MATERNAL OBESITY, NUTRITIONAL STATUS AND HYPERGLYCEMIA

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Maternal hyperglycemia is a common condition with a profound effect on prenatal and maternal health. We used two complementing cohorts to estimate the associations between: 1) gestational weight gain (GWG), adiposity distribution, and maternal hyperglycemia; 2) pre-pregnancy body mass index (BMI) and an array of maternal nutritional biomarkers; and 3) maternal vitamin D status and maternal hyperglycemia. In the Study of Nutrition and Pregnancy (SNAP), biceps and triceps skinfolds and waist circumference were measured at <13 weeks gestation and 25-hydroxyvitamin D [25(OH)D] concentrations were measured at <16 weeks gestation. Serial weight measurements and post-load glucose concentrations were abstracted from medical records. Because a full array of nutritional biomarkers was not available in SNAP, we also used the Antidepressant Use During Pregnancy (ADUP) study. In ADUP, height and nutritional biomarkers were measured and pre-pregnancy weight was self-reported at ≤ 20 weeks gestation. In the SNAP study, each 0.3-kg/week increase in first trimester GWG rate was associated with a 2.2 (95% CI: 0.1, 4.3)-mg/dl increase in glucose concentration. Each 8.6-mm increase in biceps skinfold thickness and 11.7-mm increase in triceps skinfold thickness was associated with 4.3 (95% CI: 0.2, 8.5)-mg/dl increases in glucose. In the ADUP study, principal component analysis of the biomarkers resulted in an EFA component, a Micronutrient component, and a Carotenoid component. Obese pregnant women were 3.0 (95% CI: 1.1, 7.7) times as likely of being in the lowest tertile of the EFA component and 4.5 (95% CI: 1.7, 12.3) times as likely of being in the

lowest tertile of the Carotenoid component as their lean counterparts. Among non-smokers in SNAP, each 21-nmol/L increase in serum 25(OH)D was associated with a 4.1 (95% CI: 0.9, 7.2)-mg/dl increase in maternal post-load glucose concentration. Among smokers, each 21-nmol/L increase in serum 25(OH)D was associated with a 7.3-(95% CI: 11.4, 3.1) mg/dl decrease in maternal glucose concentration after confounder adjustment. This dissertation is important to public health because hyperglycemia has a major impact on the health of mothers and infants and these data may lead to nutritional interventions that are safe, inexpensive, and acceptable to women.

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PREFACE

I would like to acknowledge the instruction and encouragement provided by my mentor, Lisa Bodnar, throughout my doctoral training and dissertation. Dr. Bodnar challenged me to constantly improve my research methodology, writing skills, and critical thinking and her guidance was fundamental to the quality of this project. I would also like to thank my dissertation committee members, Joyce Chang, Kathleen McTigue, Rhobert Evans, and Hy Simhan, for their insights and patience. Each provided crucial guidance during the course of this project. This research would not have been possible without the staff and participants of SNAP and the ADUP study, and I would like to give them my heartfelt thanks for the time they gave to this project.

Most importantly, I am greatly indebted to my friends and family. Their encouragement and advice has been an immeasurable comfort throughout the dissertation process. I especially want to thank the loves of my life, Liz Bowden and Sprout, both of whom remind me daily of what in life matters most. To Liz: you have encouraged me through each step of my education and without your love, patience and sense of humor, this project would never have reached completion.

1.0 INTRODUCTION

1.1 BACKGROUND

Maternal hyperglycemia is a common complication of pregnancy with profound effects on the health of mothers and children. It is defined as high blood sugar during pregnancy and, when above a certain threshold, is a defining component of gestational diabetes mellitus (GDM). Rates of hyperglycemia during pregnancy are not well established, but it is estimated that as many as 4% of pregnancies are complicated by GDM (Getahun, Nath, Ananth, Chavez, & Smulian, 2008). GDM has long been associated with poor pregnancy outcomes ranging from cesarean delivery to infant macrosomia (Henriksen, 2008; Jevitt, 2005). Evidence suggests that there is a dose response relationship between maternal glucose concentrations and adverse pregnancy outcomes, even below the threshold of overt GDM (Metzger et al., 2008). Despite this, few studies have examined glucose concentrations as a continuous outcome. Studies are needed to better understand the risk factors that contribute to high glucose concentrations during pregnancy.

Current evidence suggests that maternal obesity, most commonly measured by pre-pregnancy body mass index (BMI), is one of the strongest modifiable risk factors for GDM (Chu et al., 2007). But pre-pregnancy BMI only describes one aspect of maternal adiposity. Other measures of maternal adiposity, such as gestational weight gain (GWG) (Hedderson, Gunderson,

& Ferrara, 2010; Herring et al., 2009; Saldana, Siega-Riz, Adair, & Suchindran, 2006; Tovar, Must, Bermudez, Hyatt, & Chasan-Taber, 2009), subcutaneous fat (Yilmaz, Kucuk, Ilgin, & Dagdelen, 2010) and central adiposity (Branchtein et al., 1997; Madhavan, Beena Kumari, & Sanal, 2008; Wendland, Duncan, Mengue, Nucci, & Schmidt, 2007; Yeung et al., 2010; S. Zhang, Folsom, Flack, & Liu, 1995), may be associated with maternal glucose concentrations, but have been less well researched. These measures can be easily calculated during pregnancy from basic anthropomorphic measurements and, when used with pre-pregnancy BMI, have the potential to provide a better assessment of a woman's risk for hyperglycemia during pregnancy.

An important link in the relationship between maternal adiposity and GDM is nutrient deficiency. Obese pregnant women may be more likely to be deficient in the nutrients that impact glucose metabolism, such as antioxidants, folate, essential fatty acids (EFA) and vitamin D. Previous literature suggests that obese patients may be more likely to have poor nutritional status (Andersen et al., 2006; Bodnar, Catov, Roberts, & Simhan, 2007; Karlsson et al., 2006; Kimmons, Blanck, Tohill, Zhang, & Khan, 2006), but this has not been well studied in pregnancy. Vitamin D in particular may have a substantial biological effect on glucose homeostasis (C. Zhang et al., 2008). However, the relationship between vitamin D and maternal glucose tolerance has not been extensively researched.

Exploring the interplay between maternal obesity, nutrient deficiencies and glucose intolerance in pregnancy is significant because nutrition is a modifiable risk factor. Given that GDM has a major impact on the health and well-being of mothers and infants, these data may lead to nutrition interventions to prevent GDM that are safe, inexpensive, and acceptable to women.

1.2 RESEARCH AIMS

The goal of this dissertation was to explore the interrelationship among maternal adiposity, micronutrient and EFA status, and glucose concentrations during pregnancy. We used data from two prospective cohort studies. The Study of Nutrition and Pregnancy (SNAP) was a large, prospective pregnancy cohort study with rigorous data on maternal adiposity, vitamin D, and glucose concentrations. The Antidepressant Use during Pregnancy (ADUP) Study was a smaller cohort with data on pre-pregnancy BMI and a full panel of maternal nutritional biomarkers. The two studies complemented one another to provide novel data on maternal obesity, nutrition and hyperglycemia.

The specific aims of this project were as follows:

1) Determine the independent and joint associations between maternal pre-pregnancy BMI, GWG, and adiposity distribution at <16 weeks gestation and maternal post-load glucose concentrations.

2) Use principal component analysis to discover the underlying structure of maternal nutritional biomarkers and evaluate the independent association between general adiposity and these patterns at ≤ 20 weeks gestation.

3) Determine the association between maternal vitamin D status at <16 weeks gestation and maternal post-load glucose concentrations.

2.0 LITERATURE REVIEW

Maternal hyperglycemia, or high blood sugar during pregnancy, is a defining component of GDM. Although maternal hyperglycemia below the threshold of GDM diagnosis exhibits a dose-response association with adverse birth outcomes such as primary cesarean delivery, high birth weight, neonatal hypoglycemia, preeclampsia, and shoulder dystocia (Metzger, et al., 2008), very little research has been done on this clinically important outcome. Research in the arena of maternal glucose concentrations has largely focused on GDM, which is defined as glucose intolerance, i.e. the inability to appropriately metabolize sugars, with onset or first recognition during pregnancy, as an outcome. For example, rates of hyperglycemia during pregnancy are not well established, but it is estimated that GDM occurs in 4% of pregnancies (Getahun, et al., 2008). GDM has been found to be associated with short- and long-term health consequences in both mother and infant. GDM is a major risk factor for stillbirth, infant overgrowth (Henriksen, 2008), shoulder dystocia, cesarean delivery, and neonatal hypoglycemia (Jevitt, 2005). GDM can also affect both mother and child later in life. Between 15% and 60% of women with gestational diabetes will develop type 2 diabetes five to 15 years after delivery (C. Kim, Newton, & Knopp, 2002). It is estimated that large for gestational age infants born to mothers with GDM are at an almost four times greater risk of developing metabolic disorder (including glucose intolerance)

by the age of eleven than infants born to mothers without GDM (Boney, Verma, Tucker, & Vohr, 2005).

2.1 INTRODUCTION

Although the etiology of hyperglycemia during pregnancy is still largely unknown, the etiology of GDM is estimated to be similar to the etiology of type 2 diabetes mellitus (Robitaille & Grant, 2008; Solomon et al., 1997). The etiology and pathophysiology of type 2 diabetes can be different among patients, but ultimately manifests in dysfunction of the insulin producing cells in the pancreas (β-cells) and/or insulin resistance in tissue. The possible mechanisms linking obesity to type 2 diabetes are complex. Excess fat can cause high concentrations of free fatty acids, altered adipokine expression, and low-grade inflammation, all of which are thought to increase insulin resistance and the secretion of insulin by β-cells (Ioannidis, 2008). It is strongly suggested that obesity plays a key role in the development of type 2 diabetes (Anderson, Kendall, & Jenkins, 2003). There is some evidence to suggest that similar mechanisms occur in the development of GDM (Klein et al., 2008; Sivan & Boden, 2003; Tsai et al., 2005). Increased insulin resistance is a normal physiological change during pregnancy and is thought to be a mechanism to nourish the fetus. In a normal third trimester, a mother's insulin sensitivity has decreased to 30% that of her pre-pregnant state (Buchanan & Xiang, 2005). Maternal adiposity may be a factor that explains how normal insulin resistance during pregnancy can develop to maternal hyperglycemia and GDM. The goal of this project was to explore the interrelationships

among maternal adiposity, micronutrient and EFA status, and glucose concentrations during pregnancy (Figure 1).

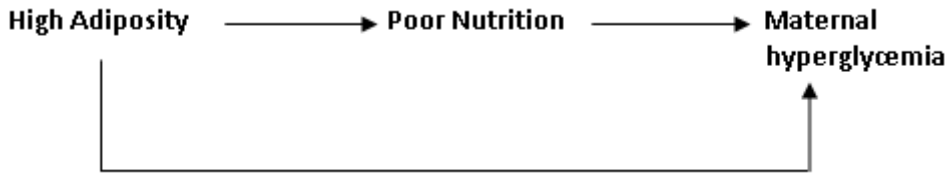

Figure 1. Proposed interrelationship between maternal adiposity, nutrition, and maternal hyperglycemia.

2.2 MATERNAL GLUCOSE CONCENTRATIONS AND ADIPOSITY

2.2.1 Pre-pregnancy body mass index

The strongest known modifiable risk factor for GDM is maternal pre-pregnancy BMI. In the United States, the rate of GDM is increasing in parallel with an epidemic rise in pre-pregnancy obesity for both black and white women (Getahun, et al., 2008; S. Y. Kim, Dietz, England, Morrow, & Callaghan, 2007) (Figure 2).

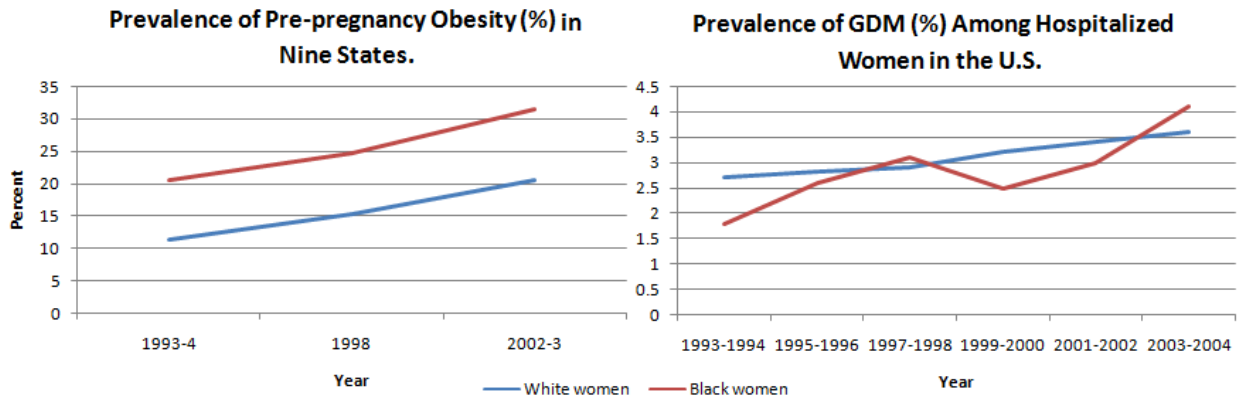

Figure 2. Increasing prevalence of pre-pregnancy obesity (S. Y. Kim, et al., 2007) and gestational diabetes (Getahun, et al., 2008), by race

The relationship between pre-pregnancy BMI, the most common measurement of maternal general adiposity, and GDM has been extensively researched in a variety of populations (Haeri, Guichard, Baker, Saddlemire, & Boggess, 2009; Hedderson, Williams, Holt, Weiss, & Ferrara, 2008; Joy, Istwan, Rhea, Desch, & Stanziano, 2009; Knight, Kurinczuk, Spark, & Brocklehurst, 2010; Leung et al., 2008; Nohr et al., 2009; Qvigstad, Voldner, Godang, Henriksen, & Bollerslev, 2010; Shirazian et al., 2009; Torloni et al., 2009; van Leeuwen et al., 2010). For example, a meta-analysis of 20 cohort studies estimated that the odds ratios for developing GDM were 2.1 (95% CI: 1.8-2.5), 3.6 (95% CI: 3.1-4.2), and 8.6 (95% CI: 5.1-16.0) among pre-pregnancy overweight, obese and severely obese women, respectively, compared with normal-weight women (Chu, et al., 2007). Researchers have recently looked beyond a woman's weight immediately before pregnancy and have begun to explore the relationship between a woman's weight changes over her life course and her risk of GDM. These studies suggest two intriguing new facets in the relationship between a woman's weight and her risk for GDM. Firstly, that women with low birth weight are at increased risk for later development of

GDM, and secondly that there is a J-shaped relationship between BMI in early adulthood and GDM (Rudra, Sorensen, Leisenring, Dashow, & Williams, 2007; Yeung, et al., 2010). This new evidence suggests that the relationship between adiposity and GDM is perhaps more complicated than simply higher BMI leads to higher risk for GDM.

Although the associations between pre-pregnancy BMI and GDM are strong and consistent, BMI has limitations as a measure of adiposity. BMI alone has poor sensitivity (49%, 95% CI: 48-50%) to measure adiposity in women in the adult general population (Romero-Corral et al., 2008) and cannot be measured during pregnancy. Although a woman may enter pregnancy with a healthy pre-pregnancy BMI, she may still excessively gain weight during the prenantal period. Additionally, pre-pregnancy BMI only measures general adiposity, and women with similar BMI values may have widely varying distribution of adipose tissue. Other measures, for example GWG and other anthropometrics, may provide valuable insight to the effect of maternal adiposity on glucose concentrations.

2.2.2 Gestational weight gain

GWG is the result of the different components of pregnancy, including the fetus and placenta, fluid, uterus, mammary glands and fat. Fat mass accumulation increases in parallel with GWG as a pregnancy progresses (Rasmussen, Yaktine, & Institute of Medicine (U.S.). Committee to Reexamine IOM Pregnancy Weight Guidelines., 2009). Despite our increasing knowledge of the physiological effect of fat mass accumulation on insulin resistance during pregnancy (Harlev & Wiznitzer, 2010), few studies have quantified the effect of weight gain during pregnancy on the risk of GDM. Additionally, pre-pregnancy BMI has an inverse relationship with GWG

(Rasmussen, et al., 2009), but it is unknown what role it may play in the relationship between GWG and GDM. A cohort study of black and white women found that adequacy of GWG ratio (observed/expected) had a borderline association with impaired glucose tolerance only among overweight women [OR=2.4, 95% CI: 0.9-6.2, p-value<0.1] and had no association with GDM (Saldana, et al., 2006). Among a Hispanic cohort of women, there was a statistically significant interaction between pre-pregnancy BMI and GWG in relation to abnormal glucose tolerance (50-g glucose tolerance test ≥ 135 mg/dl). Once accounting for this interaction, women with a pre-pregnancy BMI ≥ 35 who had a high rate of weight gain (>0.30 kg/week) were three times as likely to develop GDM as women with normal weight gain (Tovar, et al., 2009). Among a primarily white population, women in the highest quartile of GWG before GDM screening had increased odds of impaired glucose tolerance in pregnancy [OR=2.5, 95% CI: 1.3-5.2], but not GDM [OR=0.9, 95% CI: 0.5-1.7] compared to women in the lowest quartile. Pre-pregnancy BMI was not found to be a significant effect modifier (Herring, et al., 2009). A recent study found a dose response between tertiles of rate of GWG and GDM, and a significant association between exceeding the Institute of Medicine's (IOM) recommended weight guidelines and GDM [OR=1.5, 95% CI: 1.1-2.2]. This same study also found that pre-pregnancy BMI was not a significant effect modifier, and additionally suggested that risk of GDM was primarily attributed to increased weight gain in the first trimester (Hedderson, et al., 2010).

Randomized controlled trials that assessed the effect of weight control interventions on maternal glucose are inconsistent in their conclusions. An evaluation of these studies is further complicated because the interventions involved can be separated into dietary, exercise or both programs and within these categories, the interventions are widely varied. A meta-analysis of nineteen studies concluded that dietary counseling reduced the incidence of GDM, but that the

quality of these studies was poor and further research was needed to understand what interventions are most effective (Oostdam, van Poppel, Wouters, & van Mechelen, 2011). The interventions included dietary, exercise and Metformin treatment. While many interventions may be time intensive and expensive, which reduces their real-world feasibility, others are less expensive and easier to implement in a clinical setting. For example, Quinlivan et al. found that obese pregnant women who were randomized to a four step intervention which included 1) provider continuity; 2) weighing at each visit; 3) a five minute intervention by a food technologist; and 4) clinical psychology management had lower GWG (7.0 versus 13.8 kg, $p < 0.01$) and were less likely to develop GDM (OR = 0.17, 95% CI: 0.03-0.95, $p = 0.04$) than obese controls who received standard care (Quinlivan, Lam, & Fisher, 2011). These studies suggest that GWG is a modifiable risk factor and that maternal glucose can be reduced through weight management interventions, but further research is needed to develop effective interventions.

Studies have found differing relationships between GWG and glucose tolerance. Additionally, there is not agreement in previously published literature on the role that pre-pregnancy BMI plays in this relationship. These differences may be partially accounted for by the different racial make-ups of the studies and the variety of methods used to measure weight gain and glucose tolerance. The Institute of Medicine (IOM) has recently released new guidelines for the amount of weight a woman should gain during pregnancy, based on her pre-pregnancy BMI (Rasmussen, et al., 2009). Only one previous study (Hedderson, et al., 2010) explored whether women who exceeded these guidelines were at increased risk for developing GDM. Continuous glucose tolerance has not been rigorously studied and only two studies examined patterns of weight gain across trimesters of pregnancy (Hedderson, et al., 2010;

Herring, et al., 2009), therefore we are unable to fully understand the nature of the relationship between GWG and glucose tolerance. Our study aims to fill this gap in knowledge.

2.2.3 Maternal adiposity distribution

Studies of type 2 diabetes suggest that adiposity distribution, in particular central obesity, may predict insulin resistance in non-pregnant populations better than BMI (Fujimoto, Abbate, Kahn, Hokanson, & Brunzell, 1994; Huxley et al., 2008; Nyamdorj et al., 2009). For example, in a group of Japanese men, BMI was not related to the insulin sensitivity index, but central adiposity, specifically intra-abdominal adiposity, was significantly correlated (Fujimoto, et al., 1994). But the distribution of body fat in pregnant women has, for the most part, been neglected (McCarthy, Strauss, Walker, & Permezel, 2004).

Anthropometric measurements could potentially be used to describe maternal adiposity distribution. Anthropometry can be used to measure general adiposity, central adiposity and subcutaneous fat. These can be measured using a variety of techniques, ranging from the very technical, for example dual energy X-ray absorptiometry (DEXA) scans, to the very simple, for instance height, weight, waist circumference, and bicep and triceps skin folds. Anthropometric measurements such as height, weight, waist circumference, and bicep and triceps skin folds are inexpensive, objective, noninvasive, and safe. Anthropometric equations, i.e. models of body fat based on many different measurements, are predictors of body fat during pregnancy (Paxton et al., 1998) and there is evidence that anthropometric measurements can be predictive of perinatal outcomes (McCarthy, et al., 2004).

