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The Impact of Gestational Diabetes on Maternal and Cord Blood Lipids Among Prenatal Care Patients in Western Ma

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**THE IMPACT OF GESTATIONAL DIABETES ON MATERNAL AND CORD BLOOD
LIPIDS AMONG PRENATAL CARE PATIENTS IN WESTERN MA**

A Thesis Presented

by

PREETHI RAJ

Submitted to the Graduate School of the University of the Massachusetts Amherst in partial
fulfillment of the requirements for the degree of

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Biostatistics and Epidemiology

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ABSTRACT

THE IMPACT OF GESTATIONAL DIABETES ON MATERNAL AND CORD BLOOD LIPIDS AMONG PRENATAL CARE PATIENTS IN WESTERN MA

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Gestational diabetes mellitus (GDM), a pregnancy-induced metabolic disorder that affects 2-10% of pregnancies poses future risk for diabetes mellitus (DM) and cardiovascular disease in mother and child. However, few prospective studies have examined the effect of GDM on altered maternal and cord blood lipids, specifically HDL, LDL, triglycerides, and total cholesterol, both during and after pregnancy. We have evaluated the association between GDM and lipid metabolism in pregnant mothers and their infants using data from a prospective cohort study conducted at Baystate Medical Center's Wesson Women and Infant's Unit. GDM was assessed prenatally by 3-hr GTT blood samples and was confirmed by obstetrician review. Lipids were assessed via fasting and non-fasting blood samples obtained during 3-hr GTTs performed at 24-28 weeks of gestation and 6-8 weeks post-partum. Data for covariates were collected via an interview form administered at the time of recruitment. We used multivariable linear regression to evaluate the association between GDM status and maternal lipids during and after pregnancy as well as cord lipids. These study results inform future research on GDM as a risk factor for future metabolic disorders in mother and child.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	iii
ABSTRACT.....	iv
LIST OF TABLES.....	vii
LIST OF FIGURES	ix
CHAPTER	
1. INTRODUCTION.....	1
2. PHYSIOLOGY OF GDM AND LIPID OUTCOMES	4
2.1. Physiology of GDM and Maternal Blood Lipid Relationship	4
2.2. Physiology of GDM and Cord Blood Lipid Relationship.....	5
3. EPIDEMIOLOGY OF GDM AND LIPID OUTCOMES.....	7
3.1. Epidemiology of GDM and Maternal Blood Lipid Relationship.....	7
3.2. Epidemiology of GDM and Cord Blood Lipid Relationship.....	12
3.3. Summary	14
4. HYPOTHESIS AND AIMS	16
5. METHODS	17
5.1. Source Data	17
5.2. Exposure Assessment.....	18
5.3. Validity of Exposure Assessment	19
5.4. Outcome Assessment	19
5.5. Validity of Outcome Assessment.....	20
5.6. Covariate Assessment	21
6. STATISTICAL ANALYSIS	22
6.1. Data Analysis Plan for Maternal Blood Lipids Outcome	22
6.1.1. Univariate Analysis.....	22
6.1.2. Bivariate Analysis.....	23
6.1.3. Multivariable Analysis.....	23
6.2. Data Analysis Plan for Cord Blood Lipids Outcome.....	24
6.2.1. Univariate Analysis.....	24

6.2.2.	Bivariate Analysis.....	24
6.2.3.	Multivariable Analysis.....	24
7.	RESULTS.....	25
7.1.	Analysis Cohort.....	25
7.2.	Distribution of Lipids by Covariates.....	26
7.3.	Multivariable Linear and Logistic Regression.....	27
8.	DISCUSSION.....	30
9.	STUDY LIMITATIONS.....	32
9.1.1.	Nondifferential Misclassification of Exposure (Gestational Diabetes Mellitus)....	32
9.1.2.	Nondifferential Misclassification of Outcome I – Maternal Lipids.....	32
9.1.3.	Nondifferential Misclassification of Outcome II- Cord Blood Lipids.....	33
9.2.	Selection Bias.....	34
9.3.	Differential Misclassification.....	34
9.4.	Confounding.....	35
9.5.	Cross Sectional Limitations.....	37
9.6.	Generalizability.....	37
10.	SIGNIFICANCE.....	38
11.	HUMAN SUBJECT PROTECTION.....	39
12.	PERMISSION TO ACCESS DATA.....	40
	REFERENCES.....	42

LIST OF TABLES

Table	Page
1. Enrollment, Withdrawals Prior to Delivery, and Availability of Blood Lipid Values in Baystate Gestational diabetes mellitus (GDM) & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
2. Gestational diabetes mellitus (GDM) in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
3. Comparison of Women with GDM and NonGDM, Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
4a. Crude Associations of Covariates with Baseline Maternal Lipids ^c in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
4b. Crude Associations of Covariates with Baseline+3rd Hr GTT Maternal Lipids ^c in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
4c. Crude Associations of Covariates with Postpartum Maternal Lipids ^c in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
5. Crude Associations of Covariates with Cord Blood Lipids ^c in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
6a. Crude and Adjusted Mean Baseline Maternal Blood Lipids by GDM status in the Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
6b. Crude and Adjusted Mean Baseline+3rd Hr GTT Maternal Lipids by GDM status in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
6c. Crude and Adjusted Mean Postpartum Blood Lipids by GDM status in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
6d. Crude and Adjusted Mean Cord Blood Lipids by GDM status in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
7a. Variations in Crude Associations of GDM (Beta, SE) with Lipids by Patterns of Missingness in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
7b. Variations in Adjusted ^a Associations of GDM (Beta, SE) with Blood Lipids by Patterns of Missingness in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary
8a. Relative Odds with GDM (OR, 95%CI) of Elevated Baseline Fasting Maternal Lipids at Baseline in Baystate GDM & Lipid Metabolism Study, 2007-2009.....	Please see Supplementary

8b. Relative Odds with GDM (OR, 95%CI) of Elevated Baseline+3rd Hour Maternal Lipids in Baystate GDM & Lipid Metabolism Study, 2007-2009.....Please see Supplementary

8c. Relative Odds with GDM (OR, 95%CI) of Elevated Postpartum Maternal Lipids in Baystate GDM & Lipid Metabolism Study, 2007-2009.....Please see Supplementary

LIST OF FIGURES

Figure	Page
1. Study Design: Baystate GDM and Lipid Metabolism Study, 2007-2009	41

CHAPTER 1

INTRODUCTION

Diabetes mellitus is defined as a metabolic disorder in which a person experiences high levels of blood sugar either due to insufficient insulin production (Type I) or sensitivity (Type II). Gestational diabetes mellitus (GDM) is distinguished from diabetes mellitus as the advent of Type II-like, impaired musculoskeletal insulin sensitivity during pregnancy and has been recently reported to affect 18% of pregnancies due to changes in diagnostic criteria compared to previous prevalence estimates of 2-10% of pregnancies (1). Women diagnosed with gestational diabetes have a 35% to 60% chance of developing diabetes in the next 10-20 years (1) and the estimated healthcare costs are high. In the year 2007 alone, GDM increased national medical costs by \$636 million-- \$596 for maternal costs and \$40 million for neonatal costs (2). These costs can only be expected to rise.

Hyperlipidemia, the condition of elevated blood lipids, is of concern because of its association with metabolic syndrome, a complex collection of metabolic disorders including obesity, hypertension, hyperlipidemia, insulin resistance, and elevated glucose concentrations (3). Metabolic syndrome is currently estimated to affect 34% of the United States population and is known to increase an individual's future risk for cardiovascular disorders (4). For a formal diagnosis of metabolic syndrome, 3 out of 5 of the following criteria must be met: waist circumference >88cm; HDL cholesterol less than 40 mg/dL; serum triglyceride levels \geq 150 mg/dL; systolic blood pressure >130 mm/Hg or diastolic blood pressure \geq 85 mm/Hg, and a fasting blood glucose level \geq 100 mg/dL (5). Physiologic changes during pregnancy result in a naturally transient hyperlipidemic and hyperglycemic state, and can often serve as an antecedent to the development of Type II diabetes and cardiovascular disease (6).

