sufficient serum 25(OH)D has been associated with decreased risk of cardiovascular disease (29). A large scale observational study using NHANES data found that when adjusting for other confounders, those with the lowest serum 25(OH)D had a 40% increase in risk of cardiovascular mortality (36). Moreno et al's review of observational studies agrees there is an increased risk of cardiovascular disease at serum 25(OH)D concentrations less than 37.5 nmol/L (34). A meta-analysis of randomized-control trials by Witham et al saw a significant decrease in

diastolic blood pressure among those supplemented with vitamin D. This association was only seen in those whose baseline blood pressure was elevated (38).

Vitamin D may affect cardiovascular health via the renin-angiotensin-aldosterone system and parathyroid hormone (1, 37). The rate limiting step of the renin-angiotensin-aldosterone system is the formation of renin (39). Using knockout mice, Yuan et al concluded that calcitriol attached to VDR blocked the binding of proteins that stimulate the production of renin (39). Thus vitamin D prevents the initiation of the blood pressure raising system. Calcitriol also decreases expression of parathyroid hormone, by blocking transcription of pre-parathyroid hormone within parathyroid tissue (1). Explanation of the relationship between elevated parathyroid hormone and hypertension has not been established, although positive associations have been observed (35).

Type 2 Diabetes

The presence of VDR within pancreatic β-cells and localized production of calcitriol suggest that insufficient vitamin D may affect risk of type 2 diabetes (41). A long term, large scale observational study by Pittas et al found that women who consumed > 800 IU of vitamin D per day had a 23% lower risk of developing type 2 diabetes (45). Furthermore, a recent meta-analysis of observational studies agreed that there was an inverse relationship between serum 25(OH)D and risk for diabetes or metabolic syndrome (42). Direct effect of vitamin D supplementation on insulin sensitivity and secretion is difficult to assess due to its association with decreasing overall weight and FM (46-48). However, after adjusting for race, BMI and age, vitamin D supplementation of 2,000 IU/day in a pre-diabetic population was associated with increased insulin secretion and slower increase in HbA1C (43). Nikooyeh et al found similar results when comparing the same supplementation amount in diabetics, after adjusting for FM. In

this study, there was no association between serum 25(OH)D and any glycemic markers after adjusting for confounding variables (44).

The mechanism by which vitamin D may affect insulin sensitivity is not well understood. It has been suggested that is serum 25(OH)D is negatively correlated to β-cell secretion and positively correlated to insulin sensitivity (1). However, others propose that it indirectly effects glycemic control via regulation of inflammation. Bock et al supplemented healthy subjects with 140,00 IU per month and discovered that it caused an increase in the percentage of regulatory T cells, but did not affect β-cell function (40).

Vitamin D and maternal health during pregnancy

In addition to the health effects in a non-pregnant state, growing evidence suggests a role for an effect of vitamin D deficiency during pregnancy. Pregnancy results in an increased risk of vitamin D insufficiency due to the increased demand for calcium to support fetal bone deposition (7). Current research suggests vitamin D deficiency is related to the development of gestational diabetes (49-53) and pre-eclampsia (54). Assessment of vitamin D sufficiency for mom and the baby in pregnancy is best measured by 25(OH)D as this is the form that crosses the placenta. Calcitriol is formed from 25(OH)D within the fetal kidney (12).

Gestational diabetes

Many risk factors associated with gestational diabetes are non-modifiable: first degree relative with diabetes, history of glucose intolerance, increased maternal age and previous infant with macrosomia (55). Obesity is another risk of gestational diabetes (55) but not one that can be

safely modified during gestation (56). Impaired glucose tolerance in pregnancy increases glucose availability within the infant causing baby to increase production of insulin to control blood glucose (55). This can result in babies that are born large. Twenty percent of babies born to mothers with gestational diabetes are macrosomic, compared to 12% of babies born to mothers without gestational diabetes (57). Furthermore, the offspring of mother with gestational diabetes have a significantly higher risk of developing type 2 diabetes later in life (50, 51).

Like type 2 diabetes, vitamin D status may affect severity/incidence of gestational diabetes (6, 40-45, 49, 52, 53). Zhang et al observed that the risk of gestational diabetes increased 2.66 fold in participants that had serum 25(OH)D levels <50nmol/L. Serum 25(OH)D and glucose levels after a glucose tolerance test were inversely correlated (6, 52).

Causes for the correlation of vitamin D deficiency and gestational diabetes are unknown. Using the homeostasis model assessment index to assess insulin resistance, Maghbooli et al found that vitamin D deficient women have 43% higher insulin insensitivity (52). Intervention studies need to be conducted to provide more insight into the relationship. A large scale European intervention currently underway aims to assess the impact of healthy eating, physical activity and vitamin D supplementation on pregnancy outcomes (58).

Pre-eclampsia

Vitamin D's association with cardiovascular health in pregnancy is seen in pre-eclampsia. A prospective cohort study of 697 pregnant women, found that a serum 25(OH)D levels less than 50nmol/L at 24-26 weeks gestation were significantly associated with an increased risk of developing pre-eclampsia. No association was seen in early pregnancy (54). Few studies have a primary aim to assess pre-eclampsia. The mechanism for this association is thought to be due to

poor regulation of calcium within the blood or possibly interactions between vitamin D, rennin and PTH production (35, 39).

Maternal obesity and vitamin D deficiency

In the United States 47.5% of women who are of childbearing age are overweight or obese (59). Studies have consistently found that those who are obese have an increased risk of vitamin D deficiency (46-48). No definite explanation has been determined however there are two theories, the sequestration of vitamin D by adipose and the regulation of adipose tissue by vitamin D (46). Wortsman et al did an intervention where non-pregnant obese and lean participants had serum 25(OH)D drawn 24 hours after they were either exposed to UV radiation or given a 50,000 IU dose of Vitamin D_3. The study found that BMI was inversely correlated with serum vitamin D_3 after exposure to equal amounts of UV light. Since subcutaneous fat stores vitamin D_3, it is thought that more vitamin D was sequestered in the obese than the non-obese subject due to increased FM (48). The second hypothesis suggests that vitamin D regulates adipose tissue. This theory is more difficult to explore in human studies. A recent 12 week, double-blind, randomized, controlled trial in non-pregnant adults discovered that daily supplementation with 1000 IU of vitamin D was significantly associated with a decrease in body FM when compared to placebo. There were no associations between body weight or waist circumference. It is hypothesized that the mechanism is related to regulation of PTH and intracellular calcium concentration. The thought is that when vitamin D is increased, de novo lipogenesis is increased as well (47).

Recommendations for vitamin D intake during pregnancy

Vitamin D's role in disease states has become popular in scientific research. This has led to a need for standardization of recommendations and classification of status for the general population and for pregnant women. Debate has arisen as to what should be considered when determining these recommendations for pregnancy. The IOM defines adequacy as a serum level of 25(OH)D >50nmol/L (2). It is estimated that an intake of 600 IU/d of vitamin D is needed to obtain this level (2). The Endocrine Society defines adequate serum 25(OH)D as that >75nmol/L and estimates that a much greater intake of 1500 IU-2000 IU/d of vitamin D is needed to reach this blood level (3). (See **Table 1** for IOM and Endocrine Society Classifications)

TABLE 1
Recommended serum 25(OH)D in pregnancy and daily vitamin D intake to achieve those levels

	Serum 25(OH)D	Intake	Upper limit
Institute of Medicine	>50 nmol/L	600 IU	4,000 IU
Endocrine Society	>75 nmol/L	1,500-2,000 IU	10,000 IU

Rates of insufficiency or deficiency during pregnancy

Using data from the 2001-2006 NHANES survey, Ginde et al analyzed prevalence of inadequate serum 25(OH)D using both IOM and Endocrine Society criteria. Thirty-three percent of pregnant women had serum 25(OH)D levels <50nmol/L, considered less than adequate by the IOM. Sixty-nine percent had serum levels <75nmol/L, the amount considered inadequate by the Endocrine Society. Percentages of those women who were inadequate decreased as trimester increased. This is thought to be related to the increased duration of supplementation which also led to a positive association with the amount of women with adequate serum levels (60). In

another dataset from the United States, mean serum levels for pregnant and non-pregnant women were 65nmol/L and 59nmol/L respectively with 42% of non-pregnant women having inadequate serum 25(OH)D. Throughout the data, a larger percent of non-pregnant women had serum 25(OH)D levels lower than pregnant women (60). This is hypothesized to be due to the increased use of vitamin D supplementation found in prenatal vitamins.