Anthropometric measurements may be a good predictor of glucose intolerance in pregnancy (Figure 3). BMI has been widely studied as a predictor of GDM, but other measurements of maternal adiposity have not been researched as rigorously. The few studies available suggest that there is a potential association between GDM and other measures of maternal adiposity, such as waist circumference and skin fold thicknesses.

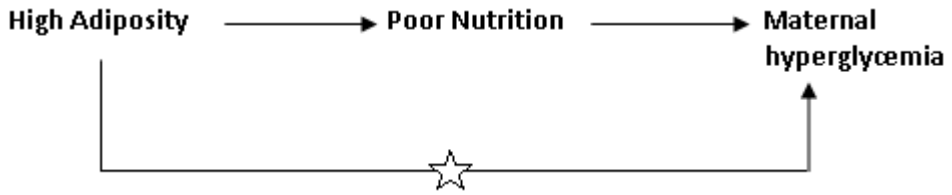

Figure 3. Proposed relationship between maternal adiposity (as measured by gestational weight gain and adiposity distribution) and maternal hyperglycemia.

Evidence suggests that maternal waist circumference measurements in early pregnancy are associated with GDM diagnosis, but there is insufficient evidence to understand the temporality, magnitude and nuances of this relationship. Cross-sectional analyses suggest that there are significant linear relationships between waist circumference and blood glucose (Branchtein, et al., 1997) and that percent body fat between 26 and 36 weeks, calculated using skin fold thicknesses, is higher in women with GDM then in women with normal glucose tolerance (Yilmaz, et al., 2010). Although these associations were significant, both of these studies lack the ability to give us information regarding the temporality of this relationship. A prospective analysis estimated that women with waist circumference measurements in the highest tertile before pregnancy were twice as likely to develop GDM as women in the lowest tertile (S. Zhang, et al., 1995). Other prospective studies have found that waist circumference

measured during pregnancy can predict later development of GDM diagnosis with a sensitivity ranging from 63 to 75% and a specificity ranging from 57 to 81% (Madhavan, et al., 2008; Wendland, et al., 2007). More compelling results from the Nurses' Cohort II Study support this hypothesis, suggesting that waist circumference has a dose response relationship with GDM diagnosis (Yeung, et al., 2010).

These conclusions are intriguing, but these studies are limited by several factors. For example, measuring anthropometric measurements several years before pregnancy and using self-reported GDM status (Yeung, et al., 2010; S. Zhang, et al., 1995) may introduce misclassification. A low sample size and a racially homogeneous population (Madhavan, et al., 2008; Yeung, et al., 2010) limit our ability to generalize these results to other populations and explore the potential for effect modification by racial status. Finally, collecting anthropometric measurements late in pregnancy, i.e. > 26 weeks gestation reduces their accuracy (Wendland, et al., 2007). Additionally, only one study assessed the effect of central adiposity on continuous glucose tolerance (Branchtein, et al., 1997).

Excessive GWG and distribution of adiposity may be important predictors of GDM and using these measures may give us unique information about how adiposity affects glucose intolerance. Previous studies have suggested that there may be a relationship between GDM and these measures of adiposity, but there are gaps in our knowledge of these associations. Studies of these relationships need to be conducted among a racially diverse population to assess the potential interaction of race. Anthropometric measurements must be taken directly before or early in pregnancy and new IOM recommendations for GWG should be used. Finally, we suggest that future studies in this arena include an assessment of continuous glucose measurements as well as risk of GDM diagnosis. As discussed above, this is because women

with glucose intolerance below the threshold of GDM diagnosis are also at increased risk for poor pregnancy outcomes (Metzger, et al., 2008).

2.3 OBESITY, MICRONUTRIENTS AND ESSENTIAL FATTY ACIDS

High adiposity may be an important predictor of GDM on its own, but we are also interested in other pathways that may explain how high adiposity affects glucose tolerance. For example, high maternal adiposity may be associated with poor micronutrient and EFA status (Figure 4).

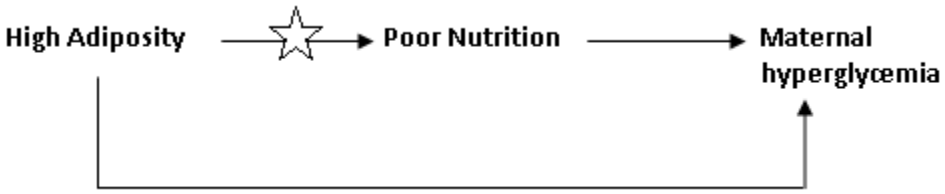

Figure 4. Proposed relationship between high adiposity (measured by pre-pregnancy BMI) and poor nutrition.

Obese women may consume a diet low in key micronutrients and EFAs, but there is also the possibility that high adiposity mass alters micronutrient and EFA status by obstructing absorption and modifying the metabolism of nutrients. Recent evidence supports the hypothesis that high adiposity may be associated with poor micronutrient and EFA status. These micronutrients and EFAs are discussed in detail below. For ease of review, our analysis of these papers is summarized in Table 1.

Table 1. Summary of evidence supporting a relationship between maternal adiposity and nutritional biomarker status.

Nutrient	Biology	Evidence Quality	Prenatal evidence
Antioxidants	Antioxidants may prevent the damage caused to β-cells by oxidative stress.	Carotenoids: Strong Vitamin E: Weak Vitamin C: Suggestive	Carotenoids: No Vitamin E: No Vitamin C: No
Folate	Folate metabolizes homocysteine, which may inhibit insulin secretion.	Strong	No
EFA	n-3 EFAs increase insulin sensitivity in tissues.	Suggestive	Yes
Vitamin D	β-cells require vitamin D to release insulin	Strong	Yes
*Strong=more than three studies in different populations, at least one having an N≥100, with agreement of a significant negative association or correlation (as BMI increases, nutrient decreases) and at least one study is a case-control or cohort; Suggestive=one to three studies in different populations with agreement of a significant association or correlation; Weak=One third or more of the available literature has results that either show a positive correlation (as BMI increases, nutrient increases) or has non-significant results. Evidence will also be classified as weak if studies showing a negative correlation are poor quality (very low N, significant potential bias, etc.)			

2.3.1 Antioxidants

Oxidative stress is more common in women with GDM than in healthy pregnant women (Chaudhari, Tandon, Vaney, & Agarwal, 2003; Coughlan, Permezel, Georgiou, & Rice, 2004; Coughlan, Vervaart, Permezel, Georgiou, & Rice, 2004; Kinalski et al., 2001; Lappas, Permezel, & Rice, 2004; Peuchant et al., 2004; Toescu et al., 2004). Pancreatic β-cells are especially vulnerable to free radicals and, in times of oxidative stress, can become damaged (Evans, Goldfine, Maddux, & Grodsky, 2003). Supplementation with antioxidants may decrease insulin resistance in obese patients (Vincent et al., 2009). By raising low-density lipoproteins, increased

fat mass may lead to oxidative stress, which in turn may damage pancreatic β-cells and impair insulin secretion. Dietary antioxidants, including carotenoids, alpha-tocopherol, and ascorbic acid, may prevent oxidative stress during pregnancy and therefore protect against GDM. High adiposity may be a moderator in this relationship, but we do not yet understand the relationship between high adiposity and antioxidant status.

Fat mass may contribute to poor antioxidant status in pregnant women by sequestering fat-soluble vitamins (carotenoids and vitamin E) or by creating an increased need for antioxidants by creating an excess of free radicals. For example, increased plasma low-density lipoprotein (LDL) levels lead to an increase in free radicals in normal pregnancy (Toescu, Nuttall, Martin, Kendall, & Dunne, 2002; Toescu, et al., 2004). Numerous studies, discussed in more detail below, suggest that obese individuals may be at increased risk of antioxidant deficiency.

Carotenoids (e.g. α-carotene, β-carotene, lycopene, lutein/zeanthin, and β-cryptoxanthin): Decreased serum carotenoid levels have been significantly correlated with increased BMI (Ford, Gillespie, Ballew, Sowell, & Mannino, 2002; Galan et al., 2005; Kimmons, et al., 2006; Neuhouser et al., 2001) and metabolic syndrome (Coyne, Ibiebele, Baade, McClintock, & Shaw, 2009) in a variety of non-pregnant populations. In a more rigorous analysis of adiposity, decreased serum β-carotene was significantly associated with general adiposity [e.g. bioelectrical impedance analysis (BIA) and BMI] and central adiposity (e.g. waist circumference and waist to hip ratios) (Wallstrom et al., 2001). Interestingly, a large prospective study reported that adiposity (BMI) at study entry predicted serum carotenoid levels seven years later (Andersen, et al., 2006). Similarly, older women with high body fat [as measured by BMI and BIA] exhibited smaller increases in plasma carotenoid levels after consuming a high-

carotenoid diet then lean older women (Yeum, Booth, Roubenoff, & Russell, 1998). These last two studies are of special interest because they support the pathway that body fat causes a reduction in plasma carotenoid levels.

Vitamin E: The relationship between vitamin E and fat mass has not, to our knowledge, been studied in pregnancy. But, decreasing serum α-tocopherol levels were correlated with increasing BMI in two different non-pregnant populations (Kimmons, et al., 2006; Neuhouser, et al., 2001). In contrast, Wallström P et al. found that decreasing serum α-tocopherol was significantly correlated with *decreasing* central adiposity among older women (Wallstrom, et al., 2001).

Ascorbic acid (vitamin C): In a large cross-sectional analysis of 10,384 healthy women, increasing waist-hip ratio was significantly correlated with plasma ascorbic acid (Canoy et al., 2005). Overweight and obese individuals, have significantly lower levels of serum ascorbic acid than normal weight women in some populations (Kimmons, et al., 2006; Tungtrongchitr et al., 2003) and *higher* serum ascorbic acid levels in others (Galan, et al., 2005).

2.3.2 Folate and Homocysteine

Folic acid plasma concentrations have been associated with endothelial dysfunction in type 2 diabetics (Mangoni et al., 2005). Additionally, folate is essential for the metabolism of homocysteine (Selhub, 2008), an amino acid that inhibits the secretion of insulin in vitro (Patterson, Flatt, & McClenaghan, 2007). In non-pregnant populations, there is strong evidence that decreased serum folate is associated with increased adiposity, even in a post-folic acid fortification era. There is a significant inverse dose response relationship between serum folate

and fat mass (BMI and DEXA scans) in post-menopausal women (Mahabir et al., 2008). Obese and overweight women have significantly lower levels of serum folate then normal weight women in a small Thai population (Tungtrongchitr, et al., 2003) and U.S. women of childbearing age (Kimmons, et al., 2006; Mojtabai, 2004).

2.3.3 Essential fatty acids

N-3 EFAs, such as linoleic acid, and n-6 EFAs, such as alpha-linolenic acid, have recently created a lot of interest because of their potential to increase insulin sensitivity in tissues (Flachs, Rossmeisl, Bryhn, & Kopecky, 2009). Increasing visceral fat mass is negatively correlated to serum levels of linoleic acid and total EFAs (Kishino et al.). Similarly, increased abdominal adipose tissue is associated with decreased serum n-3 EFAs and increased n-6:n-3 ratios in adolescent girls (Karlsson, et al., 2006). In healthy pregnant women, increased pregravid BMI was significantly associated with decreased plasma DHA (an n-3 EFA) (Wijendran et al., 1999).

2.3.4 Vitamin D

Obesity is a well-known risk factor for vitamin D deficiency. In non-pregnant populations, serum metabolites of vitamin D have been found to be significantly associated to general and central adiposity, measured using body weight, BMI, BIA, DEXA, waist circumference, and skin folds, in a variety of populations (Alemzadeh, Kichler, Babar, & Calhoun, 2008; Arunabh, Pollack, Yeh, & Aloia, 2003; Bell et al., 1985; S. Cheng et al., 2010; Compston et al., 1981; Hahn et al., 2006; Hey, Stokholm, Lund, & Sorensen, 1982; Holvik, Meyer, Haug, & Brunvand, 2005;

Hypponen & Power, 2006; Kimmons, et al., 2006; Looker, 2005; Nesby-O'Dell et al., 2002; Parikh et al., 2004; Snijder et al., 2005; Yanoff et al., 2006; Young et al., 2009). Vitamin D is likely to be regulated in the body differently in obese subjects versus lean subjects (Moan, Lagunova, Lindberg, & Porojnicu, 2009). In randomized control trials, overweight and obese subjects had a significantly reduced response to UV-B irradiation (Wortsman, Matsuoka, Chen, Lu, & Holick, 2000) (artificial sunlight) as well as a reduced response to vitamin D supplementation when compared to lean subjects (Blum, Dallal, & Dawson-Hughes, 2008; Wortsman, et al., 2000). For example, Wortsman et al. exposed 13 obese and 13 lean individuals to UV-B irradiation and 11 obese and 11 lean individuals to vitamin D supplements and evaluated their serum vitamin D3 before and after. Vitamin D3 levels increased in both groups after the treatments, but were significantly lower in the obese groups in both treatments (Wortsman, et al., 2000). The relationship between adiposity and vitamin D is further complicated by the interaction of race. 25- Hydroxyvitamin D levels are generally lower in black women than in white women, but may not be strongly associated with adiposity in black women, though an association is seen in white women (Looker, 2005; Winters, Chennubhatla, Wang, & Miller, 2009). Obese and overweight pregnant women may be increased risk for vitamin D deficiency. Obese pregnant women have been shown to have a higher prevalence (61% versus 36%; $p<0.01$) of vitamin D deficiency as measured by 25-hydroxyvitamin D when compared to lean pregnant women (Bodnar, Catov, et al., 2007).

2.3.5 Summary

There is strong evidence that obesity is associated with deficiencies of carotenoids, folate, and vitamin D. There is suggestive evidence that obese women may be at increased risk for low levels of vitamin C and EFA. There is weak evidence that obese women are at increased risk for low levels of vitamin E (Table 1). Unfortunately, a large percentage of this literature was conducted in non-pregnant populations and, due to the unique nutritional requirements of pregnancy (Institute of Medicine (U.S.). Subcommittee on Nutritional Status and Weight Gain during Pregnancy. & Institute of Medicine (U.S.). Subcommittee on Dietary Intake and Nutrient Supplements during Pregnancy., 1990), cannot be generalized to a pregnant population. Overweight and obese pregnant women may have inadequate micronutrient and EFA intake through diet and supplements and/or excess fat in overweight and obese women may cause alterations in nutrient absorption or metabolism. In an attempt to better understand how poor nutrition contributes to glucose intolerance, we will explore vitamin D, the micronutrient with the strongest evidence of a relationship to maternal fat mass, in more detail.

2.4 VITAMIN D AND MATERNAL GLUCOSE CONCENTRATIONS

Maternal vitamin D deficiency may be a link between obesity and hyperglycemia (Figure 5). The most important source of vitamin D is production in the skin through exposure to sunlight (Holick, 2004). Vitamin D is also naturally present in fish, shiitake mushrooms, and egg yolk and is fortified in many dairy products, breakfast cereals and orange juice (Holick, 2007).

Vitamin D obtained from these sources is biologically inert and must first be converted to 25-hydroxyvitamin D [25(OH)D] in the liver, and then to the physiologically active 1,25-dihydroxyvitamin D [1,25(OH)2D] in the kidney (van den Berg, 1997). The importance of vitamin D to the body is evidenced by the fact that all human tissues that have vitamin D receptors (Holick, 2007). Despite the important role vitamin D plays in a woman's health, poor vitamin D status is common among pregnant women. It is estimated that 3% of pregnant women in the U.S. are deficient in vitamin D [25(OH)D < 25nmol/L], and 63% have insufficient vitamin D status (<75nmol/L) (Looker et al., 2008). Insufficiencies are even more common in black pregnant women; it is estimated that up to 92% of black pregnant women have insufficient vitamin D status (Looker, et al., 2008). The relatively high prevalence of poor vitamin D status, as well as the sizeable disparity in status between black and white pregnant women suggests that further investigation of the relationship between adverse birth outcomes, such as maternal glucose intolerance, and poor vitamin D status are warranted.

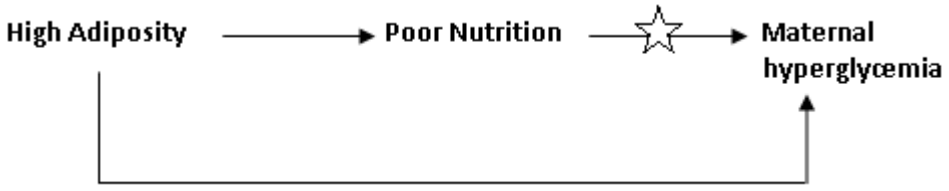

Figure 5. Proposed relationship between poor nutrition (vitamin D) and hyperglycemia

2.4.1 Biological plausibility

There are several potential mechanisms for poor maternal vitamin D status to increase maternal glucose concentrations. Vitamin D is best known for its role in calcium homeostasis, promoting bone mineralization and the prevention of rickets. However, vitamin D has many roles in human

health. 1,25(OH)2D is required for the pancreas to release insulin (Kadowaki & Norman, 1985). 1,25(OH)2D binds to receptors on the pancreatic β-cell and may regulate β-cell calcium homeostasis (Norman, Frankel, Heldt, & Grodsky, 1980; Sooy et al., 1999). Also, vitamin D in the body ensures an adequate intracellular cytosolic calcium pool, which is essential for glucose uptake by insulin-responsive tissues (Draznin et al., 1988).

Vitamin D deficiency and β-cell dysfunction were strongly associated in healthy, non-diabetic and diabetic populations (Chiu, Chu, Go, & Saad, 2004; Gedik & Akalin, 1986). Cross-sectional studies in varying non-pregnant populations have found strong correlations between blood glucose levels and 25(OH)D, the primary serum form of vitamin D (Baynes, Boucher, Feskens, & Kromhout, 1997; Isaia, Giorgino, & Adami, 2001; Scragg, Sowers, & Bell, 2004). Vitamin D supplementation improved blood glucose levels in vitamin D deficient populations (Boucher, Mannan, Noonan, Hales, & Evans, 1995). More importantly, a large prospective cohort study found that low serum 25(OH)D predicted glycemic status, insulin resistance and metabolic disorder in a non-pregnant population (Forouhi, Luan, Cooper, Boucher, & Wareham, 2008).

2.4.2 Previous literature

Recent evidence has explored the possibility that vitamin D is linked to the development of GDM. Cross-sectional data suggest that mean serum concentrations of 25(OH)D are significantly lower in women with GDM (16.49 nmol/L) than in healthy pregnant women (22.97 nmol/L, $p<0.05$) (Maghbooli et al., 2007) and similar results were found in other cross-sectional analysis (Clifton-Bligh, McElduff, & McElduff, 2008). In contrast, Farrant et al reported that maternal

hypovitaminosis D (25(OH)D <50 nmol/L) measured at 30 weeks gestation was not associated with GDM (Farrant et al., 2009), but they did find that glucose intolerance increased significantly as vitamin D concentrations decreased. These cross-sectional studies provide us with a glimpse of the potential relationship between vitamin D and maternal glucose concentrations, but they do not provide us with any information regarding temporality.

The best research to date is a large, nested case-control study (C. Zhang, et al., 2008) that found women with vitamin D deficiency at 16 weeks gestation were at 2.66-fold (95% CI: 1.01-7.02) increased risk for GDM than women with normal vitamin D status. This association was significant even after adjustment for maternal age, race/ethnicity, family history of type 2 diabetes, and pre-pregnancy BMI. This paper was an important contribution to the literature, but it was limited by a racially homogenous population and failing to examine continuous glucose tolerance, a clinically important outcome measure.

2.5 SUMMARY OF LITERATURE REVIEW

Maternal hyperglycemia causes significant maternal and infant morbidity, but is not well researched. This has motivated the search for modifiable risk factors for high glucose concentrations. Pre-pregnancy BMI is a strong predictor of GDM, but promising literature of type 2 diabetes in non-pregnant populations, and limited studies of GDM, have suggested that other facets of maternal adiposity may play a more important role in the prediction and prevention of maternal hyperglycemia. Exploring patterns of maternal adiposity, such as adiposity distribution, central adiposity, and GWG will give us valuable insight into the pathway

between maternal adiposity and glucose concentrations. Anthropometric measurements like BMI, waist circumference and skin fold thicknesses are accurate, safe methods to describe adiposity and adiposity distribution in pregnant women and may predict the risk of maternal hyperglycemia. Poor nutrition is likely to play a key role in the relationship between obesity and maternal hyperglycemia. There are many studies suggesting that obesity may increase the likelihood of poor nutritional status, but there are very few studies that research its role in the nutrition of pregnant women, when nutrition is of upmost importance. Further, there are no studies known in pregnant women that look at patterns of nutrition across a profile of key nutrients. Deficiencies in many nutrients may contribute to the development of maternal hyperglycemia. Furthermore, there is compelling biological and epidemiologic evidence to suggest that low levels of vitamin D, which is common in pregnant populations, could potentially increase the risk of developing high glucose concentrations. These studies, while highly suggestive, do not include an analysis of continuous glucose concentrations and a racially diverse population.