Established risk factors for elevated blood lipid levels during pregnancy include family history of hypercholesterolemia (7) and maternal BMI (8). Maternal diet during pregnancy has also been implicated in altering the amounts of endogenous lipids in the mother that are probably conferred to the child (9). Additionally, gestational diabetes mellitus has been proposed as a possible risk factor for elevated maternal and cord blood lipids due to its enhancement of existing altered metabolism during pregnancy (10).

The distinctive hormonal milieu of pregnancy is thought to cause insulin resistance, promoting oxidative stress which encourages the development of endothelial dysfunction and future cardiometabolic risk for both mother and child (11, 12, 13, 14). Excess endogenous glucose during the GDM pregnancy has been proposed to induce an imbalance of HDL and LDL particles in maternal blood circulation, in which LDL dominates (6, 10, 15). Furthermore, accumulation of body fat in early pregnancy and subsequent lipolytic activity in the third trimester, combined with an increased maternal-fetal gradient, have been hypothesized to cause the accelerated transfer of various cholesterol particles from mother to child (16, 17, 18). Although these physiologic mechanisms are not definitive, they provide a conceptual framework from which GDM can affect maternal and cord blood lipids.

Few epidemiologic studies (21, 22, 23, 24, 25, 26, 27, 28, 31) have examined the effect of GDM on lipid levels during pregnancy and those that have produced conflicting results. No prospective longitudinal investigations have evaluated whether women experience blood lipid alterations subsequent to GDM diagnosis. Studies are needed to explore the possibility of GDM conferring future metabolic risk for mother and child postpartum.

We therefore conducted a prospective study among pregnant women seen at Baystate Medical Center in Springfield, MA to assess the effect of their GDM status on their maternal

lipids both during and after pregnancy as well as cord blood lipids after delivery. Study findings will help to elucidate whether the effects of hyperglycemia during pregnancy are long-standing for mother and child.

CHAPTER 2

PHYSIOLOGY OF GDM AND LIPID OUTCOMES

Gestational diabetes has been shown to alter maternal and cord blood lipids simultaneously, such that cord blood lipid levels reflect maternal blood lipid levels. Previous studies comparing maternal blood and cord blood serum levels of adiponectin, a protein hormone involved in glucose regulation and fatty acid metabolism, provide conflicting explanations of how maternal and cord blood serum is related. While one study found no correlation between maternal blood and cord blood serum adiponectin levels in healthy mothers along with higher adiponectin levels in cord blood, and significant correlations between cord blood adiponectin levels and neonatal birthweight (19), another study found maternal blood adiponectin levels to be independent predictors of cord blood adiponectin levels, regardless of whether women had GDM or not (20). These findings warrant separate consideration of maternal and cord blood serum lipids in GDM and non-GDM women.

2.1. Physiology of GDM and Maternal Blood Lipid Relationship

The mechanism for how GDM affects maternal blood lipids requires further elucidation, however, several hypotheses exist. Pregnancy-induced changes in steroid hormones, estrogen and progesterone, cortisol, human chorionic somatotropin (HCS), and sex hormone binding globulin (SHBG) are further disrupted in GDM resulting in lower than normal estrogen levels accompanied by insulin resistance, or the inability to utilize insulin. Insulin resistance in turn causes inflammation, endothelial dysfunction, and the formation of reactive oxygen species, markers of oxidative stress (11, 12, 13, 14). Oxidative stress during pregnancy can cause an imbalance of HDL and LDL particles, resulting in predominant amounts of the latter, less

favorable form of cholesterol (10). This phenomenon is particularly enhanced during gestational diabetes where (excess) glucose acts as a principal oxidative substrate used by the fetus for growth (10). Secondly, excess endogenous glucose in pregnant woman stimulates the liver to metabolize fats into cholesterol, hence resulting in elevated lipid levels (15). Thirdly, while the first and second trimester of normal pregnancy is characterized by increased accumulation of maternal fat stores, breakdown and conversion of maternal fats and cholesterol for growth of the fetus occurs in the third trimester. Pronounced insulin resistance in GDM, however, inhibits third trimester lipoprotein lipase (LPL) enzyme activity, allowing for higher levels of endogenous triglycerides to which both mother and child are vulnerable (14). Lastly, the increase of lipids during pregnancy in response to GDM exposure may be a precursor to sustained postpartum dyslipidemia, much like how GDM is considered a precursor to future, if not immediate postpartum diabetes (21). Overall, the mechanisms for how GDM causes elevated maternal blood lipids are not clearly understood, yet, the often synchronous occurrence of the two purports a possible causal link.

2.2. Physiology of GDM and Cord Blood Lipid Relationship

Diabetes during pregnancy has been shown to increase the transfer of non-esterified fatty acids (NEFAs), total cholesterol, triglycerides, phospholipids, and cholesterol esters from the mother to her child. This is possibly due to an increased maternal-fetal gradient, or increased permeability of blood and organic matter between the mother and child across the placental barrier (19, 22). Maternal body fat accumulation during the early stages of pregnancy allows for the storage of long-chain polyunsaturated fatty acids, which the pregnant woman obtains via diet and her own metabolism (20). Towards the end of the pregnancy the pregnant woman experiences increased lipolytic (“fat breaking”) activity in these accumulated fat cells, which are converted into cholesterol, as well as NEFAs, glycerol, and ketone bodies for fetal growth. The

degree of maternal blood lipids found in GDM cord vein blood is dependent upon several factors such as fetal consumption, efficient or impaired placental transfer, and/or altered fetal metabolism (14). Accelerated fetal growth in the 3rd trimester demands large and frequent transfers of nutrients from the mother. In women with GDM, it has been therefore hypothesized that the excess presence of glucose within endogenous maternal blood, characteristic of GDM, provides ample opportunity for elevated levels of glucose and cholesterol to cross the placental barrier into the cord blood, resulting in metabolically challenged babies (20). Excess glucose conferred to the child produces fetal hyperinsulinemia (12, 13), which further alters the child's cardiometabolic profile. In sum, during pregnancy the mother's body acts as an exposure for their child, such that the metabolic repercussions of GDM and high cholesterol levels could possibly affect the child.

CHAPTER 3

EPIDEMIOLOGY OF GDM AND LIPID OUTCOMES

3.1. Epidemiology of GDM and Maternal Blood Lipid Relationship

Several epidemiologic studies have specifically examined the impact of GDM on maternal blood lipids (21, 23, 24, 25, 26, 27, 28). Seven prospective cohort studies, by Sokup et al (2012), Akinci et. al (2011), Marseille-Tremblay et al (2008), Lauenborg et al. (2005), Sobki et al. (2004), Couch et. al (1998), and Meyers-Seifer et al. (1996) have considered maternal blood lipids and GDM while addressing the role of cardiovascular biomarkers in the development of metabolic syndrome in women several years after they had GDM. These studies have generally observed modest differences in blood lipids comparing women with and without GDM, with women with GDM have slightly higher levels of serum total cholesterol, LDL, and HDL (measured in units of mmol/L or mg/dL, where 1 mmol=38.67 mg/dL) and triglycerides, (also measured in units of mmol/L or mg/dL, where 1 mmol=88.57 mg/dL) (29, 30).

Sokup et al. (21) conducted a prospective post-pregnancy cohort study in Poland in 2011, where they sought to investigate whether women with a previous history of gestational diabetes from 2005-2007 were at a higher and premature risk for elevated triglyceride levels and glucose dysregulation. The authors defined their exposure groups as 125 prior GDM pregnancies and 40 prior normal pregnancies. Diagnosis of GDM was made using 50g and 75g oral glucose tolerance tests and according to World Health Organization criteria. Blood samples that were profiled for total cholesterol, TG, LDL, and HDL were obtained during post-partum visits between 2-24 months after the index pregnancy. While none of the women were diet-managed or on glucose or lipid-lowering medications during their pregnancy, most of the women were breastfeeding at the time of their postpartum visit. The authors found the following median values in previous GDM vs. non-GDM mothers (in mg/dL): slightly higher TC: (195.67 vs.