Across the globe, many studies have found large portions of pregnant women with low serum 25(OH)D. Of the women participating in England's South Hampton Women's Survey, 33% had serum 25(OH) levels <50nmol/L, and 63% were <75nmol/L (8). In India, 66% of the 559 participants had serum levels <66%nmol/L (61). However, a study of 125 Gambian women did not contain any women <50nmol/L, and only 11% <80nmol/L (19).

Trends of deficiency continue in non-pregnant women and men. Countries close to the equator that receive ample sunlight, such as those in the middle east, have a large portions of women who are vitamin D deficient (62). In a sample of healthy Asian Indians 78% were considered inadequate by IOM standards (63). Even across Australia 40-67% of individuals are estimated to have serum levels <25nmol/L (64).

Classification issues

As shown in Table 1, each society presents different levels for classification of deficient, insufficient, sufficient and possible adverse effects. The IOM (who set official RDA) bases recommendations on research pertaining only to what is needed for adequate bone health and does not consider the extra-skeletal effects of vitamin D (2). The Endocrine Society includes research that addresses other adverse outcomes associated with decreased serum 25(OH)D such as increased risk for pre-eclampsia and cesarean section (2, 3). The main focus of the debate

whether the information on extra skeletal effects of vitamin D is strong enough to change recommendations. This results in recommendations that are quite different due to each society having aims that are intended to address different outcomes. Further complicating the vitamin D story are different recommendations being suggested by researchers. Hollis et al defines a vitamin D serum level between 100-150 nmol/L to be ideal. According to his research, this requires an intake of 4000 IU/d of vitamin D to achieve sufficiency (4).

Among the articles reviewed in regard to vitamin D and pregnancy outcomes, there was inconsistent classification of vitamin D intake and serum status. Only one study done in 2006 used a cut off for adequacy that is less than the current IOM recommendations. Ruth Morley et al defined serum levels <28nmol/L to be deficient, while Gale et al and Leffelaar et al used current IOM recommendations (13, 18, 61). More recent studies done in 2011, and 2012 used the Endocrine Society's classification serum adequacy or even higher (10, 15, 16, 19).

Potential reasons for increased rates of deficiency and insufficiency

It is possible that changes in lifestyle and eating habits may be why vitamin D deficiency has increased. The amount of skin exposed has been significantly associated with serum 25(OH)D levels (21, 65). Perampalam et al studied pregnant women and found that as the amount of skin exposed to the sun increased, so did the average 25(OH)D (65). This trend was also seen in an analysis of the 2003-2006 NHANES data in which lower levels of 25(OH)D were found in those who wore hats, long sleeves and stayed in the shade on sunny days (21). Another concern is the use of sunscreen, which may block UVB exposure and synthesis of vitamin D (66). Many studies that assess the effect of sunscreen on vitamin D status are conflicting. Some randomized controlled trials found decreased serum levels with sunscreen use. However, based

on observational data, the manner in which the public uses sunscreen is not associated with deficiency (24).

Eating habits may also contribute to decrease vitamin D. According to NHANES data from 1999-2004, milk provided 44% of Americans vitamin D intake. Unfortunately milk intake is on the decline (33). The percentage of individuals in all age groups who drink milk has significantly decreased from 1977-1978 to 2005-2006 (67). Historical vitamin D intake is limited because vitamin D intake was not included in NHANES What We Eat in America data tables until 2007 (68). The Minnesota Heart Survey, however, has collected information on vitamin D intake back to 1980. In 1980-1982, the average intake was 197.6 IU while in 2007-2009 this dropped significantly to 174 IU (69). Recent NHANES data reveals that although vitamin D intake from food is not exactly as it was in the 1980s, intake of supplements has increased from 33% in 2007-2008 to 36% in 2009-2010 (68, 70).

Appropriate marker of maternal vitamin D level

The serum measurement of 25(OH)D is ideal in this type of research because it is the form of vitamin D that is passed from mother to fetus (23). Also 25(OH)D is measured instead of the active form of vitamin D calcitriol, because it is not effected by vitamin D intake and it has a very short half-life (1). Two methods are used to assess serum 25(OH)D, liquid chromatography and antibody based (71). Liquid chromatography is sensitive, specific and reproducible and is considered the "gold standard" for analysis. It has the ability to differentiate between $25(OH)D_3$ and $25(OH)D_2$. This is further supported by the production of a calibration solution to assess accuracy of measures (71). Antibody based analysis only detects total 25(OH)D. This assay is more commercial and is the most common in literature (2).

Of antibody analyses, two are most common, enzyme-linked immunosorbent assay (ELISIA) and Radioimmunoassay (RIA). ELISA has a 23% cross-reactivity for 25(OH)D and radioimmunoassay has a 75% cross-reactivity for 25(OH)D (72). Due to these different assay measures, method of serum analysis should be considered when evaluating research.

Relationship between serum measured vitamin D and vitamin D from dietary assessments

Both the IOM and Endocrine Society agree that dietary intake of vitamin D has the ability to effect serum levels (2, 3). A dose response reaction is undecided. Although many studies have evaluated supplementation and intake, many factors influence vitamin D status that an increase in 1 nmol/L has not been associated with a supplemental amount of vitamin D. However, multiple studies have found and positive association between serum 25(OH)D and intake from food or supplemental vitamin D (13, 15, 73, 74).

Sources of variation in maternal vitamin D levels

Variability in maternal vitamin D due to location

The amount of UVB rays available to the skin effects its ability to synthesize previtamin D_3 (1). As latitude increases, the angle at which the sun hits the earth decreases and the amount of UVB photons available decreases, which leads to decreased synthesis of Vitamin D_3 in the skin (75). Due to rotation of the earth, this angle changes seasonal as well. Webb et al observed that skin exposed to direct sunlight in Boston, Massachusetts, 42.2 degree north, produced no pre-vitamin D_3 between the months of November and February. While further south at 34 and 18 degrees North, the skin produced previtamin D_3 year round (76).

Variability due *to pregnancy*

The effect of pregnancy on 25(OH)D is hard to determine due to increased use of supplements during gestation. According to 2001-2006 NHANES data 73% of women who were pregnant, were taking a supplement with vitamin D, compared to 32% of non-pregnant women (60). Furthermore, an observational study compared 25(OH)D of pregnant and non-pregnant women found that levels rose and fell in similarly in association with season of measurement (17). NHANES data however found that 25(OH)D tended to increase with GA. The mean 25(OH)D levels for the first, second and third trimesters were 55 nmol/L, 62 nmol/L and 80 nmol/L, respectively. The increase across trimesters could be related to increased use of vitamin D supplementation across the pregnancy (60).

Maternal vitamin D status related to fetal growth

Within the articles reviewed, serum measurements were taken throughout all stages of pregnancy (9-19). When first trimester serum measures were taken, an association was found between small-for-gestational age and serum measures in 2 of 3 studies. Hossain et al found a positive correlation between maternal serum status and fetal cord blood. Furthermore there was an inverse relationship between cord blood serum 25(OH)D and birth weight (16). It is possible that serum measures throughout gestation are inconsistently correlated to fetal growth. The growth trajectory of the infant is determined in the early stages of pregnancy so measurements at this time should be studied closely (61). No articles were found that compared estimated fetal growth to serum 25(OH) measures within stages of pregnancy. Measurement of EFW would be beneficial to understanding how serum measures effect fetal growth trajectory throughout pregnancy.