3.0 METHODS

Using a prospective design, a full panel of maternal micronutrients and EFA biomarkers, a population of African American and white pregnant women, and measurement of continuous glucose concentrations, our study was better able to describe the contribution of nutrition and obesity to maternal hyperglycemia (Figure 6). This is of vital importance because, at a time when the rate of GDM is dramatically increasing in the United States (Getahun, et al., 2008), the development of effective prevention strategies has the potential to improve the health of many women and children. Maternal nutrition and adiposity are modifiable risk factors, and prevention strategies, such as nutrition counseling and supplementation, are inexpensive, safe, and acceptable to women. Given the profound and long term impact hyperglycemia has on maternal and neonatal morbidity, this project has tremendous capacity to benefit public health.

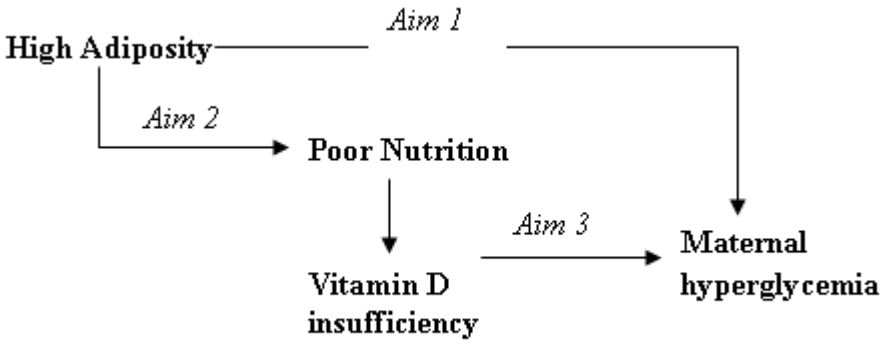

Figure 6. Proposed interrelationship between maternal adiposity, poor nutrition, and hyperglycemia

3.1 OVERVIEW OF STUDY DESIGN AND STRUCTURE

We had the unique opportunity to study the interplay between maternal adiposity, micronutrient and EFA status, and glucose tolerance using data and specimens from two pregnancy cohort studies: the Antidepressant Use during Pregnancy (ADUP) Study and the Study of Nutrition and Pregnancy (SNAP). A complete panel of nutritional biomarkers required for Specific Aim 2 was not available from the SNAP study within the constraints of our research timeframe, but these nutritional biomarkers of interest were collected in total in the ADUP study. Therefore, we employed both of these cohorts to achieve the aims of our study.

3.1.1 The Study of Nutrition and Pregnancy (SNAP)

SNAP is an ongoing prospective cohort study of the gene-environment interactions that contribute to a high-risk phenotype for preterm birth (R01 HD052732, PI: Simhan). In this study, women were recruited at Magee-Womens Hospital at <16 weeks gestation and followed through labor and delivery to assess birth outcome. Women were approached at their first prenatal visit before 16 weeks gestation and asked to participate. At that time, women participated in visit one which includes: informed consent, interview, anthropometric measurements and blood draw for 25(OH)D. Women were asked to return for visit two between 24-28 weeks gestation, which includes a second blood draw. For this analysis, we used data from visit one. Women were also

routinely screened for GDM using a 50-g 1-hour oral glucose challenge test at 24-28 weeks gestation. At delivery, pregnancy outcomes were collected and post-load glucose concentration values were obtained from the hospital laboratory electronic database.

3.1.2 The Antidepressant Use during Pregnancy (ADUP) Study

The ADUP Study was a prospective cohort study of the effects of antidepressant use and major depressive disorder on pregnancy outcomes and child development (R01 MH60335; K Wisner, PI). ADUP has completed recruitment and is in the follow-up stage. Pregnant women were recruited at < 20 weeks gestation and were followed to 2 years postpartum. Women were asked to participate in three visits during pregnancy. The first visit, at approximately 20 weeks, included informed consent, interview, and blood draw for nutritional biomarkers. Visits 2 and 3, at 30 and 36 weeks gestation, respectively, repeated the blood draw. We used data from the 20 weeks gestation visit for this analysis. At delivery, pregnancy outcomes were collected. Women were also asked to continue participation after delivery, but that data was not included in this analysis.

3.2 DESCRIPTION OF STUDY POPULATIONS

3.2.1 Description of SNAP Population

Eligible women were less than 16 weeks gestation, with a singleton pregnancy, and were self reported non-Hispanic black or non-Hispanic white. Women were excluded if they have any of

the following: pre-gestational diabetes, vaginal bleeding, known thrombophilias, chronic hypertension requiring medication, current or planned cervical cerclage, compromised immune system (HIV+, use of systemic steroids within 6 months, use of posttransplant immunosuppressants), autoimmune disease (e.g. inflammatory bowel disease, systemic lupus erythematosus), or known use of illegal drugs or controlled substances. SNAP began recruiting women in June of 2003. Data collection for this project ended on May 2010 and blood samples were assayed November 2009. As a result, 724 eligible women were included in our analysis of adiposity and glucose concentrations and 672 eligible women were included in our analysis of vitamin D status and maternal glucose concentrations.

3.2.2 Description of ADUP Population

Eligible women had singleton gestations. Women were excluded if they had any of the following: psychosis, bipolar disorder, active substance use disorder (identified by self report or urine drug screen), gestational exposure to benzodiazepines or prescription drugs in the FDA-defined category of D or X (other than SSRI) or chronic diseases (such as insulin-dependent diabetes). Between 2004 and 2007, ADUP enrolled 197 eligible women who were included in our analysis of micronutrient and essential fatty acid status and pre-pregnancy BMI. Women in ADUP are primarily older, non-Hispanic white and well educated.

3.3 DEFINITIONS AND ASSESSMENT OF MEASURES

3.3.1 Maternal Adiposity

Adiposity was measured using pre-pregnancy BMI, biceps and triceps skinfolds thicknesses, waist circumferences, and gestational weight gain.

Pre-pregnancy BMI: Pre-pregnancy weight and height were collected through self-report at 20 weeks gestation in ADUP and at <16 weeks gestation in SNAP. BMI was calculated as weight (kg) divided by height (m^2). A BMI of <18.5 was defined as underweight, 18.5-24.9 as normal, 25.0-29.9 as obese, and ≥30 as obese ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998).

Skinfolds: Skinfolds were only measured in SNAP. Biceps skinfold thickness and triceps skin fold thickness were collected at <13 weeks gestation. Biceps skinfold thickness was measured in the front of the upper arm at the mid-point of the biceps muscle and triceps skinfolds thickness was measured at the back of the upper arm on the mid-point of the triceps muscles. The skinfolds were measured in mm using calipers by a trained research nurse and measurements were taken three times to reduce measurement error. Skinfold thicknesses are measures of subcutaneous fat, and therefore can be altered by the changes in water retention experienced during pregnancy. As a result, skin folds overestimate subcutaneous fat in pregnancy when compared to more rigorous techniques (Sohlstrom & Forsum, 1997; Stevens-Simon, Thureen, Barrett, & Stamm, 2001). Skinfold thickness measurements also have the

potential to be influenced by the fatigue or distraction of the nurse collecting the measurements (Norgan, 1992).

Waist Circumference: Waist circumference was only measured in SNAP. A trained research nurse collected waist circumference measurements at <13 weeks gestation. Waist circumference is measured at the smallest point of the waist. This measurement was used to estimate central adiposity. We also categorized high waist circumference as greater than 88 cm ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998) and high waist-to-hip ratio as greater than 0.85 (Madhavan, et al., 2008). Outside of pregnancy, waist circumference is well validated (Lapidus et al., 1984; Larsson et al., 1984), but may lose accuracy in patients with BMI greater than 35 ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998).

Gestational Weight Gain: Weight gain during pregnancy was measured only in SNAP. To maintain temporality, we measured GWG up to the visit where women were screened for GDM. Weight in pounds before pregnancy and at each prenatal visit was abstracted from medical records, and recalculated into kilograms. Pre-pregnancy weight was self-reported and weight at each prenatal visit was measured with clothes on by clinic staff. “Adequate weight gain” during pregnancy has been defined using a variety of methods in the previous literature (Hedderson, et al., 2010; Herring, et al., 2009; Saldana, et al., 2006; Tovar, et al., 2009). We will calculate both the rate of GWG in kg/week and quantify the adequacy of GWG as a ratio of observed GWG to expected GWG, similar to the method described by Bodnar et al. (Bodnar, Siega-Riz, Simhan, Himes, & Abrams, 2010).

Observed GWG was calculated as weight at GDM screening visit minus pre-pregnancy weight. Expected GWG will defined as 100% of the 2009 IOM recommendations at the gestational age of screening visit (Rasmussen, et al., 2009), which can be found in Table 2.

Table 2. 2009 IOM recommendations for total and rate of maternal weight gain, by pre-pregnancy BMI

Pre-pregnancy BMI	BMI (kg/m^2)	Total Weight Gain Range (kg)	Mean and (Range) of Rates of Weight Gain[1] in 2nd and 3rd Trimester (kg/week)
Underweight	< 18.5	12.7 – 18.1	0.45 (0.45-0.59)
Normal weight	18.5 – 24.9	11.3 – 15.9	0.45 (0.36-0.45)
Overweight	25.0 – 29.9	6.8 – 11.3	0.27 (0.23-0.32)
Obese	≥ 30	5.0 – 9.1	0.23 (0.18-0.27)
IOM, Institute of Medicine; BMI, body mass index [1]Assuming a 0.5–2 kg weight gain in the first trimester (Abrams, Carmichael, & Selvin, 1995; Carmichael, Abrams, & Selvin, 1997; Siega-Riz, Adair, & Hobel, 1994)			

We categorized GWG into inadequate, adequate, and excessive. There is not a recommended range of total GWG up to GDM screening, but we calculated this range using the equation for expected GWG and the range of total recommended weight gain in the first trimester. For example, if a woman was obese and was screened for GDM at 24 weeks, then the range of recommended total weight gain was: lower limit is 2.5 kg = [0.5 + (24-13) x 0.18] and upper limit is 5.0 kg = [2 + (24-13) x 0.27]. We then divided the lower and upper limits of the recommended total weight gain range by the expected total weight gain at the gestational age of GDM screening for each BMI group and multiply by 100 to calculate ranges of recommended percentage of expected weight gain. Using the example above, the expected GWG for an obese woman at 24 weeks gestation was 3.0 kg = [0.5 + (24-13) x 0.23]. Therefore, the ranges of

percent of recommended GWG were 83% = 2.5/3 to 166%. These ranges of percent of recommended GWG were used to categorize GWG adequacy as inadequate if a woman's calculated "% of GWG recommendations met" is less than the lower cutoff of recommendation, adequate if it is within recommended range, or excessive if it is greater than the upper cutoff of recommendation.

3.3.2 Nutritional biomarkers

Micronutrient and EFA status were measured using a panel of maternal nutritional biomarkers. The ADUP study previously measured serum carotenoids, serum vitamin E, plasma vitamin C, plasma folate, plasma homocysteine, red cell EFAs, serum ferritin, serum soluble transferrin receptors, and serum 25(OH)D. Red cell EFA biomarkers were quantified in Dr. Rhobert Evan's Heinz Laboratory and measures of folate and homocysteine were quantified at Dr. Nader Rifai's laboratory at the Boston Children's hospital. All other biomarkers were quantified by Dr. Powers' laboratory at the Magee-Womens Research Institute. Biomarkers were quantified using a variety of methods which are described in detail below and summarized in Table 3.

Table 3. Information on nutritional biomarkers collected in the SNAP and ADUP studies

Nutrient	Biomarker	Time integration[1]	Method of measurement
EFA	Red cell membrane EFA	Past 2-3 months	Gas-liquid chromatography
Folate	Plasma folate	Past few weeks	LC/MS/MS
Homocysteine	Plasma homocysteine	Past few weeks	LC/MS/MS
Vitamin A	Retinol	Past few weeks	HPLC
Vitamin C	Plasma ascorbic acid	Past few weeks	HPLC
Vitamin D (SNAP)	Serum 25-hydroxyvitamin D	Past few weeks	RIA
Vitamin D (ADUP)	Serum 25-hydroxyvitamin D	Past few weeks	ELISA/HPLC

Table 3 continued.

Vitamin E	Serum α-tocopherol	Past few weeks	HPLC
Carotenoids[2]	Serum carotenoids	Past few weeks	HPLC
Iron	Serum ferritin	Past few weeks	RIA
Iron	Serum soluble transferrin receptors	Past few weeks	ELISA

EFA = essential fatty acids; LC/MS/MS = liquid chromatography, tandem mass spectrometry; HPLC = high-performance liquid chromatography; ELISA = enzyme-linked immunosorbent assay; RIA = radioimmunoassay
[1]Indicates the time in the past that the biomarker reflects nutritional status (Hunter, 1998).
[2]Carotenoids measured will be: α-carotene, β-carotene, lycopene, lutein/zeanthin, and β-cryptoxanthin.

Carotenoids, Vitamin E, and Vitamin A Status: Carotenoids, vitamin E and vitamin A all fall into the category of lipid soluble antioxidants. Carotenoids are the best known biomarkers of fruit and vegetable consumption, and we will measure the most prevalent carotenoids: α-carotene, β-carotene, lycopene, lutein/zeanthin, and β-cryptoxanthin (Institute of Medicine (U.S.). Panel on Dietary Antioxidants and Related Compounds., 2000). There are four tocopherols with vitamin E antioxidant activity: α, β, δ, and γ. Although we measured α-tocopherol, δ-tocopherol, and γ-tocopherol, we only used α-tocopherol to describe vitamin E status. Some measures of carotenoid status, such as α-carotene, β-carotene and β-cryptoxanthin, also have vitamin A activity.

The quantization of α-carotene, β-carotene, lycopene, lutein/zeanthin, and β-cryptoxanthin, α-tocopherol, and retinol, was determined by HPLC using a method based on that described by Browne and Armstrong(Armstrong, 1998). Samples were kept in the dark and in amber vials. All samples were analyzed in duplicate and in a blinded fashion.

We adjusted serum α-tocopherol using two methods. The first method will be by dividing α-tocopherol levels by cholesterol, the second by dividing α-tocopherol by total lipids, calculated

as cholesterol plus triglycerides. Serum cholesterol and triglyceride concentrations were determined enzymatically using specific reagents from Pointe Scientific (Canton, MI).

There are not previously defined insufficiency values for carotenoids (Institute of Medicine (U.S.). Panel on Dietary Antioxidants and Related Compounds., 2000; Monsen, 2000). Thus, we divided carotenoid concentrations into tertiles and define "low carotenoids" as being in the lowest tertile of concentration. Previous studies have found that lycopene does not follows the same patterns of association as other carotenoids, likely because lycopene response is almost entirely due to tomato consumption (Andersen, et al., 2006). We used the Dietary Reference Intake (DRI) definitions of insufficient α-tocopherol and retinol concentrations: <16 μmol/L and <0.7 μmol/L, respectively (Institute of Medicine (U.S.). Panel on Dietary Antioxidants and Related Compounds., 2000; Institute of Medicine (U.S.). Panel on Micronutrients., 2001). There are not yet insufficiency levels of either biomarker defined for pregnancy. Insufficiency levels have not yet been determined for δ-tocopherol and γ-tocopherol.

Vitamin C Status: Vitamin C is a water soluble vitamin that has two forms, ascorbic acid and dehydroascorbic acid. We used ascorbic acid to measure vitamin C status because it is the primary and functional form of vitamin C (Institute of Medicine (U.S.). Panel on Dietary Antioxidants and Related Compounds., 2000).

Maternal plasma ascorbic acid concentrations were determined by HPLC using a method based on that described by Rumelin et al. (Rumelin, Fauth, & Halmagyi, 1999). Maternal plasma samples were kept cold and in dark vials. Maternal plasma ascorbic acid peaks were separated on a Waters (Milford, MA) Atlantis C18. All samples were analyzed in duplicate and in a blinded fashion. We assessed two potential insufficiency values for ascorbic acid, <22 μmol/L and <38

μmol/L (Institute of Medicine (U.S.). Panel on Dietary Antioxidants and Related Compounds., 2000; Monsen, 2000). There is no previously value specific to pregnancy.

Folate Status: Institute of Medicine's Food and Nutrition Board lists several methods to measure folate status (Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., Institute of Medicine (U.S.). Panel on Folate Other B Vitamins and Choline., & Institute of Medicine (U.S.). Subcommittee on Upper Reference Levels of Nutrients., 1998). We used two of these measures to quantify folate status: plasma folate and plasma homocysteine. Plasma folate is a measure of short-term folate status and, therefore, cannot be used to differentiate between chronic folate deficiency and temporary low intake(Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., et al., 1998). But plasma folate is a sensitive measure of dietary folate intake and we will strengthen our estimation of folate status by also measuring plasma homocysteine. Homocysteine is an amino acid that whose metabolism requires folate. When folate levels are low, homocysteine levels rise.

Plasma folate and plasma homocysteine (Hcy) were quantified in the laboratory of Dr. Nader Rifai at the Boston Children's Hospital. Plasma folate was measured by a quantitative sandwich enzyme immunoassay technique on a 2010 Elecsys auto-immunoanalyzer (Roche Diagnostics, Indianapolis, IN). Total plasma Hcy was determined by an enzymatic assay on a Hitachi 917 analyzer (Roche Diagnostics - Indianapolis, IN), using reagents and calibrators from Catch Inc. (Seattle, WA).

We used a cut-off point of < 16.3 ng/ml to define insufficient folate status, which has been previously used as a biologically important insufficiency level in the 2nd trimester of pregnancy (Siega-Riz, Savitz, Zeisel, Thorp, & Herring, 2004). We used two sufficiency cut-

points for homocysteine as well. The value for homocysteine that reflects folate insufficiency in non-pregnant populations is >16 µmol/L, but Walker et al. suggests that mean concentration of homocysteine in a normal pregnancy are: 5.6 µmol/L at 8-16 wks gestation, 4.3 µmol/L at 20-28 weeks gestation, and 5.5 µmol/L at 36-42 weeks gestation (Walker, Smith, Perkins, Keely, & Garner, 1999).

Essential Fatty Acid Status: We will explore using n-3 and n-6 polyunsaturated fatty acids in our analysis. Lipids were extracted from red blood cells according to the general technique of Bligh and Dyer (Bligh & Dyer, 1959). The gas chromatograph was a Perkin Elmer Clarus 500.

There are not well defined insufficiency values for EFAs, although there are for n-6:n-3 ratios (Institute of Medicine (U.S.). Panel on Macronutrients. & Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., 2005). We will divide n-3 and n-6 polyunsaturated fatty acids into tertiles and define "low EFA" as being in the lowest tertile of concentration.

Iron Status: We used two measures of iron status: serum ferritin and soluble transferrin receptor (sTfR) concentration. Serum ferritin reflects the body's iron stores, and low values can be a sign of early iron deficiency. A high concentration of sTfR is also indicators of low functional iron status but, unlike ferritin concentration, is less sensitive to infections and inflammatory conditions (Institute of Medicine (U.S.). Panel on Micronutrients., 2001).

Serum ferritin levels were analyzed using an immunoradiometric assay with I-125 labeled anti-ferritin antibody in a kit obtained from DPC (catalog # IKFE1, Los Angeles, CA). Serum sTfR levels were measured using an enzyme-linked immunosorbent assay (ELISA) from

by R&D Systems (Minneapolis MN). All samples were analyzed in duplicate and in a blinded fashion.

The DRI defines insufficiency values for ferritin as <12 µg/L or <20 µg/L (Institute of Medicine (U.S.). Panel on Micronutrients., 2001), based on a non-pregnant population. Previous studies of sTfR concentrations in pregnancy have used a cut-off of >8.5 mg/L to indicate iron deficiency (Akesson, Bjellerup, Berglund, Bremme, & Vahter, 1998, 2002).

Vitamin D Status: Serum concentration of 25(OH)D is the best indicator of vitamin D status. It reflects vitamin D produced in the skin and obtained from food and supplements and has a long circulating half-life. Serum 25(OH)D levels do not indicate the amount of vitamin D stored in other body tissues.

There are many assays to measure 25 (OH) D concentrations currently in use, including competitive protein binding with vitamin D binding protein, radioimmunoassay (RIA), and enzyme-linked immunosorbent assay **(**ELISA). There is high inter- and intra- variability in the assays used to measure vitamin D status and no established standard method to assay 25-hydroxyvitmain D concentrations(Binkley et al., 2004), although HPLC is currently considered to be the gold standard (Holick, 2009).

In SNAP, concentrations of serum 25-hydroxyvitamin D were quantified using a DiaSorin radioimmunoassay (RIA). The RIA detects 100% of 25-hydroxy-ergocalciferol and 100% of 25- hydroxyl-cholecalciferol. Maternal serum samples were stored in aliquots at –80°C until they were analyzed for 25-hydroxyvitamin D [25-hydroxy-erocalciferol + 25-hydroxy-cholecalciferol]. The interassay CV was 9.5%. The detectable limits for the RIA are 3.75 to 250 nmol/L and none of the samples of 25-hydroxyvitamin D included in our analysis fell outside this range.

Concentrations of serum 25-hydroxyvitamin D in ADUP were measured using a commercial ELISA from Immunodiagnostic Systems Limited (IDS, Tyne, United Kingdom) in ADUP. The ELISA assay can detect serum concentrations of 25-hydroxyvitamin D between 5-300 nmol/L. ELISA results were validated against a HPLC method carried out in Dr. Powers' laboratory at the Magee-Womens Research Institute as described previously (Bodnar et al., 2007). All samples are analyzed in duplicate and in a blinded fashion.