176.34, $p=0.001$), slightly higher LDL: (122.58 vs. 99.38 , $p=0.001$), slightly lower HDL: (59.17 vs. 66.90 , $p=0.001$), and slightly higher TG: (90.34 vs. 76.17 , $p=0.012$). In spite of the statistical significance of these results there is a possibility for the assessment of postpartum lipids within the first two years of delivery to encompass a varied range of lipid profiles with respect to time since pregnancy and breastfeeding status.

Similarly, Akinçi et. al (23) conducted a prospective post-pregnancy cohort study in Turkey in 2011 where they sought to investigate whether women diagnosed with gestational diabetes between 2002-2008 were more likely to develop postpartum carbohydrate intolerance, metabolic syndrome, and exhibit cardiovascular risk factors than women without prior GDM. Gestational diabetes was diagnosed at 24-28 weeks of pregnancy using Carpenter and Coustan criteria and, in the case of positive diagnosis, women were prescribed diet control. Postpartum lipids, such as TC, TG, LDL, and HDL, were evaluated in 1 year intervals with an average follow-up of approximately 3 years. The authors found the following means for maternal lipids in GDM vs. non-GDM mothers (in mg/dL): slightly higher TC: (199.15 vs. 175.56, $p<0.001$), slightly higher TG: (122.23 vs. 87.68, $p=0.006$), slightly higher LDL: (123.74 vs. 96.29, $p<0.001$), and slightly higher HDL: (51.43 vs. 61.48, $p=0.01$). Higher BMI values for GDM mothers may suggest, however, a separate underlying pathophysiologic mechanism to produce the observed lipids.

Marseille-Tremblay et al. (24) conducted a small prospective, hospital-based cohort study in Canada in 2008, where they defined their exposure groups as GDM and non-GDM (healthy) women and their outcome as maternal total cholesterol, TG, LDL, and HDL levels. Their total enrollment was 14 subjects, with 7 insulin-treated GDM women and 7 non-GDM women. The authors assessed their GDM exposure by administering an interview to the women on medical

history around 10 weeks of gestation. The authors assessed maternal lipid outcomes via blood samples collected at delivery. The authors found the following means±standard errors for maternal lipids in GDM vs. non-GDM mothers (in mg/dL): slightly higher TC (265.66±23.20 vs. 260.0±17.40, nonsignificant [NS], no p-value provided); slightly higher TG (266.60±43.40 vs. 261.28±41.62, NS, no p-value provided); slightly higher LDL (141.92±16.63 vs. 141.53±17.79, NS, no p-value provided), and slightly lower HDL (63.03±6.19 vs. 69.99±3.48, NS, no p-value provided). These observed differences, however, were modest and not statistically significant. Marseille-Tremblay et al. was the only study to examine the effect of GDM on maternal lipids during gestation as well as after delivery, and the small sample size of this study challenges interpretation of these null findings.

Lauenborg et al. (25) conducted a prospective, hospital-based cohort study in Denmark in 2005, defining exposure as prior diagnosis of diet-only treated GDM, and the outcome as fasting serum HDL cholesterol and fasting serum TG. The total enrollment was 1,481, with 481 prior GDM (cases) and 1000 age matched, healthy, comparison women. GDM exposure was defined as diagnosis during 1978-1985 and 1987-1996 based on risk factor-based Dutch criteria (31). The authors assessed fasting HDL and TG levels via fasting morning blood samples during a median follow-up of 9.8 years after pregnancy. The authors found the following medians for maternal lipids in GDM vs. non-GDM mothers: In mg/dL; slightly higher TG: 115.14 vs. 88.57, $p<0.0005$, and slightly lower HDL: 54.14 vs. 58.0, $p<0.0005$. These statistically significant p-values belie the negligible difference between lipids in both groups. This study did not assess GDM exposure using the standard glucose tolerance test at the time of the study. If there had been any changes in the women's GDM status since their initial diagnosis via screening criteria this method of exposure assessment may not accurately reflect their GDM status during the

study, hence leading to misclassification of exposure. The efficiency of the Danish screening criteria that the authors used at the time of the study is, therefore, dependent upon how sensitive and accurate it was in distinguishing women who truly have GDM from those who do not. These potential limitations suggest the possibility of study results being biased towards the null.

Sobki et al. (26) conducted a prospective, hospital-based cohort study in Saudi Arabia in 2004, where they sought to examine the effect of gestational diabetes on biomarkers of oxidative stress and cardiovascular risk in maternal blood samples. GDM was diagnosed during the 22nd-28th week of gestation according to National Diabetes Data Group criteria and their exposure categories consisted of 19 GDM mothers receiving insulin treatment, 27 GDM women managed by diet, and 40 non-GDM women. Maternal serum samples were obtained during the second stage of labor. All comparisons of TC, TG, LDL, and HDL levels between diet-treated/ insulin-treated GDM women and non-GDM women were not statistically significant. The explanation for these results is unclear. Obtaining maternal blood samples during the second stage of labor, however, may provide an altered state of lipids in both GDM and non-GDM mothers, which would then underestimate any differences, biasing the results towards the null.

In the only study to evaluate the association between GDM and lipids at 37-38 weeks gestation, Couch et al. (27) conducted a prospective, hospital-based cohort study in Hartford, Connecticut in 1998, where they defined their exposure groups as GDM and non-GDM (healthy) women and their outcome as maternal total cholesterol, TG, LDL, and HDL levels. Their total enrollment was 40 subjects, with 20 diet-managed GDM women and 20 non-GDM women. The authors assessed their GDM exposure by administering a 1hr-50g glucose tolerance test, followed by a 3hr GTT, at 24-30 weeks gestation to confirm GDM diagnosis according to

O'Sullivan and National Diabetes Data Group criteria. Maternal lipid outcomes were assessed via blood samples collected at 37-38 weeks gestation. The authors found the following means for maternal lipids in GDM vs. non-GDM mothers (in mg/dL): slightly lower TC: 221.50 vs. 232.46 (NS, no p-value provided), slightly higher TG: 236.38 vs. 177.96 ($p \leq .01$), slightly higher LDL: 68.76 vs. 46.14 ($p < .01$), and slightly higher HDL: 58.05 vs. 41.34 ($p \leq .01$). Of these results, elevated TG, LDL, and HDL levels for GDM women were statistically significant. The use of older, less conservative, GDM diagnosis criteria and the enrollment of women with GDM who did not require insulin to maintain glucose control may have biased the study results toward the null.

The first of two studies to evaluate the association between GDM and postpartum lipids, Meyers-Seifer et al. (28) conducted a prospective, hospital-based cohort study in Providence, Rhode Island in 1996, defining exposure as prior diagnosis of GDM, and outcome as fasting maternal blood serum total cholesterol, TG, LDL, and HDL, 5-6 years post-partum. The total enrollment was 106, with 58 prior GDM (cases) and 48 age matched comparison subjects recruited 5-6 years postpartum from the Diabetes in Pregnancy Screening Program in Providence, Rhode Island Women and Infant's Hospital. GDM exposure was defined as confirmed diagnosis of GDM via 1-hr glucose tolerance and 3-hr GTT tests performed during 24-28 weeks of gestation. Mothers who had GDM received nutritional counseling, and were closely monitored for blood glucose levels throughout the pregnancy; insulin therapy was recommended in the case of high glucose levels. The authors assessed fasting lipid levels via fasting morning blood samples during a median follow-up of 5-6 years after pregnancy. The authors found the following results for maternal lipids in GDM vs. non-GDM mothers: In mg/dL; slightly higher mean TC (189.87 vs. 165.89, $p=0.005$); slightly higher mean TG (131.08

vs. 85.03, $p=0.02$); slightly higher mean LDL (121.81 vs. 105.18, $p=0.01$); and slightly higher mean HDL: (49.11 vs. 44.86, NS, p -value not provided). In Meyer-Seifer et al.'s study, the utilization of GDM diagnosis via past medical records to determine current GDM status raises the possibility of misclassification of exposure, possibly biasing the results towards the null. Also, in this study, GDM subjects were closely followed and advised for their dietary intake while comparison non-GDM subjects were not, challenging the interpretation of comparisons between the groups.