Maternal vitamin D status related to offspring size at birth

Maternal vitamin D status and infant birth weight

The data regarding the effect of 25(OH)D on low birth weight is difficult to analyze due to varied GA at measure of 25(OH)D, range of measures accessed and methods of analyzing weight. Large observational studies with more than 1000 multi-ethnic participants found positive associations with 25(OH)D measures at less than 28 weeks gestation and infant birth weight (10, 11, 14, 61). Small observational studies found no association in early gestational measurements (17-19, 77). When 25(OH)D was taken at greater than 28 weeks gestation or at birth, no association was found (12-14, 17, 19).

The presence of participants with low serum levels also effected outcomes. Studies containing most if not all participants above 30nmol/L tended to see no association, while those with ranges that went below 30nmol/L saw a positive correlation (9, 10, 14-17, 19, 61). This may be explained by one study that found the lowest risk for having an infant that is small for GA at serum levels between 60 and 80nmol/L (10).

Studies that measure small for GA rather than birth weight were more likely to find correlations between serum measures and birth outcomes. An infant that is small-for-gestational age if they are lower than the 10^{th} percentile (78). Small-for-gestational age is a beneficial measure because fetal weight can vary greatly across sexes and gestational periods. It allows for researchers to ensure that they are comparing infants to their peers. Within the articles, Bodnar et al found the lowest risk for small-for-gestational age in mothers that had serum levels between 60nmol/L and 80nmol/L in early gestation. Also, in a study by Lefelaar et al, women categorized as having adequate serum levels (>50nmol/L) had a significantly lower percentage of small-for-gestational age infants than those found in the deficient range (<29.9nmol/L) (10, 61).

Maternal vitamin D and infant body composition

As explained above, obesity is associated with low 25 (OH) D and it could be explained by FM. Maternal vitamin D concentrations may also have an effect on infant body composition. Crozier et al discovered that infants born to mothers who had 25 (OH) D concentrations >75nmol/L had infants with 10% greater FM than those with <50nmo/L (8). Associations with maternal vitamin D intake during pregnancy, and increased FM at 6 and 9 years old has been identified (8, 13).

Conclusion

The variation among research methods makes it difficult to determine if there is an exact correlation between maternal serum 25(OH)D and infant birth outcomes. As more research is done, professionals must consider ways to avoid pitfalls that may invoke bias or limit clarity of their research. An ideal project should involve limitations on classification of serum 25(OH)D to avoid bias based on classification. It should consider multiple fetal growth measures such as small-for-gestational age, birth weight, EFW through gestation and infant body composition and compare these to maternal serum measures that have been taken at multiple times throughout pregnancy.

The prevalence and severity of low birth weight infants is the reason why more standardized research needs to be performed. It is only then institutions like the IOM and Endocrine Society will be able to confidently decide on an ideal vitamin D intake to recommend to mothers so that they may have a health pregnancy.

Chapter III

METHODS

Study Overview

This study used the cohort from multiple Pregnancy Health Studies being conducted at the University of Kansas Medical Center. The purpose of this study is to explore the relationship between maternal serum 25(OH)D measured late in pregnancy, fetal growth and neonate body composition at birth.

Sample

Women that were included were participants in three clinical research studies (Factors affecting growth patterns and body composition of infants study (HSC# 13126), Maternal cardiometabolic health during pregnancy study (HSC# 13309), and Characterization of adiposity in pregnancy and its relationship to immunity and infant body composition (HSC#12793)) being performed at the University of Kansas Medical Center in Kansas City, Kansas. Only singleton healthy pregnancies were included in this study. Participants were recruited from the OB clinics at the KU Hospital.

Inclusion/Exclusion Criteria

Women were included in the study if they were:

1. between the ages of 18 and 45 years old
2. singleton pregnancy

3. English speaking

Women were excluded from the study if they:

1. were underweight according to their pre-pregnancy BMI
2. were under the age of 18 years old or over 45 years old
3. had known infectious diseases, diabetes mellitus, hypertension, use tobacco products or any drugs during pregnancy
4. did not speak English
5. were carrying more than one fetus

Setting

This study was conducted at the University of Kansas Medical Center March 2012 to August 2013.

Ethics

This study was approved under the existing protocol of the Factors affecting growth patterns and body composition of infants study (HSC# 13126), Maternal cardiometabolic health during pregnancy study (HSC# 13309) and Characterization of adiposity in pregnancy and its relationship to immunity and infant body composition (HSC#12793) protocols which were assessed and approved by IRB. Before participation, all subjects read through the consent form with a study coordinator who was available to answer any questions. If participant chose to participate, they were enrolled after signing the consent form. Once enrolled, participants were assigned a number, which will be used to safely identify their records and maintain anonymity.

Procedures

Participants attended a study visit at 34-38 weeks. Baseline and descriptive data were collected at the visit. This includes patient reported pre-pregnancy weight and parity. The visit included a blood draw by a registered nurse. Infant birth weight, GA at birth, and maternal weight at delivery were obtained from the patient's electronic medical chart.

Instrumentation:

Fetal biometrics by 2D ultrasound

Fetal biometrics by 2D ultrasound and the Hadlock equation were used to estimate fetal weight at the study visit. The Hadlock equation requires measurements of 3 anatomical locations: head circumference, abdominal circumference, and femur length (79). Images from which measurements are taken should contain specific anatomical locations to ensure accuracy across screening (2). The image of the head used for biparietal diameter and head circumference should be oval shaped and contain the thalami, third ventricle, and septum pellucidum (80, 81). An image that is round or contains the brainstem or cerebellum will not produce accurate measurements (80). Biparietal diameter is measured from the outer edge of the proximal skull to the inner edge of the distal skull (80). Head circumference is measured around the outer perimeter of the skull (Figure 1 A) (80). Abdominal circumference images are a rounded transverse picture that should contain the spine to the right or left (3 or 9 o'clock) of the image, the stomach on the left side of the fetal abdomen, symmetrical ribs and the junction of the left and right portal vein (80, 81). Circumference should be measured at the skin's surface (80). (Figure 1 B). Finally, images of the femur should be taken with the length of the bone visible and

perpendicular to the transducer (81). Measurement is taken along the length of the bone with assurance as to not include the distal epiphysis (Figure 1C) (80).

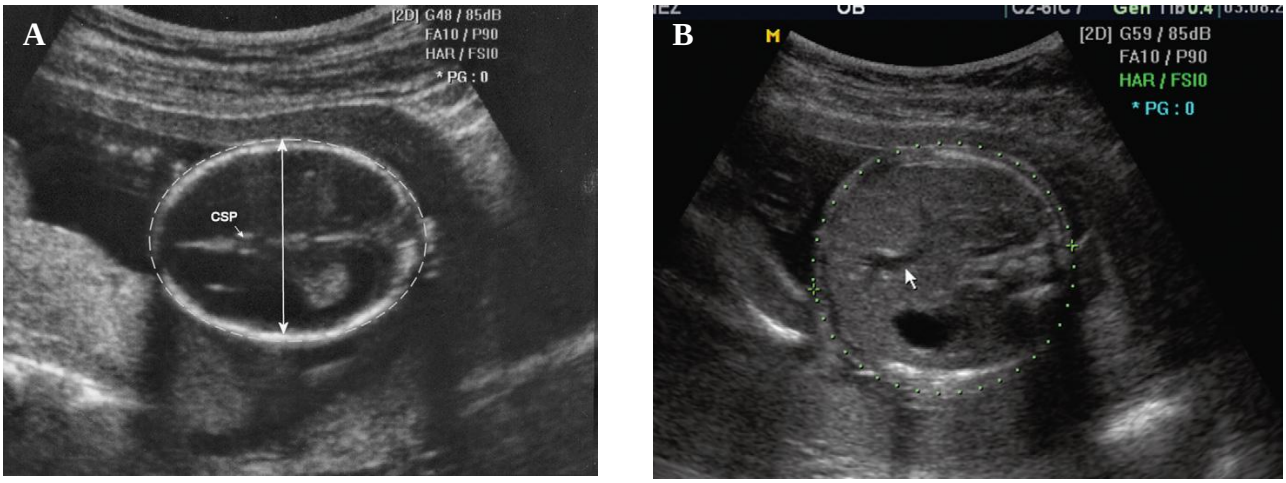


Figure 1 A-C

A. Image of fetal head, biparietal diameter represented by solid line, and circumference represented by dashed line. The cavum septum pellucidum is indicated (CSP)
B. Image for measurement of fetal abdomen circumference. An arrow indicates the junction of the umbilical vein and portal sinus. The spine is located on the right and the stomach is the dark portion at the bottom of the image.
C. Image of the fetal femur indicating a proper measurement along the length of the bone.