The appropriate cut-off for vitamin D insufficiency in non-pregnant populations is currently under debate and potential cut-offs range from <37.5 nmol/L (Institute of Medicine (U.S.). Standing Committee on the Scientific Evaluation of Dietary Reference Intakes., 1997) to <80 nmol/L(Hollis, 2005). There is not yet an established insufficiency value for pregnant women, so we explored multiple cut-offs in our analysis.

3.3.3 Post-load glucose concentration

All maternal glucose concentration values were obtained from electronic laboratory records and were only collected in SNAP. At Magee-Womens Hospital, where SNAP is conducted, post-load glucose concentrations were obtained using a 50-g, 1-hour oral glucose challenge test at approximately 24-28 weeks gestation. If the post-load glucose concentration was > 135 to 140 mg/dl, then women are also given a 100-g, 3-hour glucose challenge test Women were diagnosed with GDM if their 50-g 1-hour post-load glucose concentration was ≥ 180 mg/dl, or if two or more of their 100-g 3-hour oral glucose tolerance results were abnormal (Carpenter & Coustan, 1982). We analyzed post-load glucose concentrations because recent evidence has found that, in women below the threshold of GDM, the risk of adverse prenatal outcomes

increases significantly with increasing blood glucose levels (Metzger, et al., 2008). We did not have an adequate number of women diagnosed with GDM to assess GDM as an outcome variable.

3.3.4 Covariates

The following confounders were assessed for our analyses in both ADUP and SNAP: maternal age, race/ethnicity, parity, employment, education, marital status, and smoking status during pregnancy. Analyses in SNAP were additionally adjusted for gestational age of GDM screening, family history of gestational diabetes, income, and season of 25(OH)D measurement. Analysis in ADUP was additionally be adjusted for depression status. Maternal age, race/ethnicity, parity, income, employment, education, marital status, and smoking status were obtained by self-report at the first visit in both SNAP and ADUP. These socioeconomic and social factors were important to include in our analysis because these have been shown to be significant risk factors for obesity, nutrition, and gestational diabetes (Anna, van der Ploeg, Cheung, Huxley, & Bauman, 2008; James, Nelson, Ralph, & Leather, 1997). Depression status in ADUP was determined by trained research clinical staff. Family history of diabetes was abstracted from medical records after delivery in SNAP only. For the evaluation of the association between vitamin D status and maternal glucose concentration (specific aim 3), we also controlled for season of serum 25(OH)D sample to account for the amount of available sunlight and its affect on serum 25(OH)D status.

There are some potential covariates that will not be measured in our study. For example, measures of physical activity, type of adiposity (e.g. fat mass versus fat-free mass), genetic

factors, inflammation, and water retention were not collected in either study and income was not measured in ADUP and these unmeasured confounders may influence our analyses. Additionally, data on dietary intake, supplements and sunlight exposure were not included in our analyses. Women who are considered to be at “high risk” for gestational diabetes may be referred to nutritional counseling, and the affect of this intervention is also not measured in our study. Although all women in SNAP were screened for GDM during pregnancy we did not measure glucose levels before pregnancy. Therefore, we are not able to differentiate between women who had undiagnosed type 2 diabetes before pregnancy and women for whom glucose intolerance began during pregnancy. For some of these unmeasured covariates, we have measured variables that can be used as proxy measurements. For example, while we do not have income measurements in ADUP, we do have other related measures of socioeconomic status. Other covariates, such as physical activity, are strongly related to adiposity, nutrition, and glucose tolerance, and will have more of an effect on our analysis.

4.0 THE ROLE OF GESTATIONAL WEIGHT GAIN AND EARLY PREGNANCY MATERNAL ADIPOSITY DISTRIBUTION AND THE DEVELOPMENT OF MATERNAL HYPERGLYCEMIA

4.1 ABSTRACT

Gestational weight gain (GWG) and maternal adiposity distribution may be important risk factors for maternal hyperglycemia. The objective of our study was to estimate the effects of GWG, central adiposity and subcutaneous fat on maternal post-load glucose concentration. Pregnant women (n = 413, 62% black, 57% with BMI≥25) enrolled in a cohort study at <16 weeks gestation. GWG was abstracted from medical records. In a sub-sample of women (n = 214), waist circumference, subcutaneous fat biceps and triceps skinfold thicknesses were measured at enrollment. At 24-28 weeks gestation, post-load glucose concentration was measured using a 50-g 1-hour oral glucose tolerance test. After adjustment for confounders, including pre-pregnancy BMI, each 0.3-kg/week increase in weight at ≤ 13 weeks gestation was associated with a 2.2 (95% CI: 0.1, 4.3)-mg/dl increase in glucose concentration. After adjustment for confounders, each 8.6-mm increase in biceps skinfold thickness and 11.7-mm increase in triceps skinfold thickness was associated with a 4.3 (95% CI: 0.2, 8.5)-mg/dl increases in maternal glucose. Neither GWG in the second trimester nor waist circumference at ≤ 13 weeks was significantly associated with glucose concentration after confounder adjustment.

Independent of pre-pregnancy BMI, high early pregnancy GWG and maternal subcutaneous body fat may elevate the risk of gestational hyperglycemia, which in turn, increases the risk of adverse birth outcomes.

4.2 INTRODUCTION

Gestational diabetes mellitus (GDM), defined as glucose intolerance with onset or first recognition during pregnancy, occurs in 4% of pregnancies (Getahun, et al., 2008) and is a major risk factor for a variety of adverse maternal and child outcomes including birth trauma, macrosomia, and later development of type 2 diabetes mellitus (Henriksen, 2008; Jevitt, 2005; C. Kim, et al., 2002). Importantly, maternal hyperglycemia, defined as high blood sugar during pregnancy, below the threshold of GDM is associated with similar poor birth outcomes (Metzger, et al., 2008). Clearly, a better understanding of the risk factors of high glucose in the prenatal period is imperative to improving the health of women and their infants.

Obesity, often measured by pre-pregnancy body mass index (BMI), is strongly associated with GDM in a variety of populations (Chu, et al., 2007; Haeri, et al., 2009; Hedderson, et al., 2008; Joy, et al., 2009; Knight, et al., 2010; Leung, et al., 2008; Nohr, et al., 2009; Qvigstad, et al., 2010; Shirazian, et al., 2009; Torloni, et al., 2009; van Leeuwen, et al., 2010). However, BMI has limitations as a measure of adiposity (Romero-Corral, et al., 2008) and cannot be measured during pregnancy because of the increase in weight not related to adiposity. Additionally, pre-pregnancy BMI only measures general adiposity, and women with similar BMI values may have widely varying distribution of their adipose tissue (Romero-Corral, et al., 2008).

Excessive adiposity during pregnancy, as measured by gestational weight gain (GWG) and maternal fat distribution, may be important risk factors of GDM. Despite our increasing knowledge of the physiological effect of excess fat accumulation on insulin resistance during pregnancy (Harlev & Wiznitzer, 2010), few studies have quantified the effects of GWG and fat distribution on the risk of GDM. A recent case-control study suggested that excessive weight gain, especially in the first trimester, is associated with GDM (Hedderson, et al., 2010), and studies of maternal fat distribution have suggested that central adiposity and subcutaneous adipose tissue may be associated with maternal post-load glucose concentration and GDM (Branchtein, et al., 1997; Wendland, et al., 2007; Yeung, et al., 2010; Yilmaz, et al., 2010). These studies were limited by the use of anthropometric measures taken years before pregnancy and self-reported GDM (Yeung, et al., 2010; S. Zhang, et al., 1995), low sample sizes and a racially homogeneous populations (Madhavan, et al., 2008; Yeung, et al., 2010), and anthropometrics measured late in pregnancy, which reduces the accuracy of measurement (Wendland, et al., 2007).

The objective of our study was to determine the association between GWG up to the time of GDM screening, first trimester central adiposity and subcutaneous fat on maternal post-load glucose concentration.

4.3 METHODS

We analyzed data from the Study of Nutrition and Pregnancy (SNAP), a prospective pregnancy cohort study of women receiving care in the prenatal clinics at Magee-Womens Hospital of

UPMC in Pittsburgh, PA. Women seeking care at the clinics are primarily low-income, publically insured, and approximately 50% African American. Eligible women had singleton pregnancies and were self-reported non-Hispanic black or non-Hispanic white. Participants were excluded if they had pre-gestational diabetes, vaginal bleeding, thrombophilias, chronic hypertension requiring medication, current or planned cervical cerclage, compromised immune system, autoimmune diseases, or known use of illegal drugs or controlled substances. At enrollment participants provided informed, written consent. The study was approved by the University of Pittsburgh Institutional Review Board.

Between June 2003 and May 2010, 724 eligible pregnant women were enrolled at < 16 weeks' gestation [mean (SD) gestational age = 9.1 (2.9) weeks]. At enrollment, women completed a structured interview that included questions on socio-demographic factors and medical history. Of those eligible, we excluded women who had a spontaneous or therapeutic abortion (n = 85), implausible weight measurements (n = 2), or a first prenatal visit after the first trimester (n = 53). Women were also excluded if the prenatal visit closest to their GDM screening visit was > 30 days before the screening (n = 8) or because of GDM screening < 24 weeks gestation (n = 43). A total of 82 women were excluded because measured weight at the last visit before GDM screening was missing from their medical record and GWG could not be calculated. Of these 82 women, 22 women transferred care to another facility. There were no missing glucose concentration values. An additional 38 women were excluded because they were missing data on one of the covariates included in the final model. A total of 413 women were included in the final analysis. Women excluded from our analysis were more likely to be smokers (56% versus 44%, $p < 0.05$) and nulliparous (39% versus 21%, $p < 0.01$) than women

included in the analysis. There were no other meaningful differences in post-load glucose concentrations and other maternal characteristics (data not shown).

In June 2006, waist circumference and skinfolds measurements were added to the study protocol at ≤ 13 weeks gestation [mean (SD) = 8.5 (2.0) weeks gestation] and, therefore, these measurements were available only in a sub-sample of women (n = 436). After the exclusions made above, a total of 214 were available for analysis of adiposity distribution.

A 50-g 1-hour oral glucose challenge test was performed as part of routine clinical care at approximately 24-28 weeks gestation to screen for GDM. The post-load glucose concentration values were abstracted from medical records. Women with post-load glucose concentration values >135 mg/dL additionally received a 3-hour 100-g oral glucose tolerance test. Women with 50-g 1-hour post-load glucose concentration values ≥ 180 mg/dL, or 2 or more abnormal 3-hour 100-gram oral glucose tolerance results were classified as having GDM (Carpenter & Coustan, 1982). Additionally, in our sample, a total of 45 women considered by their clinician to be at high risk for GDM underwent early screening [< 24 weeks gestation, mean (SD), 17.2 (6.9)]. To ensure that our results were comparable to other studies, women with early GDM screening were excluded from our analysis.

We calculated the first trimester rate of GWG as the difference between weight measured at the last prenatal visit in the first trimester (≥ 8 weeks gestation and < 14 weeks gestation, “first trimester visit”) and the pre-pregnancy weight divided by the weeks of gestation at the first trimester visit. The second trimester rate of GWG was defined as the difference between the weight at GDM screening test and the weight at the first trimester visit divided by the number of weeks between measurements. The total average rate of GWG up to GDM screening was calculated as the difference between the measured weight at or before GDM screening and the

self-reported pre-pregnancy weight, divided by the weeks of gestation at the time of the weight measurement. The difference between the measured weight and GDM screening was, on average, less than 1 week [mean (SD) = 0.5 (1.6) weeks]. We defined adequacy of weight gain up to GDM screening using the 2009 IOM recommendations (Rasmussen, et al., 2009). The ratio of the observed to expected GWG was classified as inadequate, adequate, or excessive, as described previously (Bodnar, Siega-Riz, Simhan, Himes, et al., 2010).

For the women whose self-reported pre-pregnancy weight was deemed biologically improbable (n = 45, ≥ 6.8 kg gained or lost between pre-pregnancy weight and first prenatal visit ≤ 13 weeks gestation), we imputed pre-pregnancy weight based on a back calculation using the rate of weight gain between the first two prenatal visits, similarly to previously used methods (Laraia, Siega-Riz, & Gundersen, 2010). If the second weight measurement was after 16 weeks' gestation, we did not impute a pre-pregnancy weight (n = 5).

Central adiposity was assessed using waist circumference measured at < 13 weeks gestation. Waist circumference was measured at the smallest point of the waist by a trained research nurse. "At risk" waist circumference was defined as greater than 88 cm ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998). Because waist circumference measurements lose accuracy in patients with BMI ≥ 35 ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998), we performed a sensitivity analysis by excluding women with a BMI ≥ 35. Subcutaneous fat was described using biceps and triceps skinfolds, measured by a trained research nurse at < 13 weeks. Biceps skinfold thickness was measured in the front of the upper arm at the mid-point of the biceps muscle using calipers. The

triceps skinfolds were measured at the back of the upper arm on the mid-point of the triceps muscle ("National Heart, Lung, and Blood Institute: Obesity Education Initiative: "The Practical Guide: Identification, Evaluation, and Treatment of Overweight and Obesity in Adults.","). To reduce measurement error, biceps and triceps skinfolds measures were taken three times and the mean was used for analysis. If women had an outlying value, defined as a difference between the lowest and highest of the triplicate that was greater than two standard deviations from the mean of all three measurements, we calculated the mean of the two remaining values (triceps: n = 10; biceps: n = 14).

Maternal demographics and pre-pregnancy weight were obtained by self-report at enrollment. Family history of diabetes was abstracted from medical records after delivery. Pre-pregnancy BMI was calculated as pre-pregnancy weight (kg) divided by height (m) squared. A BMI of <18.5 was defined as underweight, 18.5-24.9 as normal weight, 25.0-29.9 as overweight, and ≥ 30 as obese ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998).

Maternal post-load glucose concentration was used as a continuous outcome variable because elevated post-load glucose concentrations, even below the threshold of GDM diagnosis, have been shown to be strongly associated with adverse pregnancy outcomes (Metzger, et al., 2008). Separate multivariable linear regression models were used to assess the association between each independent variable (average rate of GWG, GWG adequacy, waist circumference, and skinfolds) and post-load glucose concentration. The relationship of each independent variable with post-load glucose was assessed by visual inspection of lowess smoothing plots. Because no meaningful deviations from linearity were observed, independent variables were specified as continuous. GWG adequacy was categorized to ensure greater interpretability.

Potential confounders were maternal age, parity, race/ethnicity, smoking status, marital status, annual family income, education, family history of diabetes and gestational age of glucose challenge test. Pre-pregnancy BMI was included in all models out of convention. We defined confounding as a > 10% change in the adjusted beta coefficient after exclusion of the covariate from the full model. To maintain comparability across models, the full model was used for all four of the final GWG models. Because of the smaller sample size, we assessed confounding in the adiposity distribution measurements separately from the GWG models. Pre-pregnancy BMI, maternal age, parity, race/ethnicity, maternal education, and family history of diabetes were all found to be confounders in the skinfolds and waist circumference models.

Pre-pregnancy BMI and race/ethnicity were evaluated as effect modifiers in each model by including the interaction term in the model and testing for significance using the Wald test ($\alpha < 0.05$). To explore the interaction between first- and second-trimester rates of GWG on post-load glucose concentrations, we stratified women based on whether they were above or below the mean rates of weight gain in each trimester. We used Student's t-test to compare the differences in mean post-load glucose concentrations between these four groups, using women with GWG rate below the mean in both the first and second trimesters as the reference. Our sample size was not large enough to test this interaction using regression analysis. Data were analyzed using STATA software version 11.0 (StataCorp, College Station, TX).

4.4 RESULTS

Women in our cohort tended to be young, multiparous, African American, unmarried, high school educated or less, and had no family history of diabetes (Table 4). Approximately half the women were smokers, low income, and unemployed and more than a third were obese.

The mean (SD) post-load glucose concentration was 99.7 (24.3) mg/dl and ranged from 43 to 200 mg/dl. GDM screening occurred at a mean (SD) of 27.3 (2.3) weeks gestation. A total of 45 (9.0%) of women had post-load glucose concentrations ≥ 135 mg/dl, and of these women, eleven women (2.7% of total cohort) were diagnosed with GDM.

The mean (SD) weeks gestation for the first trimester visit was 11.7 (1.3) weeks, with a range of 8.2-13.6 weeks. On average, women gained approximately 1.4 (SD: 4.1) kg in the first trimester and 6.0 (SD: 4.4) kg in the second trimester. More than half of women had excessive weight gain before GDM screening (Table 5). In the unadjusted analysis, first trimester average rate of GWG and total average rate of GWG were linearly associated with post-load glucose. After adjustment for age, parity, race/ethnicity, smoking status, marital status, income, education, family history of diabetes, gestational age at GDM screening and pre-pregnancy BMI, each 0.3 kg/week of GWG (4 kg total) in the first trimester was associated with nearly a 2 mg/dL increase in maternal blood glucose. In both the unadjusted and adjusted models, total average rate of weight gain, second trimester specific rate of gain, and GWG adequacy were not significantly associated with maternal glucose. Pre-pregnancy BMI and race/ethnicity did not modify any of these associations.

We also examined the interaction between first and second trimester average rate of GWG in unadjusted analysis. Overall, the inclusion of this interaction did not significantly

modify the relationship between GWG rate and maternal post-load glucose concentration ($p = 0.07$). Women whose first trimester rate of GWG was ≥ 0.12 kg/week, regardless of the second trimester rate of gain, tended to have higher mean post-load glucose concentrations than women whose rate of GWG was below the mean in both trimesters (Table 6). This difference, however, did not reach statistical significance.

In unadjusted analyses biceps and triceps skinfolds had positive, linear associations with post-load glucose concentration (Table 7). After adjustment for pre-pregnancy BMI, maternal age, parity, race/ethnicity, maternal education, and family history of diabetes, each standard deviation increase in biceps and triceps skinfold thicknesses was associated with an approximately 4.3 mg/dl increase in maternal glucose. Each standard deviation increase in waist circumference was associated with an approximately 4.5 mg/dl increase in maternal glucose concentration in unadjusted analysis. However, after adjustment, the relationship was no longer statistically significant. Pre-pregnancy BMI and race/ethnicity did not modify any of these associations. No meaningful differences were detected when we excluded women with a BMI $\geq$ 35 from the analysis or when we excluded women whose waist circumference measurements were obtained > 10 weeks gestation.

4.5 DISCUSSION

We found that a high rate of GWG in the first trimester and elevated biceps and triceps skinfold thicknesses were positively associated with high post-load glucose concentrations at the GDM

screening. These associations were independent of important confounders, such as pre-pregnancy BMI, socioeconomic factors and medical history.

The 2009 Institute of Medicine Committee to Revise Gestational Weight Gain Guidelines highlighted that more evidence was needed to determine the role that GWG plays in the development of GDM (Rasmussen, et al., 2009), but few studies have been published to examine this association. And, given the increased risk to pregnant women for adverse outcomes (Metzger, et al., 2008), a rigorous examination of the relationship between adiposity and maternal hyperglycemia, and not just GDM, is warranted. Interventions to decrease maternal glucose concentrations by controlling maternal GWG through programs of exercise, dietary, pharmaceutical or a combination of the three had inconsistent results (Callaway et al., 2010; Oostdam, et al., 2011). However, there are effective interventions that suggest that not only is GWG a modifiable risk factor, but that controlling GWG can reduce incidence of GDM (Quinlivan, et al., 2011).

Our conclusion that first trimester rate of GWG is independently associated with maternal hyperglycemia is in agreement with a recent nested case-control study that found that women who gained in the highest tertile of first trimester weight gain rate were more likely to develop GDM then women who gained in the lowest tertile of weight (Hedderson, et al., 2010). A second study examined the relationship between weight gain and impaired glucose tolerance (IGT) as well as GDM, stratified by early and mid-pregnancy (Herring, et al., 2009). Interestingly, the authors found that women who had high weight gain in both early and mid-pregnancy were more likely to develop IGT, but not GDM, compared with women who had low weight gain in both periods. These researchers found a significant interaction between early and mid-pregnancy, whereas we did not have the power to include this interaction.

Our conclusions are in agreement with several previously published studies that have found no association between total weight gain up to GDM screening and GDM (Herring, et al., 2009; Tovar, et al., 2009), and adequacy of gain and GDM (Saldana, et al., 2006; Tovar, et al., 2009). Although, Tovar et al. found that among women with a pre-pregnancy BMI $\geq$ 35 kg/m^2, women who exceeded their target weight (expected weight gain based on IOM criteria) were approximately four times as likely to have abnormal glucose tolerance than women who met their target weight (Tovar, et al., 2009). It is important to note, however, that the researchers studied abnormal glucose tolerance rather than post-load glucose concentrations or overt GDM. More compellingly, Hedderson et al. found that women who exceeded the 2009 IOM weight gain recommendations were approximately 1.5 times as likely to have GDM than women who were below or within the recommendations (Hedderson, et al., 2010). The differences in our two studies may be explained by the small effect size that Hedderson et al. found, and our sample may not have been large enough to assess this effect.