3.2. Epidemiology of GDM and Cord Blood Lipid Relationship

Few studies have examined the impact of GDM on cord blood lipids (24, 26, 27, 31). Marseille-Tremblay et al (2008), Sobki et al. (2004), and Abou Ghalia et al. (2003), and Couch et al. (1998) have examined this association with mixed findings for total cholesterol, triglycerides, and LDL cord blood lipids. As previously noted, blood lipid concentrations are expressed either in mmol/L or mg/dL with a conversion factor of 38.67 for serum total cholesterol, LDL, and HDL (1 mmol=38.67 mg/dL), while serum triglycerides have a conversion factor of 88.57 (1 mmol=88.57 mg/dL) (29, 30).

In their small prospective, hospital-based cohort study, Marseille-Tremblay et al. (24) assessed newborn lipid outcomes via cord blood samples collected at delivery. The authors found the following means \pm standard errors for cord blood lipids in GDM vs. non-GDM mothers, in mg/dL: slightly lower TC: 69.61 \pm 23.20 vs. 69.99 \pm 7.73, slightly lower TG: 55.80 \pm 7.09 vs. 62.0 \pm 8.86, slightly lower LDL: 29.0 \pm 2.71 vs. 30.16 \pm 4.25, slightly higher HDL: 34.03 \pm 6.19 vs. 26.30 \pm 4.64 (all comparisons were non-significant, p -values not provided). Once again, observed differences in this small study were small and not statistically significant. Also, the use of GDM

women who were exclusively treated with insulin may suggest a less representative sample of women with exceptionally altered metabolic profiles compared to the majority of women with GDM (32).

Sobki et.al (26) conducted a hospital-based cohort study in Saudi Arabia in 2004, where they sought to examine the effect of gestational diabetes on biomarkers of oxidative stress and cardiovascular risk in cord blood samples obtained at delivery. The authors found the resulting following means for cord blood lipids in GDM-diet-treated/GDM-insulin-treated vs. non-GDM women: TC: 1.51/1.51 vs. 2.03 (non-significant, no p-value provided); TG: 0.40/0.31 vs. 0.64 ($p<0.05$), LDL: 0.79/0.86 vs. 1.97 ($p<0.05$); and HDL: 0.58/0.68 vs. 0.72 ($p<0.05$). Overall, they found that cord blood lipids were significantly, albeit slightly, lower in GDM women compared to non-GDM women. Proposed rationale for the lower levels observed in cord blood lipids of GDM women involves the hypothesis that neonatal plasma lipoprotein levels are lower than in adults, and that neonates of diabetic women are subject to a higher rate of oxidative metabolism as a result of maternal hyperlipidemia and hyperglycemia. Also, all GDM women were either diet or insulin-treated, compared to non-GDM with no treatment, which may have lowered or altered their cord blood lipids, biasing their results towards the null.

Abou Ghalia et al. (31) conducted a prospective study of 77 pregnant women who were classified according to their GDM status (i.e., GDM, insulin-dependent DM, non-insulin dependent DM, or non-gestational DM). The authors assessed GDM status through verification of family/obstetric medical records. LDL: HDL ratio was assessed via a 10 mL cord blood sample obtained immediately after delivery. The authors found the following mean \pm standard deviations in cord blood samples from GDM vs. non-GDM (healthy) mothers: in mg/dL; slightly higher TC: 73.47 ± 24.36 vs. 65.74 ± 15.47 (NS, no p-value provided), slightly lower TG:

33.66±16.83 vs. 40.74 ±15.06 (NS, no p-value provided) slightly higher HDL: 34.80±13.92 vs. 29.0±11.60 (NS, no p-value provided) and slightly higher LDL: 31.32±18.17 vs. 23.59±10.05 (NS, no p-value provided). None of these findings reached statistical significance. The inclusion of women with non-gestational diabetes for comparison with control mothers in this study limits speculation upon the specific effects that GDM may have on cord blood lipid profiles. Lastly by only focusing on the LDL: HDL ratio, Abou Ghalia et al. fail to investigate the effect of GDM on other important components of blood lipids like triglycerides and total cholesterol levels in cord blood.

In Couch (27) et al.'s study, the authors assessed GDM exposure by administering a 1hr-50g glucose tolerance test, followed by a 3hr GTT, at 24-28 weeks gestation to confirm GDM diagnosis. Cord blood lipid outcomes were assessed via blood samples collected at delivery. The authors found the following means±standard deviations of maternal lipids in GDM vs. non-GDM mothers (in mg/dL): TC: 53.58±14.01 vs. 54.82±13.80 (NS, no p-value provided), TG: 36.68±12.31 vs. 43.71±14.80 (NS, no p-value provided); LDL: 32.29±12.09 vs. 32.20±13.84 (NS, no p-value provided); and HDL: 21.30±5.42 vs. 22.07±7.09 (NS, no p-value provided). None of these results were statistically significant. Although Couch et al. sought to examine the direct effect of GDM on maternal and cord blood lipid outcomes, GDM women were diet-controlled, while controls were not, and they consequently gained significantly less weight than controls which could have altered their lipid profile, biasing the results towards the null.

3.3. Summary

Gestational diabetes, a hyperglycemic manifestation of metabolic syndrome during pregnancy, is estimated to affect 18% of pregnancies and is postulated to affect the cardiovascular health of mother and child long after gestation. Metabolic syndrome is currently

estimated to affect close to half of the United States population (4) and is known to increase an individual's future risk for cardiovascular disorders (5). Hyperlipidemia, obesity, and insulin resistance, which are increasingly prevalent metabolic risk factors, are responsible for the development of metabolic syndrome. Current knowledge about the effect of gestational diabetes upon maternal and cord blood lipids is limited. Among those that have been conducted, small sample size, and issues with determination of GDM status, differences in management of GDM during pregnancy, and timing and measurement of lipid levels have resulted in ambiguity regarding this association. More studies are needed to elucidate this association, especially studies with sufficient sample size, and assessment of lipids both during gestation and after delivery. We propose to use prospective data from Baystate Medical Center's GDM and Lipid Metabolism study to examine the association between gestational diabetes and maternal blood lipids and cord blood lipids both during and after pregnancy.

CHAPTER 4

HYPOTHESIS AND AIMS

Specific Aim #1: We propose to examine the association between gestational diabetes mellitus (GDM) and maternal blood and cord lipids, namely, total cholesterol, triglycerides (TG), high density lipoproteins (HDL), and LDL (low density lipoproteins), in a prospective cohort of women from the Baystate Maternal and Fetal Medicine unit.

- a. Hypothesis 1: Compared to women without GDM, women with GDM have elevated levels of total cholesterol, TG, LDL, and lower levels of HDL, both during pregnancy and postpartum.
- b. Hypothesis 2: Compared to the cord blood of women without GDM, the cord blood of women with GDM has elevated levels of total cholesterol, TG, LDL, and lower levels of HDL.

CHAPTER 5

METHODS

5.1. Source Data

Our study evaluated the association between gestational diabetes mellitus (GDM) and lipid metabolism in pregnant mothers (maternal blood lipids) and their infants (cord blood lipids) using existing data from a prospective cohort study conducted in Springfield Massachusetts between 2007 and 2009 amongst women obtaining services from Baystate Medical Center's Wesson Women and Infant's Unit. Baystate Medical Center is a tertiary care hospital and is the largest of four Baystate Health hospitals in the Western Massachusetts region. Subjects for this study were women that came in to the Wesson Women's Baystate Reference Laboratory Satellite office to receive a 3-hour glucose tolerance test (GTT), a procedure used to test for gestational diabetes. The GTT was performed among pregnant patients at around 24-28 weeks of gestation. Eligible women were those who had failed a preliminary fasting, 50 g, glucose test (1 hr GTT) and consequently were scheduled for the follow-up 100 g, 3-hr GTT to confirm their GDM status (Table 1). A single staff member from the Maternal and Fetal Medicine visited the Wesson Lab regularly to interview and then recruit women for the study. Information on ethnicity (Table 3) and self-reported family member history of elevated lipids, diabetes, myocardial infarction, and stroke were collected at this time (Table 3).

In accordance with the study protocol, maternal lipids were first assessed around 24-28 weeks gestational age using the same blood samples utilized for the GDM screen at baseline, allowing for simultaneous assessment of exposure and outcome. With knowledge of GDM status, postpartum lipids were then obtained prospectively at 6-8 weeks post-delivery to examine the prolonged effects of GDM on maternal lipids (Figure 1). Cord blood samples were obtained from the women at the time of birth.