Images obtained from Obstetrics: Normal and Problem Pregnancies, 6th ed. © 2012 (80)

Infant body composition by air displacement plethysmography (Pea Pod©)

Infant FM, FFM and %fat were measured within 72 hours of life using the Pea Pod© (Life Measurement Inc. Concord, CA). Density measurement via air-displacement plethysmography is a fast, easy, and non-invasive procedure (82). The Pea Pod© assesses body composition using densitometry, where body density is determined by dividing body mass by

body volume. In this method thoracic gas volume is predicted in infants because measuring it is not feasible (82). The Pea Pod© is an accurate measure of infant body composition, Sainz et al validated the Pea Pod© against chemical analysis using 24 bovine tissue phantoms (83). The Pea Pod© has also been successfully validated using a 4-compartment model in 49 healthy infants by Ellis et al (84).

Maternal serum 25-hydroxyvitamin D

Four tablespoons of blood were taken by a trained registered nurse. This blood was separated within 24 hours of blood draw and stored in -80 degree freezer until time of batch analysis. Plasma was analyzed for serum 25-hydroxy vitamin D content using enzyme-linked immunosorbant assay (ELISA). Included in the batch analysis of serum 25(OH)D is a calibration serum which is used to increase accuracy of serum measures.

Statistical Analysis

Descriptive statistics were calculated for all variables of interest. Data was analyzed using multiple linear regression to explore the relationship between maternal serum 25(OH)D and EFW and infant body composition. Covariates to be explored include maternal age, parity, infant gender, infant GA, maternal GWG and maternal pre-pregnancy BMI. Only significant variables were retained in the final model. Specific models are described with each aim below. Bivariate correlations were used to explore the relationships between the outcome variables of interest and the confounding variables.

All analyses were conducted using the Statistical Package for the Social Sciences (SPSS for windows, version 20; SPSS, Chicago, IL). For tests of significance, $p<0.05$ was used.

Aim 1: Examine the relationship between maternal serum 25(OH)D levels measured late in pregnancy and fetal growth.

Statistical analysis: Multiple linear regression was used to assess the relationship between maternal serum 25(OH)D and EFW. EFW was the outcome (dependent variable) and maternal serum 25(OH)D was the predictor variable (independent variable). Maternal age, parity, infant gender, infant GA, maternal GWG up to the time the fetal measurements were obtained and maternal pre-pregnancy BMI were explored.

Aim 2: Examine the relationship between maternal serum 25(OH)D levels measured late in pregnancy and neonate body composition (%fat, FM and FFM).

Statistical analysis: Multiple linear regression was used to assess the relationship between maternal serum 25(OH)D and infant body composition (%fat, FM and FFM). Three separate models were run with each infant body composition variable as the outcome variable (dependent) and maternal serum 25(OH)D will be the predictor variable (independent variable). Maternal age, parity, infant gender, infant GA, maternal total GWG was obtained and maternal pre-pregnancy BMI was explored.

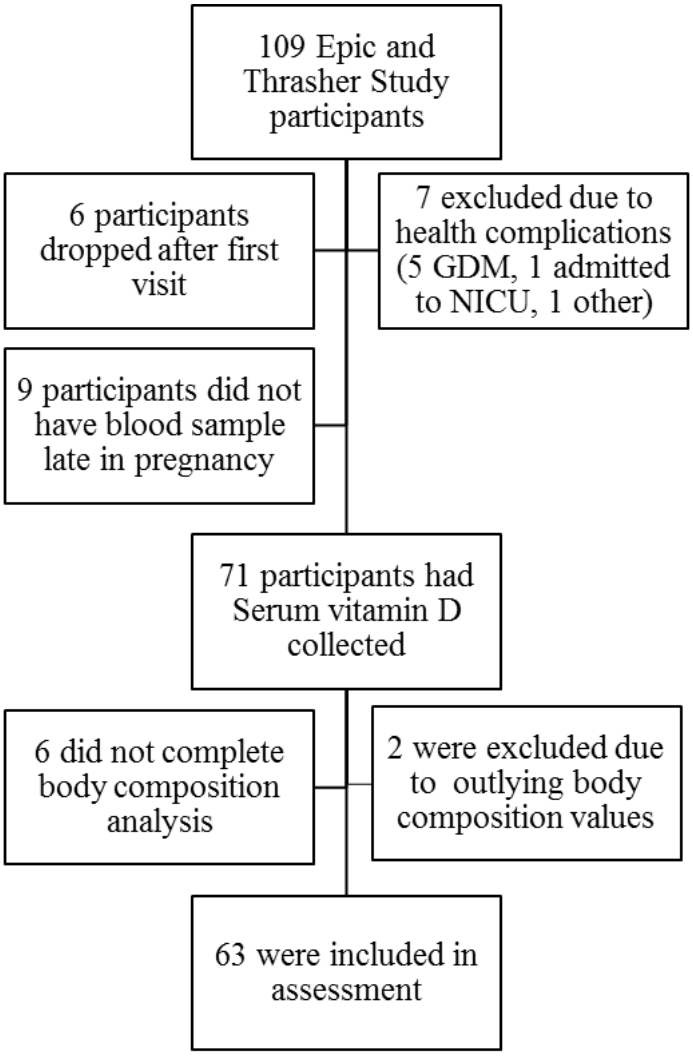
Chapter IV

Results

Study Characteristics for Body Composition Assessment

Combined, the Thrasher and the Epic Study contained 109 participants. Of these participants 45 were excluded from the study: 9 did not have a blood sample available in late pregnancy, 7 were excluded due to health complications such as gestational diabetes, 6 dropped out of the study, and 7 did not complete infant body composition analysis (**Figure 2**).

Figure 2
Inclusion and exclusion of participants from the Epic and Thrasher Study



Sixty-four participants were included in the final analysis. Characteristics of the mother infant pairs divided by serum status can be found in **Table 2**. Endocrine Society ranges for serum status were used in descriptive tables because they are determined based on various physiological effects of vitamin D, as compared to IOM which sets its ranges based solely on vitamin D's effect on bone mineralization (3, 85). The data set contained a majority of Caucasian females (23(74%), bearing male infants (36(57%)). On average participants were 29 years old at the infant's birth, had blood drawn for serum analysis at 36.5 weeks gestation and had a pre-pregnancy BMI of 25 kg/m^2. Those that were considered to have a deficient serum vitamin D status had a significantly higher pre-pregnancy BMI, while no significant difference in pre-pregnancy BMI was found between those that had insufficient and adequate serum. Furthermore, no significant differences were seen between serum status groups in terms of GA at serum analysis, GA at birth or total GWG.