Increases in weight and insulin resistance are normal physiological changes of pregnancy. But excess fat accumulation can cause high concentrations of free fatty acids, altered adipokine expression, and low-grade inflammation, all of which are thought to increase insulin resistance (Ioannidis, 2008). Consequently, this may contribute to the development of abnormally high levels of glucose concentrations during pregnancy. The differences in early pregnancy weight gain and total weight gain results may arise because early weight gain is largely fat accumulation and total weight gain is not (van Raaij, Peek, Vermaat-Miedema, Schonk, & Hautvast, 1988).

The effect of maternal adiposity distribution on risk of maternal hyperglycemia has not been extensively researched. Our result that biceps and triceps skinfolds in the first trimester are independently associated with maternal hyperglycemia is supported by a cross-sectional analysis

of Turkish women (n = 98) that suggested that percent body fat at 26 to 36 weeks gestation, calculated using skinfold thicknesses, was significantly higher in women with GDM then in women with normal glucose tolerance (Yilmaz, et al., 2010). While we found no relationship between waist circumference at < 13 weeks and glucose concentrations, others have noted positive associations between pre-pregnancy waist circumference and waist circumference at the time of GDM screening and hyperglycemia and GDM status (Branchtein, et al., 1997; Wendland, et al., 2007; Yeung, et al., 2010). The difference in our results may arise from several factors. We measured early pregnancy waist circumference, whereas other studies used self-reported pre-pregnancy waist circumference (Yeung, et al., 2010) or measured waist circumference at mid-pregnancy (Branchtein, et al., 1997; Wendland, et al., 2007). Waist circumference measured in mid-pregnancy loses accurate measure of adiposity (McCarthy, et al., 2004). Also, our study had a larger percentage of African American pregnant women than other studies. Some evidence in non-pregnant populations that suggests that relationship between waist circumference and insulin resistance may be different in African Americans than in whites (Sumner et al., 2008), although we did not find that race/ethnicity modified these associations in our population.

High subcutaneous fat has been associated with decreased insulin resistance in non-pregnant populations (Goodpaster, Thaete, Simoneau, & Kelley, 1997), and there is some evidence that diabetes and obesity during pregnancy may cause defects in the insulin-signaling pathway in subcutaneous adipose tissue in pregnant women (Colomiere, Permezel, & Lappas, 2010). Importantly, studies in non-pregnant cohorts that suggest that abdominal visceral fat thickness has a stronger association than subcutaneous fat (Bartha et al., 2007; Liu et al., 2010). Unfortunately, our methods could not differentiate abdominal visceral fat from subcutaneous fat.

More studies are needed to understand the role that adiposity distribution plays in the etiology of maternal hyperglycemia.

Strengths of our study include an assessment of continuous maternal post-load glucose concentrations. Glucose concentrations below the threshold of overt GDM have been previously associated with poor birth outcomes (Y. W. Cheng, McLaughlin, Esakoff, Block-Kurbisch, & Caughey, 2007) and were strongly associated to large-for-gestational age infants in our cohort (data not shown). Furthermore, our cohort was composed of African American and white women and we included an analysis of the effects of trimester-specific GWG and maternal fat distribution in early pregnancy on maternal post-load glucose. However, our cohort was composed of generally young, non-Hispanic women with singleton pregnancies and no previous history of GDM. Therefore, only a small percentage of women in our cohort developed GDM, and our study did not have the power needed to explore a rigorous analysis of GDM status. Our analysis of continuous glucose allowed us to examine the linearity of the relationship between adiposity and maternal post-load glucose, even in women who were not diagnosed with GDM, and high glucose in women without GDM is associated with adverse pregnancy outcomes (Metzger, et al., 2008). Our sample size was small, limiting the precision of our results and our ability to examine the interaction between trimester and GWG. Skinfold thicknesses are measures of subcutaneous fat, and therefore can be altered by the changes in hydration experienced during pregnancy. All anthropometric techniques have the potential to be influenced by human error (Norgan, 1992), but this limitation was mitigated in our study with the use of a trained research nurse taking three individual measurements.

Our results may be biased by unmeasured confounding from physical activity, genetic factors, and type of GWG (e.g. fat mass versus fat-free mass). Physical activity is associated with

reductions in weight gain (Lee, Djousse, Sesso, Wang, & Buring, 2010) and decreased insulin resistance (Church, 2011), and this would bias our results away from the null. Certain genetic factors may increase the risk of development of hyperglycemia (Freathy et al., 2010), and these if these factors also have an effect on GWG or adiposity distribution our results may be biased in either direction, depending on the effect. Although type of GWG has not been well studied, research in non-pregnant populations has suggested that gains in visceral adipose tissue and intermuscular adipose are associated with type 2 diabetes (Gallagher et al., 2009). If this association is also true in pregnancy, than the effect of GWG that we observed actually may be due, in part, to a type of adipose tissue. Because we did not measure glucose levels before pregnancy, we were not able to differentiate between women who had undiagnosed type 2 diabetes before pregnancy from women for whom glucose intolerance began during pregnancy, which limits inferences about the temporality of the associations. Future studies of continuous maternal glucose with larger sample sizes, more rigorous measures of adiposity, and analyses of trimester-specific GWG are warranted.

If confirmed by more extensive study, our results that first trimester GWG and early subcutaneous fat may play important roles in the development of hyperglycemia will provide valuable insight into the etiology of maternal hyperglycemia and the role that adiposity may play in its development. Excessive adiposity during pregnancy is a modifiable risk factor and a better understanding of its relationship with glucose concentrations made lead to more effective targeted interventions to reduce the risk of maternal hyperglycemia and improve maternal and fetal outcomes.

4.6 FIGURES AND TABLES

Table 4. Maternal characteristics of SNAP participants, n = 413

Characteristic	
Age at enrollment, mean (SD)	24.4 (4.5)
Parity, N (%)	
0	88 (21.3)
1-7	325 (78.7)
Race/ethnicity, N (%)	
White	159 (38.5)
African American	254 (61.5)
Smoking status, N (%)	
Smoker	180 (43.6)
Non-smoker	233 (56.4)
Marital status, N (%)	
Married	61 (14.8)
Not married	352 (85.2)
Annual family income, N (%)	
< $10,000	177 (42.9)
≥ $10,000	236 (57.1)
Educational status, N (%)	
Greater than a high school degree	49 (11.9)
High school educated	364 (88.1)
Family history of diabetes, N (%)	
History of diabetes	152 (36.8)
No history of diabetes	261 (63.2)
Employment status, N (%)	
Employed	224 (54.2)
Not employed	189 (45.8)
Pre-pregnancy BMI (kg/m^2), mean (SD)	28.0 (7.4)
Underweight (<18.5), N (%)	13 (3.1)
Normal weight (18.5-24.9), N (%)	165 (40.0)
Overweight (25-29.9), N (%)	91 (22.0)
Obese (30+), N (%)	144 (34.9)
BMI = body mass index	

Table 5. Unadjusted and adjusted association between maternal blood glucose < 24 weeks gestation and maternal GWG, n = 413

	mean (SD) kg/week	Unadjusted β (95% CI)	Adjusted[1] β (95% CI)
1st trimester average rate of GWG, per 0.3 kg/week increase	0.1 (0.3)	2.5 (0.4, 4.6)	2.2 (0.1, 4.3)
2nd trimester average rate of GWG, per 0.3 kg/week increase	0.4 (0.3)	0.1 (-2.5, 2.6)	-0.07 (-2.8, 2.6)
Total rate of GWG up to GDM screening, per 0.2 kg/week increase	0.3 (0.2)	2.0 (0.01, 4.0)	1.8 (-0.3, 4.0)
Adequacy of GWG up to GDM screening	N (%)	β (95% CI)	β (95% CI)
Inadequate	110 (26.6)	-3.0 (-10.1, 4.0)	-3.5 (-10.4, 3.4)
Adequate	78 (18.9)	ref	ref
Excessive	225 (54.5)	1.9 (-4.4, 8.1)	0.3 (-5.8, 6.3)

GWG = gestational weight gain
Missing: 20 women missing 1st and 2nd trimester rates of gain
[1]Adjusted for pre-pregnancy BMI, age, parity, race/ethnicity, smoking, marital status, annual family income, education, family history of diabetes, gestational age of GDM screening

Table 6. Mean post-load glucose concentration by rate of gestational weight gain in first and second trimesters of pregnancy[1], n = 393.

1st trimester	2nd trimester	N (%)	Mean (SD) glucose	p-value[2]
< Mean	< Mean	110 (28.0)	97.5 (22.5)	ref
< Mean	≥ Mean	83 (21.1)	95.4 (21.5)	0.56
≥ Mean	< Mean	89 (22.7)	103.7 (27.8)	0.07
≥ Mean	≥ Mean	111 (28.2)	102.1 (25.5)	0.16
			Overall test for interaction	0.07

[1]Mean rates of GWG rate: 1st trimester = 0.1 kg/week; 2nd trimester = 0.4 kg/week
[2]Student's t-test for categories based on mean, Wald test for overall test of interaction

Table 7. Association between maternal blood glucose < 24 weeks gestation and waist circumference, biceps and triceps skinfold thickness at < 13 weeks gestation, n = 214

Measurement	Mean (SD)	Unadjusted β (95% CI)	Adjusted[1] β (95% CI)
Biceps skin fold (mm), per 8.6 mm increase	14.6 (8.6)	5.1 (1.8, 8.3)	4.3 (0.2, 8.5)
Triceps skin fold (mm), per 11.7 mm increase	25.9 (11.7)	5.9 (2.6, 9.1)	4.3 (0.2, 8.5)
Waist circumference (cm), per 26.8 cm increase	99.9 (26.8)	4.5 (1.3, 7.8)	3.0 (-0.7, 6.8)
[1]Adjusted for pre-pregnancy BMI, age, parity, race/ethnicity, education, and family history of diabetes			

5.0 THE ASSOCIATION BETWEEN PRE-PREGNANCY OBESITY AND MATERNAL NUTRITIONAL BIOMARKER STATUS DURING PREGNANCY: A PRINCIPAL COMPONENT ANALYSIS

5.1 ABSTRACT

Pre-pregnancy obesity has been associated with an increased risk of a variety of adverse birth and pregnancy outcomes. Poor maternal essential fatty acid (EFA) and micronutrient status during pregnancy may contribute to these associations. However, these relationships have not been well researched in pregnancy. We assessed the association between maternal pre-pregnancy body mass index (BMI) and maternal micronutrient and EFA status in mid-pregnancy. Women (n=129) provided non-fasting blood samples at ≤20 weeks gestation that were assayed for red blood cell EFA, plasma folate, plasma ascorbic acid, serum retinol, serum 25-hydroxyvitamin D, serum α-tocopherol, plasma homocysteine, serum soluble transferrin receptors, and serum carotenoids. Principal component analysis of these biomarker measures was used to construct three nutritional components: an EFA component, a Micronutrient component, and a Carotenoid component. After adjustment for parity, race/ethnicity, and age, obese pregnant women were 3.0 (95% CI: 1.1, 7.7) times as likely of being in the lowest tertile of the EFA component and 4.5 (95% CI: 1.7, 12.3) times as likely of being in the lowest tertile of the Carotenoid component as their lean counterparts. We found no association between obesity and Micronutrient component

scores after confounder adjustment. Obese pregnant women may be vulnerable to having insufficient EFA and carotenoids concentrations, which may contribute to their elevated risk of adverse birth outcomes.

5.2 INTRODUCTION

In the United States, nearly a quarter of all pregnancies are complicated by pre-pregnancy obesity and the prevalence is increasing (S. Y. Kim, et al., 2007). Pregnant women who are obese are at higher risk of preeclampsia (Cedergren, 2004; Hauger, Gibbons, Vik, & Belizan, 2008), gestational diabetes mellitus (Chu, et al., 2007), birth trauma, large-for-gestational age birth (Cedergren, 2004) and stillbirth (Cedergren, 2004; Cnattingius, Bergstrom, Lipworth, & Kramer, 1998) compared with their lean counterparts. Nevertheless, the mechanism by which obesity contributes to poor outcomes remains uncertain.

Nutritional status during pregnancy may partially mediate the relation between pre-pregnancy obesity and adverse pregnancy and birth outcomes. But the relationship between obesity and nutritional biomarkers has not been thoroughly researched in pregnancy. In non-pregnant populations, obesity has been associated with insufficiencies of micronutrients, including vitamin E, vitamin C, vitamin D, folate, vitamin A and carotenoids (Andersen, et al., 2006; Kimmons, et al., 2006). Some evidence suggests that obese women may require additional supplementation of folate and vitamin C to achieve similar serum concentrations as those of lean women (Block et al., 2008; Mojtabai, 2004). Obese individuals may also have lower levels of essential fatty acids (EFA) (Karlsson, et al., 2006; Kishino, et al., 2008) than lean patients. Taken

together, these studies suggest that obesity may have a negative effect on overall micronutrient and EFA status. Because these nutrients may play important roles in maternal and fetal health (IOM, 1990; Uauy, Hoffman, Peirano, Birch, & Birch, 2001), it is critical to explore these associations in pregnancy.

The objective of our study was to evaluate the independent association between maternal adiposity, as measured by pre-pregnancy body mass index (BMI), and patterns of nutritional biomarkers at ≤ 20 weeks gestation.

5.3 METHODS

We conducted a secondary analysis of data from the Antidepressant Use during Pregnancy (ADUP) Study. ADUP is a prospective pregnancy cohort study of the effects of antidepressant use and major depressive disorder on pregnancy outcomes and child development (Wisner et al., 2009). Women were recruited at or before 20 weeks gestation after providing informed, written consent. Eligible women had singleton gestations. Women were excluded if they had psychosis, bipolar disorder, active substance use disorder (identified by self report or urine drug screen), gestational exposure to benzodiazepines or prescription drugs in the FDA-defined category of D or X (other than selective serotonin reuptake inhibitors) or chronic diseases (such as insulin-dependent diabetes). At enrollment, women reported their pre-pregnancy weight and sociodemographic information, were measured for height, and provided a non-fasting blood sample. Blood samples were assayed for red blood cell EFA, plasma folate, plasma ascorbic acid, serum retinol, serum 25-hydroxyvitamin D, serum α-tocopherol, plasma homocysteine,

serum ferritin, serum soluble transferrin receptors, and serum carotenoids (described in detail below). The study was approved by the University of Pittsburgh Institutional Review Board.

The ADUP study recruited women from 2000-2007. In 2004, the study protocol was modified to include nutrition measures, including nutritional biomarker assessment in maternal blood. Of the 197 eligible women interviewed from 2004-2007, 130 (66%) provided a non-fasting blood sample at ≤20 week that was processed for a full panel of nutritional biomarkers. We excluded one woman with missing data for pre-pregnancy weight. Our final analytic sample was 129 women. There were no meaningful differences between women included and excluded from the analysis on maternal characteristics, including race (80% versus 74% white race, p = 0.31), education (63% versus 56% college-educated, p = 0.35), and smoking (15% versus 16% smokers, p = 0.79).

Measures. General maternal adiposity before conception was measured using pre-pregnancy BMI [weight (kg)/height $(m)^2$], which was based on self-reported pre-pregnancy weight and measured height. Because only 4 women in the sample were underweight (BMI <18.5 kg/m^2), we categorized women into 3 groups: lean (<25 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30 kg/m^2) ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998).

We assayed maternal non-fasting blood samples drawn at ≤20 weeks' gestation for biomarkers of nutritional status. The methods used to assay the nutritional biomarkers in our study have been published in detail previously (Tomedi, Bogen, Hanusa, Wisner, & Bodnar, *in press*) and are described here briefly. Blood samples were immediately put on ice and wrapped in foil. They were processed within 2 hours of blood draw. Serum, plasma, and red cells were

stored at -80°C in amber vials. Lipids were extracted from red blood cells (Bligh & Dyer, 1959) and quantified using gas chromatograph (Perkin Elmer Clarus 500). Although a total of 29 EFAs were quantified, we included only the three EFAs of greatest interest to maintain ease of interpretability. The red blood cell EFAs used in this analysis were docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA). The inter-assay coefficients of variation (CV) were 14.4% for DHA, 4.2% for EPA (4.2%), and 5.5% for AA. Plasma folate was measured by a quantitative sandwich enzyme immunoassay technique (Roche Diagnostics, Indianapolis, IN) which has a CV of 2.0-3.9%. Concentrations of total homocysteine in plasma were determined by an enzymatic assay (Roche Diagnostics, Indianapolis, IN). The CV for this assay was 2.1-5.3%. Plasma ascorbic acid (vitamin C) concentrations were determined by high-performance liquid chromatography (HPLC) (Rumelin, et al., 1999), and the CV was 5.5%. Maternal vitamin D status was assessed using serum 25-hydroxyvitamin D [25(OH)D]. Serum 25(OH)D was measured using an enzyme-linked immunosorbent assay (IDS, Tyne, United Kingdom) and validated against HPLC results (Bodnar, Simhan, et al., 2007). The ELISA assay had a CV of <10.0%. Iron status was assessed using serum ferritin and serum soluble transferrin receptor (sTfR) concentrations. Serum ferritin levels were analyzed using an immunoradiometric assay with I-125 labeled anti-ferritin antibody in a kit obtained from DPC (catalog # IKFE1, Los Angeles, CA). The CV was 16.4%. Total sTfR levels were measured using an ELISA from by R&D Systems (Minneapolis MN). The CV was 8.5-17%. The quantification of serum carotenoids (β-carotene, lycopene, lutein+zeanthin, and β-cryptoxanthin), α-tocopherol (vitamin E), and retinol (vitamin A) were determined by HPLC (Armstrong, 1998). The CV's were 6.0% for serum α-tocopherol, 8.0% for serum retinol, 12.0% for serum lutein + zeaxanthin, 11.0% for

serum β-cryptoxanthin, 9.0% for serum lycopene, and 11.0% for serum β-carotene. All samples were analyzed in duplicate and in a blinded fashion.

Women identified their race/ethnicity as non-Hispanic white, non-Hispanic African American and other, but because only four women self identified as "other", we combined the African American and the "other" groups into a non-white category for analysis. Women were categorized as nulliparous or as having a previous live birth. A diagnosis of major depressive disorder was made using the Structured Clinical Interview for DSW-IV (First et al., 1996). Educational status was defined as having less than a high school education, some college, college degree and post-graduate education. Women were classified as unemployed or employed (which included women who worked full time, part time, or occasionally). For marital status, "married" included married or living as married and "unmarried" included single, separated, widowed and divorced. Women were classified as current smokers if they smoked at all during pregnancy or non-smokers if they did not.

Statistical Analysis. We used Pearson chi-square tests and Student's t-tests to determine differences in maternal characteristics by pre-pregnancy BMI categories. To describe skewed biomarkers, we calculated geometric means and log-transformed biomarkers before statistical tests were performed. We conducted a principal component analysis on the fifteen untransformed maternal dietary biomarkers listed above. Principal components analysis is a statistical method that combines many correlated variables and calculates a smaller number of components that explain much of the variation in the correlated variables (Kline, 1994). We used Spearman's rho correlation coefficients because many of the biomarkers exhibited skewed distributions. Components were orthogonally rotated using the varimax method to account for correlation between components. We used eigenvalues, scree plots, and the interpretability of the solution to

examine solutions for two to eight components, and three components were chosen. Nutritional biomarkers were considered to be loaded onto a component if the rotated component pattern value was greater than or equal 0.3. Serum ferritin did not load on any of the final components and, therefore, was not included in the final analysis. The estimated component scores were computed using a regression method. Component scores were categorized based on tertiles of the distribution.

Multivariable logistic regression was used to assess the independent associations between pre-pregnancy BMI and the likelihood of being in the lowest tertile of the each nutritional component. Potential confounders were maternal age, race/ethnicity, parity, education, marital status, smoking status, employment and depression. We defined confounding as a greater than a 10% change in the odds ratio after excluding the covariate from the full model. In the EFA component model none of the covariates met this definition of confounding. In the micronutrient component model parity, race/ethnicity, and age met this definition of confounding. In the carotenoid component model only parity and race/ethnicity were confounders. To maintain comparability across models, parity, race/ethnicity, and age were included in all three of the final models. Data were analyzed using STATA software version 11.0 (StataCorp, College Station, TX).

5.4 RESULTS

The women in this cohort were primarily well-educated, non-Hispanic white, married, non-smokers and employed (Table 8). The mean (SD) pre-pregnancy BMI was 26.6 (6.0) kg/m^2.

About half (49.6%) of women were lean, 22.5% were overweight, and 27.9% were obese. Obese women were less likely to have a college degree and more likely to be nulliparous and depressed than lean women. Maternal age, race/ethnicity, marital status, smoking, and employment did not significantly differ by pre-pregnancy BMI category.

In unadjusted analysis, women who were overweight before pregnancy had lower mean plasma folate, plasma ascorbic acid, serum β-carotene and serum β-cryptoxanthin than lean pregnant women (Table 9). Compared with lean women, obese pregnant women had lower mean red cell DHA, red cell AA, plasma ascorbic acid, serum 25(OH)D, serum β-carotene, serum lutein + zeaxanthin, and serum β-cryptoxanthin concentrations and higher mean sTfR concentrations. Red cell EPA, serum retinol, serum α-tocopherol, plasma homocysteine, and serum lycopene did not differ by pre-pregnancy BMI category.