While 398 women were initially approached for our study, 289 were excluded because they either refused, were ineligible, or terminated due to pregnancy complications. Patients were asked to identify if they fulfilled any exclusionary criteria and signed an informed consent form approved by Baystate Medical Center's Institutional Review Boards before they provided any personal/interview information. Study exclusions included: a history of diagnosis of diabetes, hypertension, heart disease, or chronic renal disease, prior history of lipid disorder or metabolic syndrome, current medications thought to adversely influence glucose tolerance, non-singleton pregnancy, < 16 or over 40 years of age, and the intention to deliver outside Baystate (Table 3). Additional caveats for termination after enrollment included having a GCT (fasting plasma glucose) ≥ 150 followed by a normal 3 hour GTT, due to the suggested possibility of an underlying insulin resistance in women that are diagnosed as non-GDM (healthy women), being unable to complete the 3-hr GTT, phlebotomy/lab errors, and withdrawal from the study prior to obtaining GTT results. Women were also excluded if they withdrew from the study due to miscarriage, subsequent pregnancy, or loss-to-follow-up (Table 1). Also, exclusions were made if a recruitment arm of the study, GDM or non-GDM was fulfilled.

5.2. Exposure Assessment

The exposure of interest is gestational diabetes mellitus, which we dichotomized into two groups of GDM (exposed) and non-GDM (unexposed) (Table 2). GDM is routinely diagnosed in a two-step process, first by patient history, clinical risk factors, or with a 50-g, 1 hour loading test at 24-28 weeks of gestation, and second by resulting values from a 100-g, 3-hr glucose tolerance test for diagnosis (33, 34). The "GDM exposed" group were defined using Carpenter and Coustan criteria (35, 36) as having elevated glucose values for two or three samples over the 3 hour (total of 3 samples) GTT, while the non-GDM, "unexposed" (healthy) group were identified as those with one or no abnormal glucose values over the 3 hour GTT. Unexposed

women were required to lack prior diagnosis of diabetes before pregnancy. Carpenter and Coustan criteria determines GDM status via the following cut-off points: a fasting value of 95 mg/dL, 1-hour value of 180 mg/dL, 2 hour value of 155 mg/dL, and 3 hour value of 140 mg/dL (37). All blood glucose assessments were performed at the Wesson Women's Baystate Reference Laboratory.

5.3. Validity of Exposure Assessment

The 3 hour 100g test is recommended by the ADA and is based on O'Sullivan's landmark studies in the 70's (33, 34) to diagnose women with GDM. More recently, the internationally sponsored HAPO study (38) used the 3 hr-GTT to evaluate if elevated glucose values were associated with greater odds of adverse pregnancy outcomes, such as hypertension (39). Using this method, the researchers observed a consistently increased risk for hypertensive disorders for elevated values at fasting (OR: 1.21; 95% CI: 1.13-1.29), 1-hour glucose (OR: 1.28; 95% CI: 1.20-1.37), and 2-hour glucose (OR: 1.28; 95% CI: 1.20-1.37). The ACOG 2001 Practice Bulletin for Gestational Diabetes acknowledges that the optimum threshold for diagnosing GDM is elusive and hence more than one threshold is acceptable (36). The American Diabetes Association ascribes approximately 90% sensitivity to the 130 mg/dL cutoff mark, and 80% sensitivity to the cutoff mark of 140 mg/dL (last abnormal value of 3 hr GTT) (40). Although both values are acceptable, a more conservative threshold is ideal to avoid false positive diagnoses of GDM.

5.4. Outcome Assessment

Our outcomes of interest were maternal and cord blood levels of four variables: total cholesterol, triglycerides, HDL, and LDL cholesterol. Women's total cholesterol, HDL, LDL, and triglyceride lipid levels were collected as fasting and 3 hour GTT-congruent measurements both at 24-28 weeks of gestation (baseline), and then at 6-8 weeks postpartum (Table 4), while

cord blood samples were collected immediately after birth. Fasting glucose, 3 hour OGTT, and cord blood levels of all four outcome variables were evaluated continuously (Table 5). National guidelines were used to classify maternal lipids into binary high and low categories.

Hyperlipidemia was diagnosed using national standards from the National Institute of Health's *Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Adult Treatment Panel III (ATP III)* (41). Hyperlipidemia is defined as the presence of excess blood lipids based on nationally-determined guidelines (41). The recommended level for total cholesterol is <200 mg/dL, for LDL is <100 mg/dL, for triglycerides is <200 mg/dL, and for HDL is between 40 mg/dL and 60 mg/dL (41).

5.5. Validity of Outcome Assessment

Our use of a fasting and then non-fasting sample while the patients came in for their regular visit is deemed one of the most acceptable and feasible ways to screen for lipid disorders (35). A study done by Bansal et al. in 2007 (42) evaluated the association of fasting and non-fasting triglyceride levels at enrollment with incident cardiovascular events over a median follow-up of 11.4 years in women. The authors found that triglyceride levels measured 2 to 4 hours post-prandially had the strongest association with cardiovascular events (fully adjusted hazard ratio [95% confidence interval] for highest vs. lowest tertiles of levels, 4.48 [1.98-10.15] [$P < .001$ for trend]), and this association progressively decreased with longer periods of fasting. This finding emphasizes the importance of non-fasting samples in reflecting the true effect of lipids resulting from meal consumption upon disease risk and incidence. Lastly, we expect the coefficient of our lab measurement to be valid and within the accepted 30% range because it is certified according to the government-monitored Clinical Laboratory Improvement Amendments (CLIA) standard.

5.6. Covariate Assessment

Data for covariates were collected via self-report through a “Lipid Metabolism and GDM Study: Patient Screening and Interview Form” administered at the time of recruitment. Specifically, date of birth, age at enrollment, expected date of delivery, gestational age at time of sample collection, BMI, race, medications, history of thyroid disease, infertility, and other serious medical conditions were collected via mostly multiple choice questions in the interview form (Table 3). Medical record abstraction was used to determine whether the women had experienced GDM in previous pregnancies. Family history, by closeness of relative, was also collected for elevated lipids, diabetes, myocardial infarction, and stroke (Table 3). Previous studies have shown that race (43), maternal BMI (44), and family history (39) are strong risk factors for future cardiometabolic disorders in mother and child, of which GDM and abnormal maternal and cord blood lipids are a proxy.

CHAPTER 6

STATISTICAL ANALYSIS

In preliminary analysis, descriptive statistics were computed to describe the characteristics of the study cohort. In the main analyses, normal theory linear regression models were used for the continuous outcomes (TC, TG, LDL, and HDL lipid values) and logistic regression approaches were used in analyses of dichotomized measures of TC, TG, LDL, and HDL. Single predictor models were fit to obtain model free estimates of associations with lipid levels. Multiple predictor models were fit to estimate associations of GDM with outcome, controlling for age, BMI, and gestational age, either at recruitment or delivery. My approach to model fitting involved a backward elimination process where all covariates of potential interest were included and sequentially removed by using partial F-tests for linear regression and likelihood ratio tests for logistic regression, with a Type I error of <0.05 .

6.1. Data Analysis Plan for Maternal Blood Lipids Outcome

6.1.1. Univariate Analysis

Hypothesis 1: Compared to women without GDM, women with GDM have elevated levels of total cholesterol, TG, LDL, and lower levels of HDL, both during pregnancy and postpartum.

In univariate analysis, descriptive statistics of the study variables were computed separately for both GDM and non-GDM mothers. The number and characteristics of the study population that were lost during follow-up are presented in Table 1 while the percent distribution of GDM/non-GDM mothers is presented in Table 2. The distributions of covariates by GDM

status are presented in Table 3. For continuous variables, means and standard deviations are presented, while for discrete variables frequencies and relative frequencies are shown.