TABLE 2
Characteristics of mother and infant pair by serum vitamin D status

	Total (n = 63)	Adequate >75nmol/L (n = 11)	Insufficient 50-75 nmol/L (n = 20)	Deficient <50nmol/L (n = 32)	p value
Mother					
Serum 25(OH)D_3 concentration (nmol/L)	52.6 ± 23.3	88.9 ± 13.6	61.9 ± 6.8	34.3 ± 11.2	.000
Pre-pregnancy BMI (kg/m^2)	25.0 ± 5.2	22.6 ±3.2	22.8 ± 4.3**	27.1 ± 5.4*	0.003
Parity	0.55 ± 1	0.20 ± 1.3	0.17 ± 0.73	0.63 ± 1.4	0.091
Race					
Caucasian (n (%))	44 (69.8)	10	16	18	
African American (n (%))	10 (15.9)	1	1	8	
Hispanic (n (%))	7 (11.1)	0	2	5	
Other (n (%))	2 (3.2)	0	1	1	
SES					
High School or no qualifications	22	10	5	7	
Some College	15	1	3	11	
4-year College	25	8	6	11	
Graduate School	11	2	6	3	
Age at child's birth (y)	29.2 ± 4.8	31.8 ± 1.7	28.9 ± 3.6	28.6 ± 5.8	0.129
Gestational age at blood draw (wk)	36.5 ± 1.2	36.4 ± 1.3	36.3 ± 1.6	36.7 ± 1.0	0.518
Total GWG (kg)	15.9 ± 6.3	15.4 ± 3.8	17 ± 4.8	15.45 ± 7.6	0.677
Infant					
Male (%)	57.1%	54.5%	55%	59.4%	
Birth weight, (g)	3481 ± 395	3365 ± 297	3495 ± 418	3512 ± 413	0.563
GA at birth, (wk)	39.6 ± 0.8	39.6 ± 0.6	39.7 ± 0.6	39.6 ± 0.9	0.868
Age at body composition testing (days)	3.4 ± 5.0	2.9 ± 2.4	3.2 ± 2.31	3.5 ± 6.7	0.927
FM (g)	377 ± 172	356 ± 133	366 ± 159	391 ± 195	0.802
%fat	11.2 ± 4.2	11.1 ± 3.5	10.8 ± 3.9	11.5 ± 4.7	0.832
FFM (g)	2904 ± 311	2819 ± 173	2930 ± 345	2917 ± 329	0.611

*value is significantly different than adequate serum group. p <0.05
** value is significantly different than deficient serum group p <0.05

Correlation matrix of variables of interest

A correlation matrix was generated to explore the relationships between the outcome and predictor variables but also to explore potential relationships between confounding variables. The date maternal vitamin D was measured was used to create a dichotomized variable: in season (May to October) and out of season (November to April) representing months when the sun can stimulate vitamin D production in the body. This variable was not related to infant %fat, FM or EFW early or late. It was negatively related to infant FFM. Therefore season of measure will only be included as a confounding variable in the model to predict infant FFM. Vitamin D was negatively related to maternal pre-pregnancy BMI (r=-0.355; p=0.003). Therefore maternal pre-pregnancy BMI was not included in any of the models.

Predictors of Infant Birth Weight

Relationships between infant birth weight and maternal serum 25(OH)D late in pregnancy were assessed using multiple linear regression analysis. The only predictor that remained significant in the model was GA at birth (β=171.05; p = 0.005) (**Table 3**). Maternal vitamin D was not related to infant birth weight.

TABLE 3
Predicting infant birth weight with linear regression (adjusted $r^2 = 0.10$)
(n = 63)

	β	*p*
GA at birth	171.05	0.005

p<0.05 considered as significant
Covariates included maternal serum 25(OH)D, GA at birth, maternal total GWG, infant age at test and infant gender

Predictors of Infant Body Composition

Percent fat

The relationship between infant %fat at birth and maternal 25(OH)D in late pregnancy was assessed using multiple linear regression analysis. Covariates included in the model were GA at birth, total maternal GWG, infant age at test and infant gender. Serum vitamin D did not remain a significant predictor of infant %fat. In the model, %fat was positively correlated with infant age (β=1.61; $p = 0.037$). An increase in age by 1 week would equal a 1.61 increase in %fat (**Table 4)**.

TABLE 4
Predicting infant %fat with linear regression (adjusted $r^2 = 0.05$)
(n = 63)

	β	*p*
Age at test	1.61	0.037

p<0.05 considered as significant
Covariates included age at test, maternal serum 25(OH)D, GA at birth, total GWG, and infant gender

Fat Mass

Infant FM was positively correlated with infant age at test (β=87.45; $p = 0.004$). Each week increase in infant age correlated with an 87.45 gram increase in FM (**Table 5**) Covariates that were assessed included serum vitamin D, GA at birth, GWG, gender, and infant age at test.

TABLE 5
Predicting infant FM with linear regression (adjusted r^2= 0.11)
(n = 63)

	β	*p*
Age at test	87.45	0.004

p<0.05 considered as significant
Covariates included age at test, maternal serum 25(OH), GA at birth, maternal total GWG, and infant gender

Fat free mass

Infant FFM was predicted by the following variables: infant age at test (β= 158.24; *p* = 0.001), infant GA at birth (β= 194.37; *p* < 0.001) and infant gender (β= -197.34; *p* = 0.004). In the dataset, males were coded as a 0 and females were coded as 1. Covariates that were assessed included serum vitamin D, season of blood draw, GA at birth, GWG, gender, and infant age at test. These results are presented in **Table 6**.

TABLE 6
Predicting infant FFM with linear regression (adjusted r^2= 0.33)
(n = 63)

	β	*p*
Age at Test	158.24	0.001
Gender	-197.34	0.004
GA at birth	194.37	<0.001

p<0.05 considered as significant
Covariates included age at test, maternal serum 25(OH), season of blood draw*, GA at birth, maternal total GWG, and infant gender
*Season of blood draw was included in this model due to association with infant fat free mass in correlation matrix

Study Characteristics for Estimated Fetal Weight Assessment

Characteristics of participants included in the analysis to predict fetal weight at the early sonogram measurement can be found in **Table 7.** Out of the 64 participants included in body

composition analysis, 8 were excluded from analysis of EFW at early sonogram due to missing data. The 56 participants included have fetal weight estimated at an average of 19.9 weeks gestation. The population included mostly Caucasian women with some education beyond a high school diploma. There were no significant differences in parity, or GA at assessment between serum classification groups. Pre-pregnancy BMI was significantly different between the adequate and deficient group, as well as the insufficient and deficient group.

TABLE 7
Characteristics of mother and fetal measurements at early sonogram measurements

	Total (n = 56)	Adequacy >75nmol/L (n = 9)	Insufficient 50-75nmol/L (n = 19)	Deficient <50nmol/L (n = 28)	p value
Mother					
Serum 25(OH)D_3 concentration (nmol/L)	52.1 ± 23.6	90.0 ± 15.0	61.4 ± 6.6	33.5 ± 11.8	0.000
Pre-pregnancy BMI (kg/m^2)	24.6 ± 4.8	23.0 ± 3.5	22.6 ± 4.4**	26.4 ± 4.8	0.015
Parity	0.70 ± 0.8	0.67 ± 0.9	0.47 ± 0.6	0.86 ± 0.8	0.261
Race					
Caucasian (n (%))	38	8	16	14	
African American (n (%))	10	1	1	8	
Hispanic (n (%))	7	0	2	5	
Other (n (%))	1	0	0	1	
SES					
High School or no qualifications	10	0	5	5	
Some College	15	1	3	11	
4-year College	22	7	6	9	
Graduate School	9	1	5	3	
Age at child's birth (y)	28.8 ± 4.7	31.7 ± 1.8	286 ± 3.6	28.1 ± 5.7	0.133
GA at blood draw (wk)	36.3 ± 1.2	36.2 ± 1.5	36.2 ± 1.5	36.7 ± 1.0	0.360
GWG up to sonogram measurement	5.4 ± 4.5	3.7 ± 2.3	5.0 ± 3.6	6.1 ± 5.5	0.389
Male (%)	58.9%	66.6%	63.2%	53.6%	
GA at Sonogram measurement	19.9 ± 1.7	19.3 ± 2.0	19.7 ± 1.4	20.2 ± 1.7	0.302
EFW	339 ± 111	298 ± 121	320 ± 88	363 ± 120	0.239

*value is significantly different than adequate serum group. p <0.05
** value is significantly different than deficient serum group p <0.05

Only 31 of the included participants had an estimation of fetal weight late in pregnancy. Characteristics of these women can be located in **Table 8.** Of these women there were no significant differences between pre-pregnancy BMI, GWG or GA at sonogram measurements.