Principle component analysis of the nutritional biomarkers resulted in three components. These components were assigned names based on the biomarkers that loaded heavily on the component and each represents biologically meaningful correlations between biomarkers. Component 1 was named "Essential Fatty Acids," component 2 was named "Micronutrients," and component 3 was named "Carotenoids" (Table 10). Although, β-carotene loaded on to both the Micronutrient and the Carotenoid components, for this analysis we will consider it to be a part of the Carotenoid component.

As pre-pregnancy BMI category increased, the percent of women in the lowest tertile of each nutritional component increased (Figure 7). In unadjusted analysis, obese women had significantly greater odds of being in the lowest tertile of all three nutritional components (Table 11). After adjustment for parity, race/ethnicity, and maternal age, obese pregnant women were three times as likely to be in the lowest tertile of the EFA component, and nearly five times as

likely to be in the lowest tertile of the carotenoid component as lean pregnant women. After adjustment, there was no association between obesity and micronutrient component scores. There was no relation between pre-pregnancy overweight and any of the nutritional components before or after adjustment.

5.5 DISCUSSION

Using three general nutritional components describing an array of nutritional biomarkers, we found that a larger percentage of obese women were in the lowest tertile of the EFA, Micronutrient, and Carotenoid components than lean women at ≤20 weeks gestation. After adjustment for maternal characteristics, pre-pregnancy obesity remained associated with poorer EFA and Carotenoid component scores.

It is important to understand the relationship between pre-pregnancy obesity and nutritional patterns during pregnancy. More than 20% of American women are obese prior to pregnancy (S. Y. Kim, et al., 2007) and high micronutrient and EFA concentrations are associated with improved birth outcomes (Helland, Smith, Saarem, Saugstad, & Drevon, 2003; Kramer et al., 2009; van Goor et al., 2010). Nutritional interventions in other high risk populations have been successful in improving maternal nutritional status (Imhoff-Kunsch, Stein, Villalpando, Martorell, & Ramakrishnan, 2011; Piirainen, Isolauri, Lagstrom, & Laitinen, 2006), suggesting that maternal nutritional status can be modified. However, very few researchers have explored micronutrient and EFA status of obese women in pregnancy. Further, we are unaware

of any other study to have examined obesity relative to a wide range of maternal biomarkers summarized using principal component analysis methods.

Our results showing poorer carotenoid status among obese women compared with lean women are in agreement with several studies in non-pregnant populations (Chai et al., 2010; Ford, et al., 2002; Galan, et al., 2005; Neuhouser, et al., 2001; Wallstrom, et al., 2001). For example, in a cross-sectional analysis of Third National Health and Nutrition Examination Survey (NHANES) that included 4,512 non-pregnant, pre-menopausal women, both obese and overweight women were significantly more likely to be in the lowest 20^{th} percentile of a sum of carotenoid concentrations (Kimmons, et al., 2006). In a longitudinal study of over 3,000 young adults, Anderson et al. evaluated the independent effect of obesity on future carotenoid status, and found that, in non-smokers, obese patients had 12-34% ($p < 0.01$) lower carotenoid concentrations seven years later compared with patients with a BMI below 22 kg/m2 at baseline (Anderson, et al., 2003).

Our result that pre-pregnancy BMI is associated with an EFA component (composed of three polyunsaturated EFAs) is supported by a small ($n = 14$) cross-sectional analysis of obese versus non-obese adolescent females. In this study, researchers found that obese girls had a significantly lower sum of polyunsaturated EFAs then normal weight girls [mean (SD): 34.9 (0.9) versus 37.0 (1.7) mole %, $p < 0.05$] (Karlsson, et al., 2006). Obesity may decrease concentrations of EFAs, but poor EFA status may also increase the likelihood of being obese. For example, in a prospective analysis of 1,250 mother-child pairs, a higher sum of umbilical cord plasma DHA and EPA concentrations was associated with lower adiposity at three years of age in the infant [OR = 0.09 (95% CI: 0.02, 0.52 for obesity)] (Donahue et al., 2011).

Concentrations of AA in umbilical cord plasma were not associated with later adiposity. Further study is required to better understand the directionality of these associations.

We found that pre-pregnancy obesity was not related to an increased likelihood of being in the lowest tertile of the micronutrient component after adjustment for maternal characteristics. It is difficult to directly compare our results to those of previous studies because we used a measure of overall micronutrient status, and many previously published studies presented one or two specific biomarkers. In one cross-sectional analysis of multiple micronutrients among non-pregnant, pre-menopausal women, researchers found that obese women are more likely to have low levels of vitamin E, vitamin C, vitamin D and serum folate than non-obese women (Kimmons, et al., 2006). They found no association between BMI and vitamin A and red blood cell folate. In another study examining multiple micronutrients, 76 obese women had lower concentrations of serum retinol, serum ascorbic acid, serum 25(OH)D, and serum alpha-tocopherol than 30 lean women, but serum folic acid concentrations were not different (Aasheim, Hofso, Hjelmesaeth, Birkeland, & Bohmer, 2008). Studies of single nutrients examined in non-pregnant populations by obesity status have reported strong associations between BMI and micronutrients such as vitamin D (Arunabh, et al., 2003; Holvik, et al., 2005; Nesby-O'Dell, et al., 2002; Parikh, et al., 2004; Snijder, et al., 2005) and folate (Mahabir, et al., 2008; Mojtabai, 2004; Tungtrongchitr, et al., 2003). But studies of other nutrients, such as α-tocopherol, have been inconsistent (Chai, et al., 2010; Galan, et al., 2005; Neuhouser, et al., 2001). Additionally, there is evidence that maternal obesity increases the likelihood of poor vitamin D (Bodnar, Catov, et al., 2007) and folate (Han, Ha, Park, Kim, & Lee, 2010) status during pregnancy.

The dissimilarities between our micronutrient findings and those of previous studies are likely due to major differences in our study populations and differences in our outcome

measurement. For example, Kimmons and colleagues studied a large, nationally representative sample of non-pregnant, non-lactating women compared with our small study of well-educated pregnant women. Prenatal vitamin use is common among pregnant women (Yu, Keppel, Singh, & Kessel, 1996), and regular use of prenatal vitamins may confound or modify the association between BMI and blood micronutrient concentrations. Unfortunately, neither our study, nor that of Kimmons et al. incorporated data on vitamin and mineral supplementation to test this hypothesis. Similar to many previous studies, we found that obese women had lower mean serum 25(OH)D than lean women. When 25(OH)D was included into a Micronutrient component, our primary outcome measure, there was no difference between obese women and lean women.

There are several potential mechanisms may explain an association between high BMI and poor nutritional biomarker status. Obese women may have a poor diet quality (Laraia, Bodnar, & Siega-Riz, 2007), which may account for low biomarker concentrations. Many of the micronutrients that loaded on to the Micronutrient component are present in prenatal vitamins, but the EFAs in the EFA Component and the carotenoids that loaded on to the carotenoid component are not always included in prenatal supplements. If obese women in our cohort were taking prenatal supplements, but consumed diets poor in fruits and vegetables and EFAs, this may account for our results. There is also the possibility that excess fat may have a direct effect on nutritional biomarker status. For example, obesity may alter the absorption and metabolism of vitamin D (Blum, et al., 2008; Moan, et al., 2009; Wortsman, et al., 2000). Other fat soluble nutrients, such as carotenoids, may also be sequestered by excess fat and rendered biologically unavailable. Lastly, obesity may contribute to decreased concentrations of antioxidants, such as carotenoids, in pregnant women by creating an increased need for antioxidants by creating an excess of oxidative stress (Keaney et al., 2003).

Several limitations should be taken into consideration when reviewing our results. Pre-pregnancy BMI is a general measure of adiposity that does not provide information on type or distribution of fat, which may be more informative for micronutrient and EFA status. Obesity is associated with an increase in total blood volume (Alpert, 2001), which could artificially lower micronutrient and EFA concentrations in the blood. Unfortunately, measures of blood volume were not collected in this study and we were unable to control for this possible covariate. Additionally, using BMI calculated from self-reported pre-pregnancy weight may have led to misclassification (Bodnar, Siega-Riz, Simhan, Diesel, & Abrams, 2010). Data on supplement use and dietary intake were not available, so we could not consider their confounding or modifying effects on the associations we reported. Ferritin concentrations did not load onto the Micronutrient component, and was not included in our analysis. This may be because ferritin concentrations can reflect inflammation as well as dietary intake and therefore may have not correlated as well with the other micronutrient concentrations. Women with chronic diseases were excluded from our study, which meant that women included in our analysis were generally healthy. Our study had a relatively small sample size of primarily white, well-educated, non-smoking women. The population's small size and homogeneity led to imprecise estimates and reduced generalizability. Furthermore, because we did not collect nutritional biomarkers before pregnancy, our understanding of the temporality of the relationship between BMI and nutritional status is limited. Concentrations of nutritional biomarkers were generally high in our cohort, decreasing the variation of our outcome measurement. As a consequence, our observed effect sizes are likely closer to the null than those that we would have seen if our cohort had a wider range of nutritional biomarker values.

Major strengths of our study include an analysis of a wide array of nutritional biomarkers measured in mid-pregnancy and the use of principle components analysis of the biomarkers to account for the inherent correlation between nutrients and reduce the likelihood of type 1 error due to multiple comparisons. In the future, large, prospective studies with a full panel of nutritional biomarkers and detailed information on distribution of fat mass, diet and supplement use during pregnancy are needed to confirm or refute our results.

Our results suggest that women who are obese before pregnancy may be a high-risk group for having poor EFA and carotenoid status during pregnancy. Maternal carotenoid and EFA concentrations have been associated with important pregnancy and birth outcomes such as neurological development and preterm delivery, and effective interventions that improve nutritional status are available. A better understanding of the effect of pre-pregnancy obesity on maternal micronutrient and EFA status may lead to more specific nutritional interventions to improve pregnancy and birth outcomes in this at-risk population.

5.6 FIGURES AND TABLES

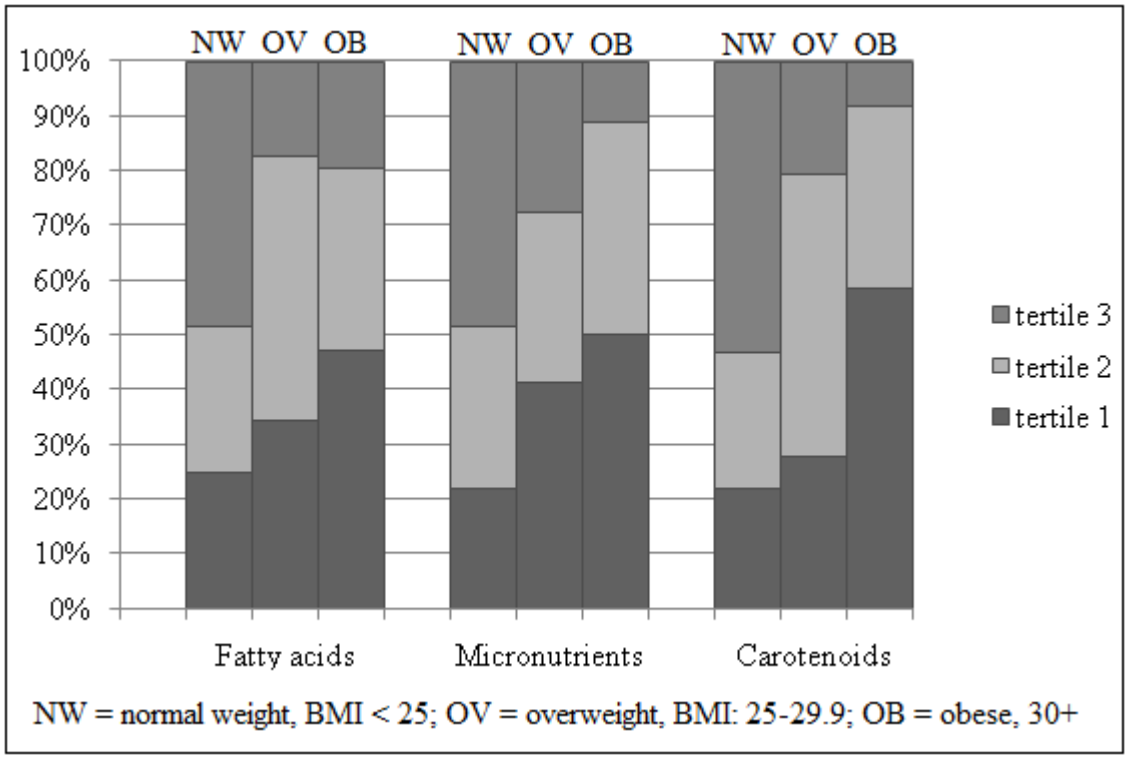

Figure 7. Percent of pregnant women in each tertile of the Fatty acids, Micronutrients, and Carotenoids components, by pre-pregnancy BMI. Tertile 1 represents the lowest component scores and tertile 3 represents the highest component scores, N =129

Table 8. Maternal characteristics of the ADUP population, overall and stratified by pre-pregnancy BMI.

Characteristic	Total (N = 129)	BMI < 25 (n = 64)	BMI 25-29.9 (n = 29)	BMI ≥ 30 (n = 36)	p-value[1]
Age, mean (SD)	30.3 (5.6)	30.6 (5.8)	31.6 (4.4)	28.7 (5.9)	0.14
Race/ethnicity, N (%)					
White	103 (79.8)	56 (87.5)	23 (79.3)	24 (66.7)	
African American	22 (17.1)	6 (9.4)	6 (20.7)	10 (27.8)	
Other	4 (3.1)	2 (3.1)	0 (0.0)	2 (5.6)	0.10
Parity, N (%)					
0	43 (33.3)	33 (51.6)	23 (79.3)	30 (83.3)	
1-6	86 (66.7)	31 (48.4)	6 (20.7)	6 (16.7)	<0.01
Educational status, N (%)					
≤ High School	23 (17.8)	5 (7.8)	4 (13.8)	14 (38.9)	
Some college	25 (19.4)	12 (18.8)	4 (13.8)	9 (25.0)	
College degree	45 (34.9)	25 (39.1)	9 (31.0)	11 (30.6)	
Post-graduate education	36 (27.9)	22 (34.4)	12 (41.4)	2 (5.6)	<0.01
Married, N (%)					
Married	95 (73.6)	48 (75.0)	25 (86.2)	22 (61.1)	
Unmarried	34 (26.4)	16 (25.0)	4 (13.8)	14 (38.9)	0.07
Smoker, N (%)					
Non-smoker	110 (85.3)	56 (87.5)	27 (93.1)	27 (75.0)	
Smoker	19 (14.7)	8 (12.5)	2 (6.9)	9 (25.0)	0.10
Employment status, N (%)					
Employed	73 (56.6)	37 (57.8)	17 (58.6)	19 (52.8)	
Unemployed	56 (43.4)	27 (42.2)	12 (41.4)	17 (47.2)	0.86
Major depresssion[2], N (%)					
Not depressed	96 (74.4)	53 (82.8)	22 (75.9)	21 (58.3)	
Depressed	33 (25.6)	11 (17.2)	7 (24.1)	15 (41.7)	0.03

BMI = body mass index
[1]Based on a student's t-test for maternal age and a chi-squared test for the other covariates
[2]As measured by the Structured Clinical Interview for DSW-IV

Table 9. Mean[1] maternal nutritional biomarkers at 20 weeks gestation, stratified by pre-pregnancy BMI

Biomarker	BMI<25 (n = 64)	BMI 25-29 (n = 29)	p-value[2]	BMI ≥ 30 (n = 36)	p-value
Red cell DHA (%)	3.4 (2.7-4.4)	3.1 (2.2-4.2)	0.58	1.9 (1.3-2.7)	<0.01
Red cell AA (%)	13.0 (11.4-14.8)	12.9 (10.5-15.8)	0.95	9.3 (7.3-11.9)	0.01
Red cell EPA (%)	0.33 (0.28-0.40)	0.28 (0.22-0.35)	0.30	0.25 (0.18-0.33)	0.06
Plasma folate (ng/ml)	15.3 (13.6-17.2)	12.5 (10.7-14.6)	0.04	13.0 (11.3-15.1)	0.08
Plasma ascorbic acid (µg/ml)	12.9 (12.1-13.7)	10.4 (8.7-12.4)	0.01	11.0 (9.7-12.4)	0.03
Serum retinol (µg/ml)	0.50 (0.48-0.53)	0.46 (0.43-0.50)	0.11	0.47 (0.43-0.51)	0.17
Serum 25(OH)D (nmol/L)	88.5 (81.3- 96.3)	77.8 (63.1-96.0)	0.20	69.9 (59.0-82.9)	0.01
Serum α-tocopherol (µg/ml)	0.07 (0.06-0.07)	0.07 (0.06-0.08)	0.72	0.07 (0.06-0.07)	0.47
Serum β-carotene (µg/ml)	0.14 (0.10-0.19)	0.06 (0.04-0.11)	0.01	0.04 (0.03-0.06)	<0.01
Serum homocysteine (µmol/L)	2.3 (2.1-2.6)	2.3 (2.1-2.6)	0.94	2.3 (1.9-2.7)	0.75
Serum sTfR (nmol/L)	14.5 (13.7-15.4)	15.9 (14.5-17.4)	0.09	16.3 (14.9-17.7)	0.03
Serum lutein+zeaxanthin (µg/ml)	0.14 (0.12-0.17)	0.12 (0.10-0.14)	0.17	0.08 (0.06-0.10)	<0.01
Serum β-cryptoxanthin (µg/ml)	0.10 (0.08-0.13)	0.06 (0.04-0.09)	0.02	0.05 (0.04-0.07)	<0.01
Serum lycopene (µg/ml)	0.30 (0.26-0.35)	0.32 (0.27-0.37)	0.76	0.28 (0.23-0.34)	0.41

BMI = body mass index; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; 25(OH)D = 25-hydroxyvitamin D; sTfR = soluble transferrin receptors

[1]Geometric means, all values presented as mean (95% CI)

[2]Student's t-test, biomarkers log transformed before tests were performed, BMI < 25 is the reference group

Table 10. Rotated pattern matrix of maternal dietary biomarkers. Biomarkers loaded on to three components: a fatty acid, a micronutrient, and a carotenoid component.

	Component		
Biomarker	Fatty Acids	Micronutrients	Carotenoids
Red cell docosahexaenoic acid	0.94	-	-
Red cell arachidonic acid	0.87	-	-
Red cell eicosapentaenoic acid	0.66	-	-
Plasma folate	-	0.55	-
Plasma ascorbic acid	-	0.55	-
Serum retinol	-	0.51	-
Serum 25-hydroxyvitamin D	-	0.47	-
Serum α-tocopherol	-	0.32	-
Serum β-carotene	-	0.30	0.68
Serum homocysteine	-	-0.58	-
Serum soluble transferrin receptors	-	-0.56	-
Serum lutein+zeaxanthin	-	-	0.77
Serum β-cryptoxanthin	-	-	0.66
Serum lycopene	-	-	0.52
Factor analysis on 14 biomarkers; reported as |factor loadings| ≥ 0.30.			

Table 11. Association between pre-pregnancy BMI and probability of being in the lowest tertile[1] of fatty acid, micronutrient and carotenoid components, N = 129

	Nutrient component, OR (95% CI)		
	Fatty acid	Micronutrient	Carotenoid
Unadjusted model			
BMI < 25	ref	ref	ref
BMI: 25-29.9	1.6 (0.6, 4.1)	2.5 (1.0, 6.5)	1.4 (0.5, 3.7)
BMI ≥ 30	2.7 (1.1, 6.4)	3.6 (1.5, 8.6)	5.0 (2.1, 12.2)
Adjusted model[2]			
BMI<25	ref	ref	ref
BMI: 25-29.9	1.5 (0.6, 4.1)	1.8 (0.6, 5.7)	1.4 (0.5, 4.3)
BMI ≥ 30	3.0 (1.1, 7.7)	1.5 (0.5, 4.5)	4.5 (1.7, 12.3)

BMI = body mass index
[1]Lowest tertile compared to the combined middle and highest tertiles of nutrient components
[2]Adjusted for parity, race/ethnicity, age. Further adjustment for other covariates had no meaningful impact on the findings.

6.0 THE ROLE OF MATERNAL VITAMIN D STATUS IN THE DEVELOPMENT OF GESTATIONAL HYPERGLYCEMIA

6.1 ABSTRACT

Maternal vitamin D status may play an important role in glucose homeostasis during pregnancy. The objective of our study was to estimate the effects of early pregnancy 25-hydroxyvitamin D [25(OH)D] concentrations on maternal post-load glucose concentration at GDM screening. Pregnant women (n = 484, 63% black, 57% with BMI≥25) enrolled in a cohort study at <16 weeks gestation, and blood samples were assayed for serum 25(OH)D. At 24-28 weeks gestation, post-load glucose concentration was measured using a 50-g 1-hour oral glucose tolerance test. Smoking during pregnancy modified the association between vitamin D and glucose concentration. Among non-smokers, each 21-nmol/L increase in serum 25(OH)D was associated with a 4.1 (95% CI: 0.9, 7.2)-mg/dl increase in maternal post-load glucose concentration after adjustment for race/ethnicity, age, pre-pregnancy BMI, and season of blood sample. Among smokers, each 21-nmol/L increase in serum 25(OH)D was associated with a 7.3 (95% CI: 11.4, 3.1)-mg/dl decrease in maternal post-load glucose concentration after confounder adjustment. In this cohort, vitamin D status was positively associated with maternal glucose concentrations in non-smokers and negatively associated with maternal glucose in smokers. Further research is

needed to understand the relationship between vitamin D status and maternal glucose concentrations among smokers and non-smokers during pregnancy.