6.1.2. Bivariate Analysis

Covariates were cross-tabulated with total cholesterol (TotChol), triglycerides (TG), HDL, and LDL for maternal blood lipids (Table 4), to evaluate potential confounders. P-values from Chi-square, ANOVA, or Student t-tests which reflect the differences in distributions are presented for all of the covariates. We used directed acyclic graphs (DAGs) to examine relationships between the covariates and the exposure and outcome. Unadjusted linear (for continuous variables) and logistic (for categorical variables) regression models were used to evaluate the effect of covariates on the association between GDM and maternal lipids (Table 6, 7a, 7b, and Table 8).

6.1.3. Multivariable Analysis

We modeled the relationship between GDM status and continuous maternal blood lipid levels first using multivariable linear regression to calculate beta coefficients, standard errors, and p-values, for both fasting , 3-hr GTT values, at baseline and 6-8 weeks postpartum (Table 6). We evaluated this relationship by treating maternal blood lipids as a dichotomous variable and evaluating the odds of observing high maternal blood lipid levels among exposed vs. unexposed using multivariable logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (Table 8). Three multivariable logistic regression models were run for fasting and 3hr GTT maternal lipids at baseline and for fasting at 6-8 weeks postpartum (Table 8).

Confounders were evaluated by running all models with and without their presence. Any covariate that changes the estimate for GDM exposure by 10% or greater was retained in the model as a confounder.

6.2. Data Analysis Plan for Cord Blood Lipids Outcome

Hypothesis 2: Compared to the cord blood of women without GDM, the cord blood of women with GDM has elevated levels of total cholesterol, TG, LDL, and lower levels of HDL.

6.2.1. Univariate Analysis

The percent distribution of GDM/non-GDM (Table 1) and the distribution of cord blood cholesterol (TotChol), triglycerides (TG), HDL, and LDL are presented as well (Table 5).

6.2.2. Bivariate Analysis

Covariates were cross-tabulated with GDM exposure (Table 3) and cord blood lipid outcomes (Table 5) to evaluate potential confounders. P-values from Chi-square, ANOVA, or Student t-tests reflect the differences in distributions for all of the covariates. We used directed acyclic graphs (DAGs) to examine relationships between the covariates and the exposure and outcome. Unadjusted linear models were also used to evaluate the effect of covariates on the association between GDM and cord blood lipids (Table 6).

6.2.3. Multivariable Analysis

We modeled the relationship between GDM status and continuous variable lipid levels first using multivariable linear regression to calculate beta coefficients, standard error, and p-values (Table 6). Insufficient low cord blood lipid levels prevented us from evaluating the odds of observing high lipid cord blood lipid levels among exposed vs. unexposed using multivariable logistic regression to calculate ORs and 95% confidence intervals (Table 8).

Confounders were evaluated by running all models with and without their presence. Any covariate that changes the estimate for GDM exposure by 10% or greater was retained in the model as a confounder.

CHAPTER 7

RESULTS

7.1. Analysis Cohort

A total of 109 women were initially enrolled in our study, with 61 non-GDM and 48 GDM mothers. Of the 109 pregnant women originally recruited for this study, 2 women without GDM and 1 woman with GDM withdrew from the study prior to delivery (n=3), resulting in a final cohort of 106 women with information available through delivery (Table 1 and Table 2). An additional 7 women withdrew after delivery, of whom 6 were without GDM. Only two GDM women were excluded due to miscarriage and subsequent pregnancy, respectively, and 54.1% of non-GDM mothers vs. 41.7% of GDM mothers were lost due to follow-up. Slightly more than half of the women in our study (55.7%) did not have gestational diabetes (Table 2). The availability of blood lipid values varied throughout the course of this study as there was significant loss-to-follow-up postpartum (Table 1).

Due to the logistical challenges of conducting a prospective pregnancy cohort, there was a substantial amount of missing data due to missed clinic visits and/or dropout in our cohort. Of the 106 in the analysis cohort, complete data was successfully obtained from 24 women (22.6%), which included having complete lipid values for maternal baseline fasting, maternal baseline+3rd hour GTT, maternal postpartum, and cord blood. Eighty-four (79.2%) participants had at least one lipid outcome missing over the course of the study. Of the 24 women with complete information, 14 were GDM women, and 10 were non-GDM women. This slight difference in participant retention by GDM status represents a potential bias for our results. Therefore, we sought to address the possibility of informative censoring by comparing the crude and adjusted association of GDM with lipids by patterns of missingness in 3 ways: in the analysis cohort as a

whole (n=106), in the women with at least one missing value (n=84), and in the women with complete information (n=24). These results are further explained as a secondary component of the linear regression analyses (Table 7a and 7b).

Table 3 presents the distribution of potential physiologically meaningful covariates according to GDM status. The mean age for mothers in this population was 30.48 years (SD±5.28), with a non-significant association between age category and GDM status (p=0.13). Mothers with gestational diabetes were slightly older than those without gestational diabetes (31.6 vs. 29.6 yrs., p=0.05), and were also recruited for the study at an earlier gestational age (27 vs. 29 weeks, p=0.02). Gestational age at delivery was similar in the two groups (38.5 vs. 38.9, p=0.27). Mothers with GDM had a higher mean BMI of 33.72 (SD±1.10) compared to a mean BMI of 30.29 (SD±.84) for mothers without GDM (p=0.01). Neither race (p=0.73) nor history of thyroid disease (p=0.82) were associated with GDM status. Six women reported past experiences of infertility, of whom 5 were GDM mothers compared with 1 without GDM (p=0.05). Similarly, GDM and non-GDM mothers reported having similar backgrounds for family history of metabolic disease, but a greater proportion of non-GDM mothers reported having no family history (p=0.09).

7.2. Distribution of Lipids by Covariates

The distribution of maternal total cholesterol (TotChol), triglycerides (TG), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) by covariates at both the baseline and postpartum visits are presented in Tables 4a-4c. Age was not associated with higher lipids for the fasting and 3rd hour of the glucose tolerance test (GTT) at the baseline visit (Tables 4a and 4b). Age, however, was associated with higher TotChol (p=0.02) and LDL (p=0.04) in the 6-8 week maternal postpartum lipid sample (Table 4c). BMI was associated with modestly higher TG as

well as modestly lower TotChol, LDL, and HDL at all timepoints. At the fasting antenatal visit, there was a significant association between overweight and obese BMI categories and lower LDL ($p=0.06$) and HDL values ($p=0.01$), while at the 3rd hour of the antenatal visit there was a significant association between overweight and obese BMI categories and higher TG ($p=0.02$) and lower HDL values ($p=0.02$) (Table 4a). Higher postpartum HDL was associated with lower BMI ($p<0.001$). Though few in our study population, mothers of Asian and other races were shown to have higher fasting ($p=0.05$) and 3hr triglyceride levels ($p=0.01$) at the baseline visit; there was no significant association between race and lipid values at the postpartum visit. Infertility and family history were not associated with maternal lipid values at any of the three possible timepoints.

The distribution of the four blood lipid outcomes by covariates as measured in cord blood samples obtained at delivery are presented in Table 5. Continuous and categorical age were not significantly associated with the lipid values, although a significant 0.50 decrease in HDL was observed for a unit increase in continuous BMI ($p=0.03$). Race was not associated with cord blood lipid values. Infertility was observed to be associated with higher TG levels ($p=0.003$), while family history of metabolic disease was significantly associated with higher LDL levels ($p=0.03$).

7.3. Multivariable Linear and Logistic Regression

Tables 6a-6d show both crude and adjusted group mean associations of blood lipids for women with GDM compared to women without GDM. At the fasting antenatal visit (Table 6a: Model 1), unadjusted linear regressions of TC, TG, LDL, and HDL levels, comparing GDM to non-GDM women, resulted in an overall lower lipid values for GDM women with total cholesterol values that were significantly lower by 27.74 mg/dL (217.35 mg/dL vs. 245.08

mg/dL, $p=0.002$) and LDL values that were lower by 22.1 mg/dL (115.15 mg/dL vs. 137.25 mg/dL, $p=0.003$). Adjusted group means for age, BMI, and gestational age at recruitment produced less pronounced lower values for GDM women compared to non-GDM women for TC (220.52 mg/dL vs. 242.53, $p=0.015$) and LDL (117.87 vs. 134.81 mg/dL, $p=0.027$).