Participants who were classified as deficient had blood analysis that was significantly later in gestation than those that were insufficient. No association was found between the adequate and deficient groups or adequate and insufficient groups.

TABLE 8
Characteristics of mother and fetal measurements at late sonogram measurements

	Total (n = 31)	Adequacy >75nmol/L (n = 6)	Insufficient 50-75nmol/L (n = 11)	Deficient <50nmol/L (n = 14)	p value
Serum 25(OH)D_3 concentration (nmol/L)	52.7 ± 24.4	87.6 ± 11.9	61.5 ± 7.0	30.8 ± 11.6	0.000
Pre-pregnancy BMI (kg/m^2)	24.8 ± 5.4	23.6 ± 4.3	22.5 ± 4.7	27.0 ± 5.6	0.084
Parity	0.58 ± 0.7	0.33 ± 0.5	0.36 ± 0.5	0.86 ± 0.7	0.113
Race					
Caucasian (n (%))	23	6	9	8	
African American (n (%))	6	0	2	4	
Hispanic (n (%))	2	0	0	2	
Other (n (%))	0	0	0	0	
SES					
High School or no qualifications	7	0	3	4	
Some College	6	0	2	4	
4-year College	10	5	1	4	
Graduate School	8	1	5	2	
Age at child's birth (y)	28.7 ± 4.6	31.4 ± 1.8	28.1 ± 3.7	28.0 ± 5.7	0.288
GA at blood draw (wk)	36.3 ± 1.2	36.3 ± 0.7	35.6 ± 1.6	36.8 ± 0.7	0.039
GWG up to sonogram measurement	14.7 ± 6.1	12.8 ± 3.0	13.7 ± 5.0	16.3 ± 7.6	0.404
Male (%)	54.8%	33.3 %	54.5 %	64.3 %	
GA at Sonogram measurement	35.3 ± 2.7	35.5 ± 2.4	35.3 ± 1.3	35.1 ± 3.7	0.952
EFW	2623 ± 663	2423 ± 502	2688 ± 584	2658 ± 795	0.722

*value is significantly different than adequate serum group. $p < 0.05$
** value is significantly different than deficient serum group $p < 0.05$

Predictors of Estimated Fetal Weight

The relationship between maternal serum 25(OH)D and EFW was assessed using multiple linear regression analysis. Covariates included were GA at sonogram measurement, GWG up to sonogram measurement and infant gender. Estimated fetal weight early in pregnancy was predicted by GA at sonogram measurement (β=62.57; p <0.001) and GWG up to the sonogram (β= -3.84; p=0.006) (**Table 9**).

TABLE 9
Predicting EFW early in pregnancy with linear regression (adjusted $r^2 = 0.86$)
(n = 56)

	β	*p*
GWG at measurement	- 3.84	0.006
GA at measurement	65.65	<0.001

p<0.05 considered as significant
Covariates included serum 25(OH)D late in pregnancy, GA at measurement, GWG at measurement, and infant gender

Fetal weight estimated later in pregnancy was predicted by GA at sonogram (β=207.81; p < 0.001). Maternal serum 25(OH)D approached significance (β=-4.42; p = 0.090). When maternal vitamin D was dropped from the model, GA at sonogram remained significant. (**Table 10**).

TABLE 10
Predicting EFW late in pregnancy with linear regression (adjusted $r^2 = 0.83$)
(n = 31

	β	*p*
GA at sonogram	208.83	<0.001

p<0.05 considered as significant
Covariates included serum 25(OH)D late in pregnancy, GA at measurement, GWG at measurement, and infant gender

Chapter V

Discussion

The purpose of this study was to explore the relationship between maternal vitamin D levels to fetal growth and infant body composition at birth. Within this data set, maternal serum 25(OH)D was not significantly correlated with EFW in pregnancy, infant birth weight or infant body composition. These results vary from the literature in multiple ways.

Birth Weight and Body Composition and Serum Vitamin D

Infant birth weight

In our sample, serum 25(OH)D was not a predictor of infant birth weight. We found that maternal pre-pregnancy BMI and GA predicted infant birth weight. The body of literature is mixed with some studies finding differences (9-11, 14, 16, 61, 73) while other studies have found no relationship between vitamin D levels and birth weight (12, 13, 15, 17-19, 77).

Gestational age at serum measurement may affect the association between serum 25(OH)D and birth weight. Gale et al analyzed serum 25(OH)D in 466 women late in pregnancy. The population of this study was similar to that of our study and it also found no association with birth weight. While Gernand et al measured serum 25(OH)D in the first and second trimester, and only found a negative relationship with birth weight in the first trimester. This is similar to results found in other studies that assessed serum 25(OH)D before 28 weeks gestation (10, 11, 61). It could be suggested that early serum vitamin D may contribute to a growth trajectory set in early gestation. This theory has been supported in other studies of physiological factors of growth (86).

Furthermore, studies that found an association between birth weight and serum 25(OH)D had over 1000 participants. Moller et al assessed serum 25(OH)D in 92 planned pregnancies at 11, 22, and 35 weeks GA and found no association (17). Leffelaar et al assessed 3730 women at less than 12 weeks gestation and also found an association (61). In multiple studies, low serum 26(OH)D increases the risk of delivering an infant that is small-for-gestational age (10, 11, 61). The relationship may not be detectable within a small cohort.

Infant body composition

In our sample, infant %fat correlated infant age at test. An association between infant age and infant %fat was expected due to the change in infant body composition after birth.
One study has analyzed serum 25(OH)D and infant body composition at birth in a similar manner. Crozier et al saw a correlation between serum vitamin D and %fat at birth. Our study and Crozier's analysis differ significantly in methodology. Crozier assessed infant FM using dual energy x-ray absorptiometry within 3 weeks of life. Our study assessed infant FM using air displacement plethysmography within 3 days of life. Variation between body composition analysis tools is likely minimal (87). However, time of analysis makes these two studies quite different. Statistical models from Crozier et al. did not account for infant weight change from birth to test, feeding methods and other covariates that may affect a child's body composition early in life (8).After a few weeks of life these factors may contribute to infant body composition changes and confound the ability to assess relationships between the maternal *in utero* environment and infant outcomes.

In our study, infant FFM at birth was significantly predicted by age, gender, and GA, which was expected. Within the last weeks of gestation and first weeks of life, infant rate of

growth is quite rapid, thus increasing FFM with age. This is further supported by research which measured infant body composition by week (88). Gender differences in %fat at birth have been reported. In 2009 Fields et al, found a difference in %fat and FFM based on gender. However, this difference no longer existed at 6 months of age (89)

Estimated Fetal Weight and Serum Vitamin D

No published research studies have assessed the relationship between EFW and serum 25(OH)D status. As suspected in both late and early sonogram measurements, GA was a significant predictor of fetal weight. Furthermore, estimation of fetal weight varies greatly after the first trimester therefore it may take a large sample size to detect differences between groups. In a systematic review, N.J Dudley emphasizes that the random errors within estimating fetal weight cause it to have limited accuracy and sensitivity (90). Factors affecting these errors include observer error, image quality, equipment calibration and measurement methods.

Estimated fetal weight early in pregnancy was negatively associated with GWG and positively associated with GA at measurement. Though this may seem like a counterintuitive relationship, with further analysis a logical explanation may be obvious. Maternal pre-pregnancy BMI was not related to early EFW. When you look at the mean maternal GWG per week at the early sonogram, there are dramatic differences by pre-pregnancy BMI. The values for normal, overweight and obese are 0.25, 0.48 and 0.17 kg/week, respectively. The negative relationship with maternal GWG may be reflective of an underlying relationship within the maternal pre-pregnancy groups where an obese maternal pre-pregnancy BMI in light of a low early weight gain still programs a larger fetus.