6.2 INTRODUCTION

Gestational diabetes mellitus (GDM), defined as glucose intolerance with onset or first recognition during pregnancy, occurs in 4% of pregnancies (Getahun, et al., 2008). GDM is a major risk factor for a variety of pregnancy complications, including macrosomia and birth trauma (Henriksen, 2008; Jevitt, 2005). Importantly, maternal hyperglycemia below the threshold of GDM has also been shown to have a linear relationship with adverse birth outcomes, such as preeclampsia, shoulder dystocia, and birth weight > 90^{th} percentile (Metzger, et al., 2008). Nevertheless, the etiology of maternal hyperglycemia remains obscure.

Vitamin D status may be associated with maternal hyperglycemia. Vitamin D is a secosteroid, obtained through diet and cutaneous synthesis after sunlight exposure (Holick, 2004). Vitamin D deficiency inhibits the secretion of insulin in animal models (Draznin, et al., 1988; Norman, et al., 1980; Sooy, et al., 1999) and has been associated with glucose concentrations in non-pregnant populations (Nikooyeh et al., 2011; von Hurst, Stonehouse, & Coad, 2010) has prompted several investigations of the role of vitamin D in the development of GDM. Nevertheless, studies are limited and results have been inconclusive (Clifton-Bligh, et al., 2008; Farrant, et al., 2009; Maghbooli, et al., 2007; C. Zhang, et al., 2008). Because maternal hyperglycemia is associated with poor outcomes, studying its association with a modifiable risk factor such as vitamin D is important for public health.

The objective of our study was to determine the independent association between early maternal vitamin D status, as measured by serum 25(OH)D and mid-pregnancy maternal post-load glucose concentration.

6.3 METHODS

We used data from the Study of Nutrition and Pregnancy (SNAP). SNAP is an ongoing prospective pregnancy cohort study conducted in the prenatal clinics at Magee-Womens Hospital (Pittsburgh, PA). Pregnant women attending the clinics are primarily low-income, publically insured, and approximately 50% African American. Eligible women had singleton pregnancies and had self reported their race/ethnicity as non-Hispanic black or non-Hispanic white. Patients were excluded if they had pre-gestational diabetes, vaginal bleeding, thrombophilias, chronic hypertension requiring medication, current or planned cervical cerclage, compromised immune system, autoimmune diseases, or known use of illegal drugs or controlled substances. All enrolled participants provided informed, written consent. The study was approved by the University of Pittsburgh Institutional Review Board.

A total of 672 eligible pregnant women were enrolled at < 16 weeks' gestation between June 2003 and November 2009. At enrollment, women completed a structured interview that included questions on socio-demographic factors and medical history. For this analysis, we excluded women who had a spontaneous or therapeutic abortion (n = 79) or had biologically implausible 25(OH)D measurements (n = 5). A total of 55 women were missing 25(OH)D measurements because they lacked sufficient serum volume to assay for 25(OH)D. An additional

49 women were excluded from our analysis because they lacked post-load glucose concentration data. Mean post-load glucose concentrations and maternal characteristics did not significantly differ between women included and excluded from the analyses (data not shown). A total of 484 women were included in the final analyses.

50-g 1-hour oral glucose challenge test was performed as part of routine clinical care at approximately 24-28 weeks gestation to screen for GDM. Women with post-load glucose concentration values >135 mg/dL additionally received a 3-hour 100-gram oral glucose tolerance test. All glucose concentrations were abstracted from medical records. Maternal hyperglycemia was defined as high blood sugar during pregnancy (Metzger, et al., 2008). We defined women with post-load glucose concentration values ≥ 180 mg/dL, or 2 or more abnormal 3-hour 100-gram oral glucose tolerance results as having GDM (Carpenter & Coustan, 1982). A total of 45 women whose health care provider classified them as high risk underwent early screening (< 24 weeks gestation). We conducted a sensitivity analysis to determine the influence of excluding these women from our analysis and found no significant difference between the models (data not shown). Therefore, women with early GDM screening were retained in our sample.

Concentrations of serum 25(OH)D were quantified using a DiaSorin radioimmunoassay (RIA). The RIA detects 100% of 25(OH)D2 and 100% of 25(OH)D3. Maternal serum samples were stored in aliquots at –80°C until they were analyzed for 25(OH)D [25(OH)D2 + 25(OH)D3]. The interassay coefficient of variation was 9.5%. The detectable limits for the RIA are 3.75 to 250 nmol/L. None of the samples of 25(OH)D included in our analysis fell outside this range. We defined vitamin D sufficiency as ≥ 50 nmol/L and also explored a cut-off of <40 nmol/L (IOM, 2011).

Maternal demographics and pre-pregnancy weight were obtained by self-report at enrollment, and family history of diabetes was abstracted from medical records after delivery. Women were classified as current smokers if they smoked any cigarettes during pregnancy or non-smokers if they did not. Beginning in July of 2006, exhaled carbon monoxide (CO) measurements were measured in the SNAP study to confirm self-reported smoking status (Pearce & Hayes, 2005) (n = 122). Exhaled CO concentration was measured using a CO breath analyzer (Micro CO; Micro Medical Limited; Chatham, Kent, England) that was sensitive to CO concentrations from 1 to 500 ppm (by volume). Participants were asked to hold their breath for 15 seconds and then exhale slowly with a constant flow into the analyzer, and this measurement was recorded.

Women were categorized as nulliparous or multiparous. For marital status, women were categorized as married if they were currently married and unmarried otherwise (single, separated, widowed and divorced). Annual family income was separated into < $10,000, $10,000-25,000, $25,000-50,000, and ≥$50,000. Women were classified as unemployed or employed (which included women who worked full time, part time, or occasionally). Educational status was defined as having a high school degree or less, a college degree, or a graduate/professional degree. A family history of diabetes included any reported history of a family member having type 1 or type 2 diabetes mellitus. Pre-pregnancy BMI was calculated as self-reported pre-pregnancy weight (kg) divided by measured height (m^2). A BMI of <18.5 was defined as underweight, 18.5-24.9 as normal weight, 25.0-29.9 as obese, and 30+ as obese ("Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health," 1998). Season of blood sampling was categorized as by winter (December-May) or summer (June-November).

We analyzed maternal post-load glucose as a continuous outcome variable because maternal hyperglycemia, even below the threshold of GDM diagnosis, is strongly associated with adverse pregnancy outcomes (Metzger, et al., 2008). The Student's t-test and Pearson chi-square test were used to determine the differences in post-load glucose and GDM status by maternal characteristics. The relationship of serum 25(OH)D concentration with post-load glucose concentration was assessed using locally weighted regression (lowess smoothing plots) (StataCorp, 2009). As no meaningful deviations from linearity were found, 25(OH)D was examined as a continuous variable in linear regression models assessing the independent association between 25(OH)D and post-load glucose. However, we also categorized 25(OH)D for ease of interpretation. Effect modification by race/ethnicity, pre-pregnancy BMI, and smoking were tested using the Wald test ($\alpha < 0.05$). We assessed maternal age, parity, race/ethnicity, smoking status, marital status, annual family income, employment status, education, family history of diabetes, pre-pregnancy BMI, season of 25(OH)D sample and gestational age of 50-g 1-hour oral glucose challenge test as potential confounders. We defined confounding as a > 10% change in the adjusted beta coefficient after exclusion of the covariate from the full model. The following confounders met this definition: pre-pregnancy BMI, race/ethnicity, maternal age, and season of 25(OH)D sample. We conducted a post-hoc analysis of the effect modification of exhaled CO values on the relationship between vitamin D and maternal glucose concentrations in a sub-sample of women (n=122). Because of the small sample of women with exhaled CO values, we assessed CO as a continuous variable using a model that contained a continuous 25(OH)D variable, exhaled CO variable and interaction term [25(OH)D times exhaled CO]. Data were analyzed using STATA software version 11.0 (StataCorp, 2009).

6.4 RESULTS

The mean (SD) gestational age at enrollment was 9.3 (2.9) weeks' gestation. The majority of women were young, unmarried, low-income, multiparous, African American and high-school educated (Table 12). Nearly half the women in the cohort smoked during pregnancy and more than a third were obese before pregnancy.

The mean (SD) 25(OH)D concentration in our cohort was 41.5 (21.4) nmol/L. A total of 263 (54.3%) women had 25(OH)D concentrations < 40 nmol/L and 325 (67.2%) women had concentrations < 50 nmol/L. Low vitamin D status (25(OH)D < 50 nmol/L) was more prevalent in multiparous, African American, unmarried, high-school educated, and obese women as well as women whose blood was drawn in winter months (Table 12). Furthermore, African American women, unmarried women and high-school educated women had significantly lower mean glucose concentrations than their counterparts. Neither 25(OH)D insufficiency nor post-load differed by smoking status, annual family income, employment, and family history of diabetes.

Women in our cohort were screened for GDM at approximately 26.7 (SD: 3.6) weeks gestation, and the mean (SD) post-load glucose concentration was 99.4 (24.9) mg/dl. Ten percent of the women in our cohort failed the GDM screening (post-load glucose concentrations ≥ 135 mg/dl) and 2.5% were later diagnosed with GDM. In the total population, vitamin D deficiency was not significantly associated with mean post-load glucose concentrations, failed GDM screening (glucose concentration ≥ 135 mg/dl), or GDM diagnosis (Table 13).

Smoking status during pregnancy modified the effect of effect of serum 25(OH)D on glucose concentrations (Figure 8, Table 14). Among non-smokers, there was a positive linear

relationship between early pregnancy serum 25 (OH) D concentrations and post-load glucose concentration.

Each standard deviation (SD) increase in 25(OH)D concentration was associated with an approximately 4.1-mg/dL increase in post-load glucose concentrations after adjustment for pre-pregnancy BMI, race/ethnicity, maternal age, and season of blood sampling. Concentrations of 25(OH)D $\geq$ 50 nmol/L were associated with an approximately 7-mg/dl increase in post-load glucose concentrations after confounder adjustment. Results were similar when we used a cut-point of 25(OH)D $\geq$ 40 nmol/L (data not shown).

Conversely, among smokers, post-load concentrations decreased as 25(OH)D concentrations increased. Each SD increase in 25(OH)D concentration was associated with an approximately 7.3-mg/dl decrease in post-load glucose after adjustment. Levels of 25(OH)D $\geq$ 50 nmol/L among smokers were associated with an approximately 13-mg/dl decrease in post-load glucose concentration, with results when a cut-point of 25(OH)D $\geq$ 40 nmol/L was used (data not shown).

In the sub-population of women with a measured exhaled CO, the mean (SD) of exhaled CO was 3.2 (4.0) ppm among self-reported non-smokers and 10.9 (6.9) ppm among self-reported smokers ($p<0.05$). To confirm the interaction of self-reported smoking, we tested this same interaction, post-hoc, using exhaled CO, a biomarker of smoking status. After accounting for the interaction between 25(OH)D and continuous exhaled CO, we observed that 25(OH)D had a positive relationship with glucose concentrations when exhaled CO values were zero, and a negative relationship with glucose concentrations as CO values increased. For example, among women who had a exhaled CO value of zero ppm, a woman who had a 25(OH)D concentration of 23 nmol/L would have a glucose concentration of 89.1 (95% CI: 79.8, 98.4) mg/dl, and if her

25(OH)D concentration increased to 58 nmol/L, then her glucose concentration would increase to 102.9 (95% CI: 95.4, 110.4) mg/dl (p = 0.01). However, among women who had an exhaled CO of 10 ppm (the mean for smokers), a woman who had a 25(OH)D concentration of 23 nmol/L would have a glucose concentration of 99.3 (95% CI: 90.6, 108.0) mg/dl, and if her 25(OH)D concentration increased to 58 nmol/L, then her glucose concentration would decrease to 97.9 (95% CI: 92.0, 103.9) mg/dl (p = 0.03).

6.5 DISCUSSION

In this cohort of African American and white pregnant women, the association between maternal serum 25(OH)D and post-load glucose concentrations was significantly modified by maternal smoking status. Among smokers, serum 25(OH)D concentrations in early pregnancy were inversely associated with mid-pregnancy post-load glucose concentration. In non-smokers, as 25(OH)D concentrations increased, post-load glucose concentrations also increased. Both of these associations remained statistically significant after adjustment for measured confounders.

In non-pregnant populations, there is a growing body of literature supporting an association between vitamin D status and glucose concentrations (Baynes, et al., 1997; Forouhi, et al., 2008; Isaia, et al., 2001; Nikooyeh, et al., 2011; von Hurst, et al., 2010). In intervention studies, a causal link between vitamin D insufficiency and impaired glucose is controversial in non-pregnant populations (Shapses & Manson, 2011). But some studies in non-pregnant populations support that treatment with vitamin D may improve glucose levels (Boucher, et al., 1995; Forouhi, et al., 2008). Results of the few previous studies of vitamin D status and GDM

have been inconsistent. Cross-sectional studies have reported poorer vitamin D status among women with GDM compared with healthy pregnant women in unadjusted analysis (Maghbooli, et al., 2007), as well as associations between vitamin D status and GDM that did not reach statistical significance (Farrant, et al., 2009) or fasting glucose after confounder adjustment (Clifton-Bligh, et al., 2008). These three studies measured vitamin D status at GDM screening or glucose concentration assessment, often in mid to late pregnancy and did not report smoking status. To our knowledge, only one study has assessed the association between vitamin D status early in pregnancy and GDM. In this nested case-control study women with vitamin D deficiency [25(OH)D < 20 ng/ml] at 16 weeks' gestation were at a nearly 3-fold increased risk for GDM than women with 25(OH)D ≥ 20 ng/ml (C. Zhang, et al., 2008). This association was significant after adjustment for maternal age, race/ethnicity, family history of type 2 diabetes, and pre-pregnancy BMI. Our results may differ because we assessed continuous post-load glucose concentration in a predominately African American cohort with a high prevalence of smoking, and Zhang et al. studied overt GDM in a cohort of mostly non-smoking non-Hispanic white women. However, our results among non-smokers were not in agreement with the results found by Zhang et al. in their primarily non-smoking population.

There are several potential mechanisms for poor maternal vitamin D status to increase the risk of maternal hyperglycemia. 1,25-hydroxyvitamin D, the hormonally active form of the vitamin, is required for the pancreas to release insulin (Kadowaki & Norman, 1985). 1, 25-hydroxyvitamin 2D binds to receptors on the pancreatic β-cell and may regulate β-cell calcium homeostasis (Norman, et al., 1980; Sooy, et al., 1999). Also, vitamin D in the body ensures an adequate intracellular cytosolic calcium pool, which is essential for glucose uptake by insulin-responsive tissues (Draznin, et al., 1988).

Our results that vitamin D status is positively associated with maternal glucose concentrations among non-smokers and negatively associated with glucose concentrations among smokers is not well supported by previous literature. To our knowledge, a positive linear relationship between maternal 25(OH)D and post-load glucose concentrations among non-smokers has not been previously observed. In an attempt to resolve this difference in conclusions, we confirmed our findings with a biomarker of self-reported smoking status. The biomarker, exhaled CO, is also a marker of oxidative stress, suggesting a potential biological explanation for our results. We are only aware of one study that examined how smoking may modify the effect between vitamin D status and glucose concentration. In a population-based study of older, non-pregnant Norwegians, researchers found that, in both smokers and non-smokers, there was no association between vitamin D and type 2 diabetes after adjustment for BMI (Grimnes et al., 2010). The possible mechanisms for positive association between vitamin D status and glucose concentrations that we observed among non-smokers are uncertain. It may be that the association between vitamin D and maternal glucose concentration differs in the presence of oxidative stress. Exhaled CO values are not only a biomarker of smoking status, but may also indicate oxidative stress among diabetics (Paredi, Biernacki, Invernizzi, Kharitonov, & Barnes, 1999). Therefore, our exhaled CO results suggest that vitamin D lowers glucose concentrations in the presence of oxidative stress, but raises glucose concentrations in the absence of oxidative stress. While this has not been well researched in vivo, in vitro cancer studies have suggested that metabolites of vitamin D may have antioxidant properties in some cell lines (Bao, Ting, Hsu, & Lee, 2008) and pro-oxidant properties in others (Ravid et al., 1999). Researchers have hypothesized that vitamin D exhibits oxidative properties in some situations and pro-oxidant properties in others because vitamin D may actually cause a small amount of

oxidative stress, stimulating a cell's detoxification mechanisms when challenged with other oxidative stress challenges (Bao, et al., 2008). More research is needed to test this hypothesis rigorously.

Our study has several limitations that merit discussion. Pre-pregnancy glucose was not measured in our study, so we cannot exclude the possibility that a limited number of women may have had undiagnosed pre-pregnancy hyperglycemia or type 2 diabetes. Therefore, we cannot assert a temporal relationship between vitamin D and maternal hyperglycemia. GDM was rare in our cohort and we were not able to study the relationship between vitamin D status and GDM diagnosis. However our analysis of continuous glucose allowed us to examine the relationship between vitamin D and hyperglycemia across a range of glucose concentrations, even in women who were not diagnosed with GDM. We were unable to assess confounding arising from physical activity, dietary interventions and water retention as these covariates were not measured in our study. Dietary interventions may have improved vitamin D status by improving supplementation or increasing dietary intake of foods like fortified milk, and may also decrease maternal glucose concentrations through healthy eating and weight control, and this could potentially bias our results away from the null. If women had undiagnosed high glucose concentrations before pregnancy, this may have affected their kidneys and caused water retention (Hryciw, Lee, Pollock, & Poronnik, 2004). This, in turn, may artificially dilute concentrations of 25(OH)D, making it seem that women with low 25(OH)D concentrations have high glucose concentrations, which would also bias our results away from the null. Future studies are needed to assess the importance of these potential covariates on the relationship between vitamin D status and post-load glucose concentration. However, our study has several strengths. Our population was composed of African American and white women and we examined post-load

glucose concentration as a continuous variable, a clinically important outcome measure that has been associated with adverse pregnancy outcomes. We measured 25(OH)D concentrations early pregnancy, which supports a temporal relationship between maternal vitamin D status and hyperglycemia. Lastly, we quantified serum 25(OH)D using a DiaSorin RIA kit, a rigorous method which detects 100% of 25(OH)D2 and 25(OH)D3.

Although further research is necessary, we conclude that vitamin D status is positively associated with maternal glucose in non-smokers and negatively associated with maternal glucose in smokers in this cohort of primarily African American, smoking pregnant women. Considering that a vast number of pregnant women are vitamin D insufficient and that rates of GDM are increasing, research is urgently needed to better understand of the role vitamin D plays in the development of maternal hyperglycemia among smokers and non-smokers. This information is needed so that effective interventions to reduce the risk of maternal hyperglycemia can be developed.

6.6 FIGURES AND TABLES

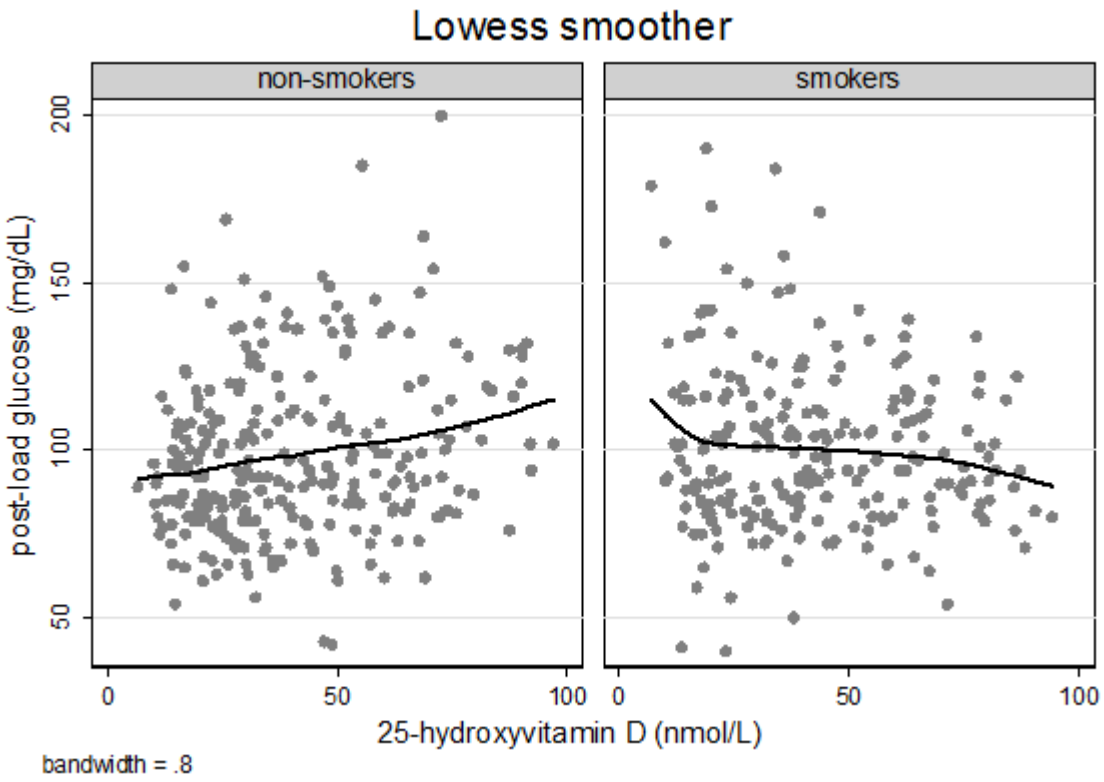

Figure 8. Univariate relationship and locally weight regression line between early pregnancy 25-hydroxyvitamin D status and post-load glucose concentration, stratified by smoking status ($p < 0.01$), N = 484.