Similarly, at the 3rd hour of the antenatal visit (Table 6b: Model 2), crude group means of TC, TG, LDL, and HDL lipid values, comparing GDM to non-GDM women, resulted in overall lower lipid values for GDM women with a significant total cholesterol values lower in GDM women by 24.29 mg/dL (209.59 vs. 233.88 mg/dL, $p=0.003$) LDL values by 20.17 mg/dL (111.85 vs. 132.02 mg/dL, $p=0.004$), and HDL values by 6.62 mg/dL (61.13 vs. 61.46 mg/dL, $p=0.014$). Group means adjusted for age, BMI, and gestational age at recruitment produced less pronounced values of TC in GDM women compared to non-GDM women that were lower by 20.11 mg/dL (211.81 vs. 231.91 mg/dL, $p=0.018$), LDL levels lower by 16.57 mg/dL (113.65 vs. 130.22 mg/dL, $p=0.024$), and HDL levels lower by 5.87 mg/dL (61.46 vs. 67.33 mg/dL, $p=0.042$).

Crude and adjusted group means for postpartum maternal lipids (Table 6c: Model 3) produced significantly higher values of TG, in GDM women compared to non-GDM women, by 37.43 mg/dL (126.88 vs. 89.45 mg/dL, $p=0.042$) and lower HDL values in GDM compared to non-GDM women by 10.86 mg/dL (49.79 vs. 60.65 mg/dL, $p=0.007$); however, the magnitude of TG and HDL difference was minimized and became insignificant after adjusting for age, BMI, and gestational age at time of delivery in weeks ($p=0.58$, and, $p=0.21$, respectively).

Crude group means for cord blood lipids (Table 6d: Model 4) at delivery, comparing GDM to non-GDM women, resulted in a significantly higher TG values for GDM women by

13.23 mg/dL (41.80 vs. 28.57 mg/dL, $p=0.043$), higher LDL values by 5.53 mg/dL (29.92 vs. 24.39 mg/dL, $p=0.047$), and lower HDL values by 5.28 mg/dL (24.76 vs. 30.04 mg/dL, $p=0.057$). Group means adjusted for age, BMI, and gestational age at time of delivery in weeks (Model 4: Adjusted_a) showed slightly higher TG values ($p=0.05$) and LDL values ($p=0.03$) for GDM women compared to non-GDM women. We were also interested in examining whether maternal fasting antenatal lipids had an intermediary effect upon cord blood lipids. However, adding the maternal fasting antenatal lipid fraction as a covariate to the adjusted cord blood lipids model (Model 4: Adjusted_b) did not significantly affect the regression coefficients.

As there was substantial missing data, in order to evaluate the possible influence of dropout as a bias, secondary crude (Table 7a) and adjusted (Table 7b) analyses were performed comparing those with complete data and those with missing data. Overall, no significant differences were observed for these analyses, though the regression coefficients for women with missing data ($n=82$) were more comparable to those of the whole cohort ($N=106$) than those for women who had complete data from all time points ($n=24$).

Crude and adjusted odds ratios for the odds of elevated lipid levels by GDM status are shown in Tables 8a-8c. In models of high fasting antenatal total cholesterol levels, compared to women without GDM, we observed 60% lower odds for high lipid levels in mothers with GDM (OR: 0.375, 95%CI: 0.153-0.922; p -value: 0.033) and ~66% lower odds for high lipid levels in maternal 3hr antenatal high total cholesterol levels for mothers with GDM (OR: 0.34, 95%CI: 0.13-0.87; p -value: 0.024). The remaining OR estimates were not statistically significant, both unadjusted as well as those adjusted for maternal age and BMI.

CHAPTER 8

DISCUSSION

In our study, we observed overall lower maternal blood lipids at 24-28 weeks gestation and higher cord blood lipids for those women with gestational diabetes; no association was observed between GDM status and maternal blood lipids postpartum. Differences in lipid levels between GDM and non-GDM mothers were small, of magnitudes comparable to previous studies, and of questionable clinical importance. Our findings are partially inconsistent with previous observations of higher total cholesterol, triglycerides, low-density lipoproteins, and lower high-density lipoproteins for maternal blood lipids (21, 22, 23, 24, 25, 26, 27, 28), and conflicting associations for cord blood lipids (24, 26, 27, 31).

Whereas we observed nonsignificantly higher postpartum TG levels in GDM women compared to non-GDM women (115.99 vs. 106.07 mg/dL, $p=0.58$) and lower HDL levels (52.11 vs. 56.70 mg/dL, $p=0.22$), Lauenborg et al. (8) found slightly higher TG: 115.14 vs. 88.57 mg/dL, $p<0.0005$, and slightly lower HDL: 54.14 vs. 58.0 mg/dL, $p<0.0005$ in prior GDM women compared to non-GDM women. Although Lauenborg et al.'s findings were deemed statistically significant; the clinical significance of their small magnitude findings is questionable. In previous studies maternal lipids were also sampled across various timepoints, ranging from during delivery to several years postpartum, which might reflect changes in the maternal lipid profile over time. Our study used Carpenter and Coustan criteria, diagnosed women for GDM with the OGTT at 24-28 weeks gestation, and obtained lipids between 6 weeks to 6 months postpartum, a much shorter follow-up period. Assuming women are more closely monitored and managed for hyperglycemia and dyslipidemia during pregnancy, and that the negative consequences of GDM worsen over time, it is possible that by assessing maternal lipids

during and soon after pregnancy the negative effects of GDM were underestimated in our study, biasing our results towards the null.

Consistent with our findings, Abou Ghalia et. al found lipid means±standard deviations with higher TC: 168 ± 55.80 vs. 150.57 ± 35.43 mg/dL; slightly higher HDL: 34.80 ± 13.92 vs. 29.0 ± 11.60 mg/dL, and slightly higher LDL: 31.32 ± 18.17 vs. 23.59 ± 10.05 mg/dL in cord blood samples of GDM women vs. non-GDM women. In contrast, Sobki et. al (27) and Marseille-Tremblay et al. (24) found overall lower cord blood lipid levels in GDM women. In both Sobki et. al and Marseille-Tremblay et al.'s studies, all the GDM women were either diet or insulin treated, whereas in Abou Ghalia et. al's study GDM women were combined with women with non-gestational (pre-pregnancy) diabetes for comparison with non-GDM women. Thus, some of the women in Abou Ghalia et al's study may have had an underlying risk for dyslipidemia because their first incidence of diabetes was prior to pregnancy, possibly biasing their results away from the null. Although differences in cord blood lipid levels in previous studies as well as our present study may have reached statistical significance, clinical significance is likely minor.

Our study had the strengths of administering OGTTs during the course of the study and access to lipid profiles from fasting and 3-hr blood samples obtained both during and after pregnancy. However, the incompatibility of our results with previous studies and the uncertainty about their clinical significance warrants an examination of possible limitations. We suspect that while multiple factors are at play, limited statistical power, methodology and timing of GDM classification, timing of lipid assessment, selection bias via non-random dropout, unmeasured confounders, and sampling variability may account for the differences between our and previous studies' results. One should consider that our result of nonsignificant lower lipids in women with GDM could represent a true association.

CHAPTER 9

STUDY LIMITATIONS

9.1. Nondifferential Misclassification

9.1.1. Nondifferential Misclassification of Exposure (Gestational Diabetes Mellitus)

Pregnant women who failed the initial GCT (glucose challenge test) were scheduled for the follow-up 3-hr GTT (glucose tolerance test), in which two or more samples of elevated glucose levels confirmed gestational diabetes mellitus (GDM) status. GDM status was determined based on results of these tests at 24-28 weeks gestation. GDM status may be misclassified due to error (e.g., due to laboratory error, unaccounted circadian variation, etc.), or because of change in status between the time it was determined and later in pregnancy, or the type of diagnostic criteria used. In the Lauenborg study, for instance, GDM assessment was attained by using risk factor based Dutch diagnostic criteria instead of the more widely used OGTT and Carpenter and Coustan criteria, possibly suggesting that pre-diabetic non-GDM may have been counted as GDM in other studies, biasing their results towards the null. However, we expect that this misclassification of GDM was minimized in our study because of the use OGTTs that were analyzed at the Wesson Women's Baystate Reference Laboratory, a CLIA (Clinical Laboratory Improvement Amendments) certified facility. This centralized processing of the samples greatly reduces variability in laboratory processes, which minimizes the likelihood and magnitude of this potential bias. Although the chances of GDM status changing after 7 months of pregnancy may be small, there is a possibility that a woman could develop GDM in the last stage of pregnancy. This misclassification of exposure would have biased our results toward the null because women who go on to develop GDM late in their pregnancy would be considered as non-GDM throughout the study. Given that we are unaware if and when women in our study were

monitored and managed for their GDM we cannot be sure of the extent of this potential misclassification.