Limitations and Strengths

Unavoidable limitations are contained within this study. First, EFW was assessed by a trained but not certified study coordinator. This is due to lack of certified sonographer availability and monetary costs associated with it. The sample size included in the study is small and we may not have enough power to detect differences caused by serum vitamin D. We were only able to assess maternal vitamin D status late in pregnancy therefore making the assumption that the maternal vitamin D levels assessed late in pregnancy were representative of the maternal vitamin D status early in pregnancy. Research has suggested that maternal vitamin D does increase across pregnancy (60). Therefore the level we measured late in pregnancy may be underestimating a true relationship as a late measured value is likely greater than what would have been measured early in pregnancy.

There are also several strengths of this study. Maternal vitamin D was analyzed by serum which accounts for vitamin D that is synthesized in the skin and obtained from the diet. Our sample included a wide range of serum vitamin D concentrations from 12 to 120 nmol/L. Another strength of the study is that body composition of infants was measured using air-displacement plethysmography. This method is accurate, safe and easy measure infant FM, FFM, and %fat (82-84). Infant body composition was also assessed within 72 hours of life. This helped to avoid any effects of infant feeding or growth that may occur within the first weeks of life.

Conclusions

In our sample, serum 25(OH)D late in pregnancy did not predict infant birth weight, EFW, or body composition at birth. A review of the literature suggests that serum 25(OH)D's role in fetal growth is observable in large cohorts and when serum 25(OH)D is assessed early in

gestation. More research is required in order to investigate the potential role of early serum 25(OH)D on programming the fetal growth trajectory. Future research should assess a large cohort of women at less than 28 weeks gestation. Inclusion of EFW in this research may provide a greater insight to how a fetus develops differently in mothers of adequate or deficient status. Early detection of insufficient fetal growth related to insufficient 25(OH)D could provide mothers with the ability to correct status and possible improve birth outcomes.

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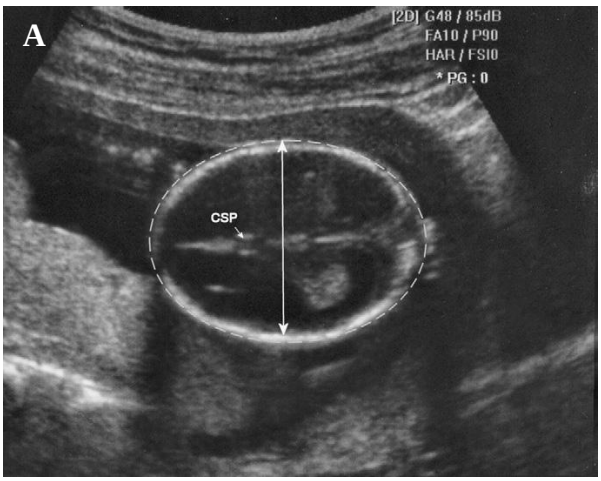
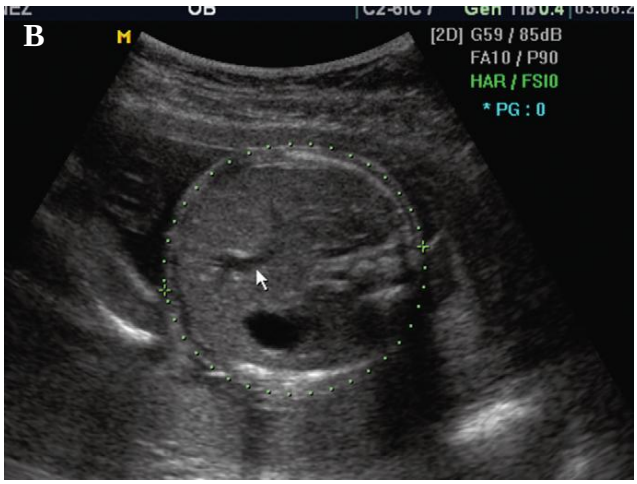
APPENDIX

TABLE 1
Recommended serum 25(OH)D in pregnancy and daily vitamin D intake to achieve those levels

	Serum 25(OH)D	Intake	Upper limit
Institute of Medicine	>50 nmol/L	600 IU	4,000 IU
Endocrine Society	>75 nmol/L	1,500-2,000 IU	10,000 IU





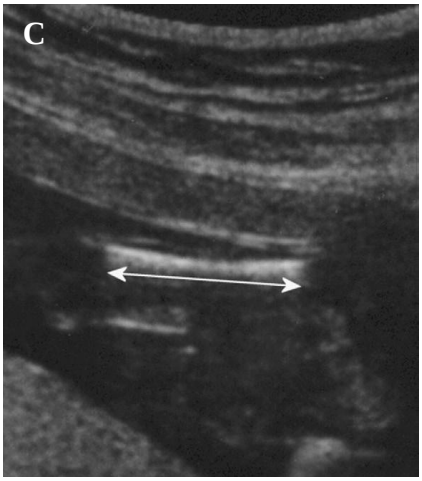


Figure 1 A-C

A. Image of fetal head, biparietal diameter represented by solid line, and circumference represented by dashed line. The cavum septum pellucidum is indicated (CSP)
B. Image for measurement of fetal abdomen circumference. An arrow indicates the junction of the umbilical vein and portal sinus. The spine is located on the right and the stomach is the dark portion at the bottom of the image.
C. Image of the fetal femur indicating a proper measurement along the length of the bone.

Images obtained from Obstetrics: Normal and Problem Pregnancies, 6th ed. © 2012 (80)