Table 12. Maternal characteristics of SNAP participants, prevalence of insufficient vitamin D status by characteristic, and mean maternal post-load glucose concentration by characteristic, n = 484

Characteristic	N (%)	25(OH)D≤50 nmol/L N (%)	p-value[1]	Glucose mg/dl mean (SD)	p-value
Parity					
0	102 (21.6)	48 (47.1)		99.2 (22.8)	
1-7	370 (78.4)	268 (72.4)	<0.01	100.0 (25.4)	0.27
Race/Ethnicity					
White	181 (37.4)	73 (40.3)		103.9 (25.5)	
African American	303 (62.6)	252 (83.2)	<0.01	96.7 (24.1)	<0.01
Smoking status					
Smoker	220 (45.5)	144 (65.5)		100.2 (24.8)	
Non-smoker	264 (54.5)	181 (68.6)	0.47	98.7 (25.0)	0.25
Marital status					
Married	71 (14.7)	35 (49.3)		110.2 (25.0)	
Not married	413 (85.3)	290 (70.2)	<0.01	97.5 (24.4)	<0.01
Annual family income					
< $10,000	207 (43.9)	146 (70.5)		98.2 (23.5)	
$10,000-25,000	161 (34.1)	115 (71.4)		97.2 (22.8)	
$25,000-50,000	89 (18.9)	53 (59.6)		106.2 (29.2)	
≥ $50,000	15 (3.2)	4 (26.7)	<0.01	104.5 (31.0)	0.01
Employment status					
Employed	249 (51.5)	168 (67.5)		99.5 (26.2)	
Not employed	234 (48.5)	156 (66.7)	0.85	99.5 (23.2)	0.88
Educational status					
≤ High school degree	427 (88.2)	294 (68.9)		98.4 (24.6)	
College degree	47 (9.7)	28 (59.6)		105.1 (25.7)	
Post-college degree	10 (2.1)	3 (30.0)	0.02	116.4 (25.8)	0.02

Table 12 continued.

Family history of diabetes					
History of diabetes	166 (36.6)	120 (72.3)		99.4 (25.9)	
No history of diabetes	288 (63.4)	187 (64.9)	0.11	99.8 (24.7)	0.92
Pre-pregnancy BMI (kg/m^2)					
Underweight (<18.5)	19 (3.9)	9 (47.4)		98.1 (21.5)	
Normal (18.5-24.9)	188 (38.8)	116 (61.7)		94.8 (24.2)	
Overweight (25-29.9)	103 (21.3)	65 (63.1)		101.8 (24.0)	
Obese (≥30)	174 (36.0)	135 (77.6)	<0.01	103.0 (25.9)	<0.01
25(OH)D season sample					
December-May	219 (45.2)	176 (80.4)		99.5 (26.0)	
June-November	265 (54.8)	149 (56.2)	<0.01	99.2 (24.0)	0.92

25(OH)D = 25-hydroxyvitamin D; BMI = body mass index
Missing: 12 women missing parity; 12 missing income; 30 missing family history of diabetes
[1]25(OH)D≤50 nmol/L: χ^2 test; mean glucose: Student's t-test, Wald test for BMI, income status and educational status

Table 13. Maternal post-load glucose concentration and GDM prevalence by vitamin D insufficiency, N=484

Vitamin D status	Total N (%)	Glucose concentration (mg/dl) Mean (SD)	p-value[1]	Glucose ≥ 135 (mg/dl) N (%)	p-value	GDM N (%)	p-value
25(OH)D < 50 nmol/L	325 (67.1)	98.6 (25.4)		35 (10.8)		9 (2.8)	
25(OH)D ≥ 50 nmol/L	159 (32.9)	101.0 (23.8)	0.32	14 (8.8)	0.50	3 (1.9)	0.56

GDM = gestational diabetes mellitus

[1]Students' t-test for glucose concentration and $\chi 2$ test for glucose ≥ 135 mg/dl and GDM

Table 14. Association between early pregnancy (< 16 weeks) vitamin D status and maternal post-load glucose concentration, stratified by smoking status, n = 484

Non-smokers	Unadjusted β (95% CI)	Adjusted[1] β (95% CI)
Serum 25(OH)D, β reflects 21 nmol/L increase	4.9 (2.0, 7.8)	4.1 (0.9, 7.2)
Vitamin D status		
25(OH)D < 50.0 nmol/L	ref	ref
25(OH)D ≥ 50.0 nmol/L	8.8 (2.3, 15.2)	6.9 (0.2, 13.6)
Smokers		
Serum 25(OH)D, β reflects 21 nmol/L increase	-7.6 (-11.9, -3.2)	-7.3 (-11.4, -3.1)
Vitamin D status		
< 50.0 nmol/L	ref	ref
≥ 50.0 nmol/L	-13.8 (-23.2, -4.4)	-13.6 (-22.7, -4.5)

BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D
[1]Adjusted for pre-pregnancy BMI, race/ethnicity, maternal age, and 25-hydroxyvitamin D sample season

7.0 SYNTHESIS

7.1 OVERVIEW OF RESEARCH FINDINGS

This dissertation used complementing data from two pregnancy cohorts, the Study of Nutrition and Pregnancy (SNAP) and the Antidepressant Use during Pregnancy (ADUP) Study, to improve our understanding of the interrelationship of maternal obesity, nutritional status, and maternal hyperglycemia, which is defined as high blood sugar during pregnancy (Metzger, et al., 2008). We established associations between early maternal adiposity measures and hyperglycemia. Principle component analysis was used to create nutritional biomarker components and these components were used to assess the association between pre-pregnancy obesity and maternal micronutrient and EFA status. We also explored the relationship between early pregnancy vitamin D status and maternal glucose concentrations. Here we outline the research findings presented in this dissertation.

1) Determine the independent and joint associations between maternal pre-pregnancy BMI, GWG, and adiposity distribution at <16 weeks gestation and maternal post-load glucose concentrations.

Measures of post-load glucose concentration and maternal adiposity from SNAP were used to assess the association between maternal adiposity and hyperglycemia during pregnancy. Patterns of GWG by trimester and GWG adequacy, as well as skinfold thicknesses and waist

circumference, were explored as independent variables. We found that high GWG in the first trimester, independent of pre-pregnancy BMI, was associated with hyperglycemia later in pregnancy. This finding was consistent with other studies that have explored trimester-specific GWG in other populations, though ours was the first study to explore this association with hyperglycemia in a population of black and white, low-income women. Pregnant women with high GWG in their first trimester may have an elevated risk of maternal hyperglycemia because early pregnancy weight gain, of which a large part is fat accumulation, may increase biological factors that can increase insulin resistance above that of the normal pregnant state.

We also found that high early pregnancy biceps and triceps skinfold thicknesses, measures of subcutaneous fat, were associated with maternal hyperglycemia. There is a paucity of information regarding the role of adiposity distribution in the development of maternal hyperglycemia a gap that our results help to ameliorate. However, there is some evidence that maternal diabetes and obesity may cause defects in the insulin-signaling pathway in maternal subcutaneous adipose tissue.

2) Using principal component analysis to discover the underlying structure of maternal nutritional biomarkers and evaluate the independent effect of general adiposity on these patterns at ≤ 20 weeks gestation.

We used pre-pregnancy BMI and nutritional biomarker data from the ADUP study cohort to explore the association between of maternal general adiposity and nutritional (micronutrient, carotenoid and EFA) status. Principal component analysis of fifteen nutritional biomarkers measured at ≤ 20 weeks gestation was performed to create three nutritional components: a Micronutrient component, a Carotenoid component, and an EFA component. Pre-pregnancy obesity was associated with the decreased Carotenoid and EFA Components scores.

The use of principal component analysis allowed us to explore the relationship between maternal adiposity and a wide array of nutritional biomarkers, and also allowed us to take into account the inherent correlation between individual nutritional biomarkers. This relationship may arise from poor diet quality among obese women or excess fat altering the absorption and metabolism of nutrients or creating an increased need for antioxidant nutrients by increasing oxidative stress.

3) Determine the effect of maternal vitamin D status at <16 weeks gestation on maternal hyperglycemia.

We used the SNAP cohort, which had a large percentage of women who smoked during pregnancy, to determine the association between maternal 25-hydroxyvitamin D status early in pregnancy and maternal hyperglycemia. In non-smokers, 25-hydroxyvitamin D and maternal post-load glucose concentrations had a positive, linear association, whereas in smokers, 25-hydroxyvitamin D and maternal post-load glucose concentrations had a negative, linear association. These findings are not consistent with previous literature. Therefore, we confirmed our results using biomarker of self-reported smoking status.

7.2 STRENGTHS AND LIMITATIONS OF THIS RESEARCH

Our proposed study has limitations that should be taken into account. Our research was challenged by small sample sizes and missing data. Because this was a secondary analysis, often using data collected outside the research setting, many women in the ADUP and SNAP cohorts were missing information on nutritional biomarker status, glucose concentrations or serial

measured weights during pregnancy. Our analysis of adiposity distribution was also limited by a small sample size because the anthropometric measurements that we used were added to the protocol mid-way through the study. As a result, our analytic sample was reduced. Although, important variables of interest rarely differed meaningfully between our analytic sample and the larger cohort, the reduction in sample size limited the precision of our effect estimates and restricted our ability to explore important covariates, such as the interaction between trimester specific GWG rates.

We were unable to use more rigorous measures of adipose tissue, such as dual-emission X-ray absorptiometry scans or bioelectrical impedance analysis because they are cumbersome and costly. This means that our analysis of adiposity were less accurate than had we used these more rigorous methods. Although we took skin fold thicknesses and waist circumference measurements three times to reduce random error, anthropometric measurements are particularly susceptible to user error. Using anthropometric measurements also impeded our ability to differentiate between different kinds of adipose tissue (e.g. fat mass versus fat-free mass). Nevertheless, the anthropometric measurements we used could potentially be more applicable in a practical clinical setting. Maternal weight at each clinic visit was measured by hospital staff in a clinical setting, without a standardized protocol. Our measures of pre-pregnancy BMI were calculated using self-reported height and weight, which may lead to misclassification of pre-pregnancy BMI status.

An additional limitation was that there is not an available “gold standard” method of measuring global nutritional status. The nutritional biomarkers we used may have varied because of many factors, such as metabolism and physical well-being. Nutritional biomarkers are correlated to a woman’s diet and likely had inter-person variability because a woman’s diet

changes from day to day. The nutritional biomarkers that we used generally reflect an average of a woman's micronutrient and EFA concentration over a period of time, but this period of time was different between assays. The assay we used to measure vitamin D in the ADUP cohort has large inter- and intra-participant variability and may have over-estimated the concentration of vitamin D, although our measurements were validated using HPLC in the ADUP cohort.

Our measure of maternal post-load glucose may also have limitations. Because the SNAP cohort population had a low prevalence of GDM, we did not have the sample size necessary to conduct an analysis of GDM status. Blood glucose measurements were collected in a clinical setting, rather than a research setting. Therefore, they may be subject to measurement error because of varying collection techniques and handling of samples, including collecting samples across a wide range of gestational ages. Variation in collection and handling of samples is likely to be uniform across women and would not differ by anthropometric or vitamin D status. This limits the potential for differential misclassification. Gestational age of screening likely differs by GDM risk. To mitigate this limitation, we assessed gestational age at screening for confounding and conducted sensitivity analysis to assess the effect of including or excluding women with early glucose screening, who may have been at higher risk, in our analysis.

There are several methodological limitations that should be taken into consideration when reviewing our results. Both ADUP and SNAP recruited participants on a volunteer basis and this may have introduced volunteer bias into results. For example, if women who were willing to volunteer for ADUP were more likely to be obese and well-nourished, our results would have been biased towards the null. In our analysis of the association between adiposity and maternal glucose concentrations, women who were excluded from our analysis were more likely to be smokers and nulliparous than women included in the analysis. If non-smoking or

multiparous women were more likely to have excess adiposity, and were more likely to develop hyperglycemia, then our results would be biased away from the null. Our results may also be susceptible to measurement bias. Our measure of GWG was calculated using weights measured in a clinical setting without a standard protocol and if clinical staff was more likely to measure weights differently in obese women, (e.g. rounding weight measurements of obese women down or up, encouraging obese women to remove shoes), then women at high risk for hyperglycemia may have an artificially high or low GWG. Women who are obese during pregnancy may also systematically recall their pre-pregnancy weight as either higher or lower than their actual weight, which may have led to misclassification in our data. Our results may also be affected by unmeasured confounding. For example, we were unable to adjust our results for dietary intake, supplement use, physical activity, genetic factors and sunlight exposure. Lastly, our study used observed data and therefore we can only suggest associations and we cannot suggest causality.

Despite these limitations, our study also has many advantages. In both SNAP and ADUP, our data was prospectively collected. In the SNAP cohort, we were able to assess exposures, i.e. adiposity distribution and vitamin D status, early in pregnancy. We were able to analyze maternal post-load glucose concentrations as a continuous variable which allowed us to explore the linear nature of our associations. Also in the SNAP population, we were able to investigate the relationship between adipose and glucose concentrations and vitamin D and glucose concentrations in a black and white population. Principal component analysis was used to assess nutrition status, allowing us to assess multiple biomarkers, and the correlations between these biomarkers, simultaneously. We used biomarkers of micronutrient (including carotenoid) and EFA status, which are objective measures of a woman's nutrient status. The assays used to quantify micronutrient and EFA concentrations have intrinsic measurement error and a limit of

detection, below which we were unable to determine the micronutrient or EFA concentration of a sample. But this limitation, as well as the skewed distributions of the biomarker concentrations, was ameliorated by the use of Spearman's rho correlation coefficients in a principal component analysis. We were able to contribute new information to the field of adiposity and nutrition, modifiable risk factors to prevent the development of maternal hyperglycemia. Lastly, our study evaluates relationships of great public health importance.

7.3 PUBLIC HEALTH SIGNIFICANCE

The findings of this dissertation have important implications for the improvement of public health among pregnant women and their infants. Many women develop GDM during pregnancy, and it is likely that even more suffer from maternal hyperglycemia. Both GDM and maternal hyperglycemia have been associated with adverse pregnancy and birth outcomes such as preeclampsia, macrosomia, and birth trauma. Understanding the factors that may be associated with the development of hyperglycemia during pregnancy is important for the formation of effective interventions to prevent maternal hyperglycemia. This, in turn, could have a major impact on the health of women and their infants.

The results of this project suggest that there are aspects of adiposity, independent of pre-pregnancy BMI that may contribute to the development of maternal hyperglycemia. Women who have a normal pre-pregnancy BMI, but high levels of subcutaneous fat or GWG early in pregnancy may be at high risk for developing maternal hyperglycemia, but misclassified as low risk. Maternal adiposity is a modifiable risk factor and screening for high adiposity can be easily

accomplished because GWG is typically measured throughout a woman's prenatal care and anthropometric measures of subcutaneous fat can be easily, safely and inexpensively measured in a clinical setting. Effective interventions to prevent excessive adiposity in early pregnancy may prevent or reduce the prevalence of maternal hyperglycemia, which could considerably decrease the burden of disease during pregnancy and delivery.

Public health can also be improved by the knowledge that women who are obese before pregnancy may be a high-risk group for having poor EFA and carotenoid status during pregnancy. Pre-pregnancy obesity rates in the United States, much like rates of obesity in non-pregnant populations, have reached epidemic proportions and are increasing. Insufficient levels of carotenoids and EFAs, both essential for a healthy pregnancy and normal fetal development, among obese women could be detrimental to maternal and child health. Interventions, for example targeted multi-micronutrient supplementation, have been effective in improving the nutritional status in other high risk pregnancy populations, and may Nutritional interventions are safe, acceptable and effective and targeted nutritional interventions could be developed to improve pregnancy and birth outcomes in this at-risk population.

Our results that vitamin D status is significantly associated with maternal hyperglycemia have great public health impact. A fuller understanding of the relationship between maternal vitamin D status and glucose concentrations, and the potential interaction between vitamin D and the oxidative stress caused by smoking, can be used to develop effective interventions to reduce the prevalence of hyperglycemia during pregnancy. A large percentage of pregnant women, for example more than 50% in our study, have insufficient levels of vitamin D, and nutritional interventions to improve vitamin D status could potentially have a great impact on the number of women affected by high glucose concentrations.

This research also has important clinical implications. We found that first trimester GWG and subcutaneous fat are associated with maternal glucose concentrations, independent of family history of diabetes and pre-pregnancy BMI. If clinicians are using high pre-pregnancy BMI and a family history of diabetes as their only identifying criteria for increased hyperglycemia and GDM risk, then many at-risk women may be overlooked in early screening and intervention. Our results that obese pregnant women may have poorer carotenoid and EFA status may inform targeted nutritional interventions that can be easily incorporated into clinical practice. The result that vitamin D status is positively associated with maternal glucose in non-smokers and negatively associated with maternal glucose in smokers needs to be confirmed with further research before these conclusions can be incorporated into clinical practice. But our results do suggest that the relationship between vitamin D and glucose may be more complex than previously thought.

7.4 DIRECTIONS FOR FUTURE RESEARCH

The conclusions of this dissertation can be used to inform the planning of randomized controlled trials of weight control early in pregnancy to prevent hyperglycemia and of targeted nutritional interventions in obese pregnant women. Additionally, our results that subcutaneous fat may be independently associated with maternal glucose concentrations can be used to develop investigations of the role adiposity plays in the pathogenesis of maternal hyperglycemia and GDM. Lastly, our results revealed a potential new aspect of the relationship between vitamin D and maternal glucose concentration: that smoking, perhaps by causing oxidative stress, may

modify the effect of this relationship. But the post-hoc nature of our results necessitates further study to confirm these conclusions.

Future studies with larger sample sizes will be able to assess important aspects of this research that we were unable to explore due to data collection and time constraints. In a larger cohort, researchers will be able to test if trimester of gain modifies the relationship between GWG and post-load glucose concentrations. Because composition of maternal weight gain differs by trimester, this information is crucial in order to develop timely interventions. Waist circumference was only collected in a sub-sample of our cohort and if the effect size of waist circumference is small, larger studies would be required to observe this effect. Similarly, our estimates of the effect of pre-pregnancy BMI on micronutrient (including carotenoid) and EFA status would be more precise with a larger cohort of women with a full array of nutritional biomarkers.

The arena of maternal adiposity, nutrition and maternal hyperglycemia would also benefit from studies that include several variables that were not available to us in this study. There are more advanced imaging technologies, for example MRI or ultrasounds, which are considered safe during pregnancy. These methods will provide more accurate information of adiposity quantity and distribution, but are also expensive and cumbersome and do not lend themselves to clinical screening interventions. Alternatively, there is a wide array of other inexpensive and simple anthropometric techniques that future studies can explore to better understand different aspects of maternal adiposity distribution, such as percent of body fat, which we could not calculate with the measures at hand. Future studies would also benefit from the collection of glucose concentrations before pregnancy so that women with undiagnosed type 2 diabetes can be excluded. Lastly, there are many additional covariates that we were unable to measure that are of

interest, but the two that we most strongly recommend being included in future research are supplement information and physical activity. Both of which are likely to have a considerable impact on this research field. Regular supplement use and physical activity are likely to decrease obesity, improve nutritional status and decrease the likelihood of developing hyperglycemia.

Future studies of the role of oxidative stress and its interaction with vitamin D in the development of maternal hyperglycemia are warranted. We have suggested that these studies are especially important in cohorts with high rates of smoking. Here we were able to conduct a post-hoc analysis of one measure of oxidative stress, exhaled CO, but there are other markers of oxidative stress that may give researchers more information about this relationship. Also, we were only able to conduct this analysis in a sub-set of our cohort.

We also recommend that our results be confirmed in populations at higher risk for GDM. For example, cohorts of older women from higher risk racial/ethnic groups. We observed linear relationships between maternal post-load glucose concentrations and measures of adiposity and vitamin D status in a primarily low risk population. It is important to establish if this relationship is similar in higher risk populations. A higher risk population would also allow for an analysis of overt GDM as well as maternal hyperglycemia.

Observational studies provide valuable insights of associations between maternal adiposity, nutrition and hyperglycemia, but we cannot assert causality from these studies. To truly understand the effect that these factors have on the development of maternal hyperglycemia, future researchers must develop effective interventions to improve obesity and nutritional status in pregnant women. Furthermore, these interventions must be tested for their ability to prevent and control maternal hyperglycemia.

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