Figure 2

Inclusion and exclusion of participants from the Epic and Thrasher Study

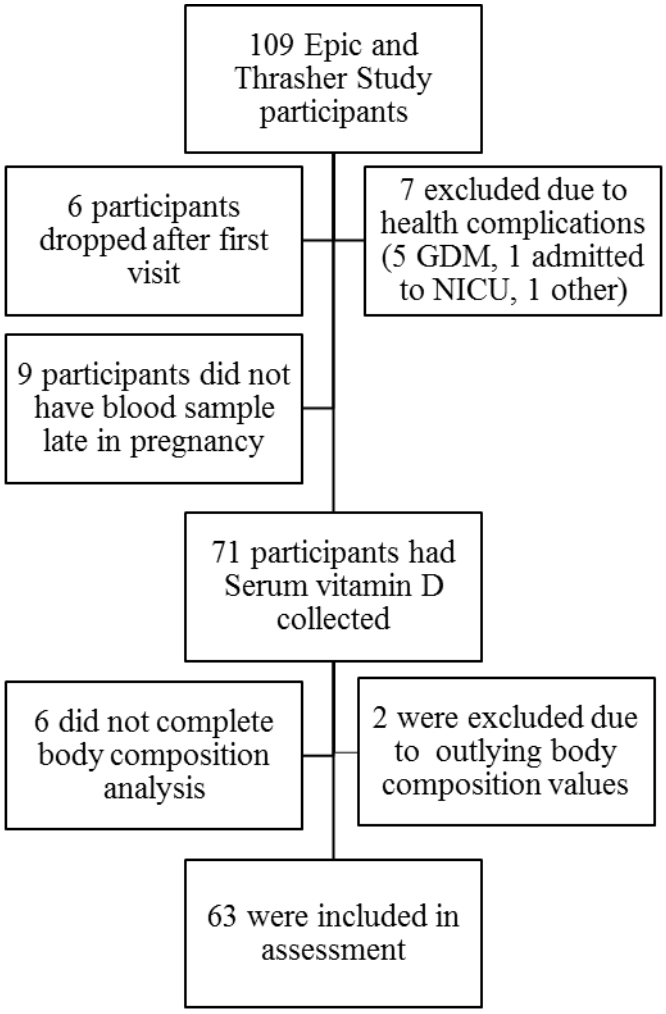


TABLE 2
Characteristics of mother and infant pair by serum vitamin D status

	Total (n = 63)	Adequate >75nmol/L (n = 11)	Insufficient 50-75 nmol/L (n = 20)	Deficient <50nmol/L (n = 32)	p value
Mother					
Serum 25(OH)D_3 concentration (nmol/L)	52.6 ± 23.3	88.9 ± 13.6	61.9 ± 6.8	34.3 ± 11.2	.000
Pre-pregnancy BMI (kg/m^2)	25.0 ± 5.2	22.6 ±3.2	22.8 ± 4.3**	27.1 ± 5.4*	0.003
Parity	0.55 ± 1	0.20 ± 1.3	0.17 ± 0.73	0.63 ± 1.4	0.091
Race					
Caucasian (n (%))	44 (69.8)	10	16	18	
African American (n (%))	10 (15.9)	1	1	8	
Hispanic (n (%))	7 (11.1)	0	2	5	
Other (n (%))	2 (3.2)	0	1	1	
SES					
High School or no qualifications	22	10	5	7	
Some College	15	1	3	11	
4-year College	25	8	6	11	
Graduate School	11	2	6	3	
Age at child's birth (y)	29.2 ± 4.8	31.8 ± 1.7	28.9 ± 3.6	28.6 ± 5.8	0.129
Gestational age at blood draw (wk)	36.5 ± 1.2	36.4 ± 1.3	36.3 ± 1.6	36.7 ± 1.0	0.518
Total GWG (kg)	15.9 ± 6.3	15.4 ± 3.8	17 ± 4.8	15.45 ± 7.6	0.677
Infant					
Male (%)	57.1%	54.5%	55%	59.4%	
Birth weight, (g)	3481 ± 395	3365 ± 297	3495 ± 418	3512 ± 413	0.563
GA at birth, (wk)	39.6 ± 0.8	39.6 ± 0.6	39.7 ± 0.6	39.6 ± 0.9	0.868
Age at body composition testing (days)	3.4 ± 5.0	2.9 ± 2.4	3.2 ± 2.31	3.5 ± 6.7	0.927
FM (g)	377 ± 172	356 ± 133	366 ± 159	391 ± 195	0.802
%fat	11.2 ± 4.2	11.1 ± 3.5	10.8 ± 3.9	11.5 ± 4.7	0.832
FFM (g)	2904 ± 311	2819 ± 173	2930 ± 345	2917 ± 329	0.611

*value is significantly different than adequate serum group. p <0.05
** value is significantly different than deficient serum group p <0.05

TABLE 3
Predicting infant birth weight with linear regression (adjusted $r^2 = 0.10$)
(n = 63)

	β	*p*
GA at birth	171.05	0.005

p<0.05 considered as significant
Covariates included maternal serum 25(OH)D, GA at birth, maternal total GWG, infant age at test and infant gender

TABLE 4
Predicting infant %fat with linear regression (adjusted $r^2 = 0.05$)
(n = 63)

	β	*p*
Age at test	1.61	0.037

p<0.05 considered as significant
Covariates included age at test, maternal serum 25(OH)D, GA at birth, total GWG, and infant gender

TABLE 5
Predicting infant FM with linear regression (adjusted $r^2 = 0.11$)
(n = 63)

	β	*p*
Age at test	87.45	0.004

p<0.05 considered as significant
Covariates included age at test, maternal serum 25(OH), GA at birth, maternal total GWG, and infant gender

TABLE 6
Predicting infant FFM with linear regression (adjusted r^2= 0.33)
(n = 63)

	β	*p*
Age at Test	158.24	0.001
Gender	-197.34	0.004
GA at birth	194.37	<0.001

p<0.05 considered as significant
Covariates included age at test, maternal serum 25(OH), season of blood draw*, GA at birth, maternal total GWG, and infant gender
*Season of blood draw was included in this model due to association with infant fat free mass in correlation matrix

TABLE 7
Characteristics of mother and fetal measurements at early sonogram measurements

	Total (n = 56)	Adequacy >75nmol/L (n = 9)	Insufficient 50-75nmol/L (n = 19)	Deficient <50nmol/L (n = 28)	p value
Mother					
Serum 25(OH)D_3 concentration (nmol/L)	52.1 ± 23.6	90.0 ± 15.0	61.4 ± 6.6	33.5 ± 11.8	0.000
Pre-pregnancy BMI (kg/m^2)	24.6 ± 4.8	23.0 ± 3.5	22.6 ± 4.4**	26.4 ± 4.8	0.015
Parity	0.70 ± 0.8	0.67 ± 0.9	0.47 ± 0.6	0.86 ± 0.8	0.261
Race					
Caucasian (n (%))	38	8	16	14	
African American (n (%))	10	1	1	8	
Hispanic (n (%))	7	0	2	5	
Other (n (%))	1	0	0	1	
SES					
High School or no qualifications	10	0	5	5	
Some College	15	1	3	11	
4-year College	22	7	6	9	
Graduate School	9	1	5	3	
Age at child's birth (y)	28.8 ± 4.7	31.7 ± 1.8	286 ± 3.6	28.1 ± 5.7	0.133
GA at blood draw (wk)	36.3 ± 1.2	36.2 ± 1.5	36.2 ± 1.5	36.7 ± 1.0	0.360
GWG up to sonogram measurement	5.4 ± 4.5	3.7 ± 2.3	5.0 ± 3.6	6.1 ± 5.5	0.389
Male (%)	58.9%	66.6%	63.2%	53.6%	
GA at Sonogram measurement	19.9 ± 1.7	19.3 ± 2.0	19.7 ± 1.4	20.2 ± 1.7	0.302
EFW	339 ± 111	298 ± 121	320 ± 88	363 ± 120	0.239

*value is significantly different than adequate serum group. p <0.05
** value is significantly different than deficient serum group p <0.05

TABLE 8
Characteristics of mother and fetal measurements at late sonogram measurements

	Total (n = 31)	Adequacy >75nmol/L (n = 6)	Insufficient 50-75nmol/L (n = 11)	Deficient <50nmol/L (n = 14)	p value
Serum 25(OH)D_3 concentration (nmol/L)	52.7 ± 24.4	87.6 ± 11.9	61.5 ± 7.0	30.8 ± 11.6	0.000
Pre-pregnancy BMI (kg/m^2)	24.8 ± 5.4	23.6 ± 4.3	22.5 ± 4.7	27.0 ± 5.6	0.084
Parity	0.58 ± 0.7	0.33 ± 0.5	0.36 ± 0.5	0.86 ± 0.7	0.113
Race					
Caucasian (n (%))	23	6	9	8	
African American (n (%))	6	0	2	4	
Hispanic (n (%))	2	0	0	2	
Other (n (%))	0	0	0	0	
SES					
High School or no qualifications	7	0	3	4	
Some College	6	0	2	4	
4-year College	10	5	1	4	
Graduate School	8	1	5	2	
Age at child's birth (y)	28.7 ± 4.6	31.4 ± 1.8	28.1 ± 3.7	28.0 ± 5.7	0.288
GA at blood draw (wk)	36.3 ± 1.2	36.3 ± 0.7	35.6 ± 1.6	36.8 ± 0.7	0.039
GWG up to sonogram measurement	14.7 ± 6.1	12.8 ± 3.0	13.7 ± 5.0	16.3 ± 7.6	0.404
Male (%)	54.8%	33.3 %	54.5 %	64.3 %	
GA at Sonogram measurement	35.3 ± 2.7	35.5 ± 2.4	35.3 ± 1.3	35.1 ± 3.7	0.952
EFW	2623 ± 663	2423 ± 502	2688 ± 584	2658 ± 795	0.722

*value is significantly different than adequate serum group. p <0.05
** value is significantly different than deficient serum group p <0.05

TABLE 9
Predicting EFW early in pregnancy with linear regression (adjusted $r^2 = 0.86$)
(n = 56)

	β	*p*
GWG at measurement	- 3.84	0.006
GA at measurement	65.65	<0.001

p<0.05 considered as significant
Covariates included serum 25(OH)D late in pregnancy, GA at measurement, GWG at measurement, and infant gender

TABLE 10
Predicting EFW late in pregnancy with linear regression (adjusted $r^2 = 0.83$)
(n = 31

	β	*p*
GA at sonogram	208.83	<0.001

p<0.05 considered as significant
Covariates included serum 25(OH)D late in pregnancy, GA at measurement, GWG at measurement, and infant gender