9.1.2. Nondifferential Misclassification of Outcome I – Maternal Lipids

Variability in how the samples were collected, transferred, and analyzed could have resulted in lab error with measurement of maternal lipids values. This laboratory error could have therefore lead to misclassification of the outcome. Because laboratory personnel were not aware of GDM status for biospecimens used to determine lipid levels, we expect any such misclassification to be non-differential and therefore would have biased our results towards the null. However, we expect that this misclassification of maternal lipid levels was minimized because all lipid assessments were performed at the Wesson Women’s Baystate Reference Laboratory, a CLIA certified facility. This centralized processing of the samples greatly reduces variability in laboratory processes, which minimizes the likelihood and magnitude of this potential bias.

9.1.3. Nondifferential Misclassification of Outcome II- Cord Blood Lipids

Cord blood lipids were obtained from mothers shortly after delivery, via a 5 cc sample of blood. Given the immediate and precise collection of blood after delivery, misclassification of the outcome is unlikely. However, differences in how the samples were collected, transferred, and analyzed could lead to lab error and variability in assessing maternal lipids values. This laboratory error could therefore have led to nondifferential misclassification of this outcome, which may bias our results towards the null. However, we expect that this misclassification of high and low cord blood lipids was minimized because all lipid assessments were performed at the Wesson Women’s Baystate Reference Laboratory, a CLIA (Clinical Laboratory Improvement Amendments) certified facility. This centralized processing of the samples greatly reduces the likelihood and magnitude of this potential bias.

9.2. Selection Bias

Due to the prospective nature of this study, likelihood of selection bias related to recruitment is minimal, because exposure status (GDM) was determined before lipid levels were analyzed. However, selection bias may have occurred due to loss-to-follow-up in the pregnancy cohort, especially postpartum. The new stresses of motherhood can deter women from coming in for their postpartum lipid assessment, which could have either biased our results towards or away from the null, depending on the GDM status of those women who were lost. For example, if women lost to follow-up were more likely to have GDM and with higher levels of blood lipids, a selection bias would have occurred that would cause the results of this study to be biased towards the null. Also, we observed an earlier time of enrollment (i.e., lower gestational age at recruitment) among GDM mothers compared to non-GDM mothers. Perhaps, this slightly earlier recruitment and reduced loss-to-follow-up of GDM mothers reflects more intensive management of women with GDM, with the limitation of not knowing whether GDM mothers were managed for glucose or lipids during pregnancy. On the other hand, if women who did not have GDM and had elevated lipids were more likely to be lost to follow-up, perhaps due to the presumption of being healthy, this selection bias would have biased our results away from the null. In our study, women lost to follow-up were similar to those with complete (including postpartum) information and therefore any selection bias that occurred in this cohort due to loss to follow-up appears to be minimal.

9.3. Differential Misclassification

Women in this study were followed from the time of GDM ascertainment (24-28 weeks) until about 2 months after delivery. Although within 2 months after delivery was the ideal follow-up period for postpartum lipids, many women were recontacted up to 6-8 months after delivery. Thus, the postpartum lipid profiles of mothers are likely to have varied by their time of

follow-up. The laboratory technicians who analyzed these women's lipid profiles were blinded to the women's GDM status, so any differential treatment of the blood samples is unlikely. It is equally unlikely that the women's lipid levels were inaccurately or differentially assessed, as long as the same tests/methods and personnel were used to analyze all the samples. If different laboratory lipid assessment methods were utilized by different technicians across the samples, our results could potentially be biased in an unknown direction, depending on the nature of the method and technician. However, we expect minimal bias to have occurred due to the blinding of GDM status, standardized, CLIA-certified laboratory procedures, and adherence to lab protocol.

9.4. Confounding

We evaluated measured confounders by controlling for them in our analysis, using multivariable linear regression and stratification. Three measured confounders in our study were gestational age, maternal BMI, and gestational age at recruitment, and gestational age at delivery.

A number of additional potential confounders would have been beneficial to consider in our study. Information was not collected on whether the GDM mother was primiparous or had experienced GDM in prior pregnancies, whether the mother was managed for GDM or dyslipidemia during the study, the mothers' change in BMI over the course of the study, (clinical) history of familial hypercholesterolemia, and maternal diet both before and throughout the course of pregnancy.

It is useful to know whether this was the first GDM pregnancy experienced by the mother because it has been proven that prior experience of GDM is predictive of future GDM pregnancies and long-term cardiovascular risk (45). For instance, if most of our study participants had experienced GDM in a previous pregnancy, we are likely to have overestimated

the independent influence of GDM on lipids, as they are likely to be other underlying risk factors for dyslipidemia.

Treatment for GDM or dyslipidemia during pregnancy is an important covariate for which we lacked information. By not knowing which women may have been diet-controlled or medicated for their GDM we are prone to either under or overestimating the association between GDM and maternal and cord blood lipids. Also, the fact that maternal serum glucose values only provide an instantaneous snapshot of the mother's glucose control, in contrast to the HBA1C measure that captures information on glucose bound to hemoglobin RBCs for the past 3 months, suggests that we cannot speculate on the relative severity of GDM in our cohort.

Maternal change in BMI over the course of the study (most likely weight gain) positively confounds the relationship between GDM and lipid outcomes, which means that because we failed to adjust for BMI changes during pregnancy we may have overestimated the association between GDM and blood lipids. Clinical history of familial hypercholesterolemia would predispose a woman to have elevated lipid levels, especially if she was prediabetic prior to her pregnancy, and therefore likely to develop GDM. This means that because we failed to adjust for familial hypercholesterolemia, the association between GDM and blood lipids would be biased away from the null. Lastly, a high-fat maternal diet both before and throughout the course of the pregnancy is positively associated with GDM and future metabolic disease risk. By not adjusting for maternal diet over the course of the study our results are likely biased away from the null.

Given that GDM and abnormal lipids fall under an umbrella of metabolic risk factors, one must consider the possibility of reverse causality, or, a third common cause affecting the

association between GDM and lipid levels such that both exposure and outcome behave as a confounders of each other.

9.5. Cross Sectional Limitations

Simultaneous assessment of GDM and fasting/3hr maternal lipids at baseline creates several cross-sectional limitations for our study.

First, the fact that both GDM status and maternal lipids are assessed at the same point at baseline, makes it unclear whether GDM precedes or is caused by high maternal blood lipids. In other words, a reverse causal mechanism is possible where the high lipids resulting from other metabolic comorbidities and risk factors can lead to the development of gestational diabetes. It is equally possible for women who have GDM and elevated blood lipids to be put on medically prescribed dietary interventions that would underestimate the causal effects of GDM. Lastly, the survivor bias of women dying from severe levels of GDM exposure, and therefore being unavailable to participate in the study, would underestimate the association between GDM and maternal and cord blood lipid outcomes.

9.6. Generalizability

The results of this study may apply for other populations of pregnant women with singleton pregnancies and who are at risk for metabolic syndrome/disorders. Generalizability of these results is possible if the biologic mechanism that describes how GDM affects cardiovascular risk is similar across women of different socioeconomic status and ethnicity, among other factors.

CHAPTER 10

SIGNIFICANCE

Few studies have evaluated the changes that occur in TG, HDL, LDL, and total cholesterol levels during the diabetic pregnancy. Gestational diabetes is identified as a precursor of cardiovascular and metabolic disease in both mother and child (46). In our study, we observed small differences in blood lipids between women with GDM and women without GDM. However, it is possible that studies with ample power, greater sampling variability, information on diabetic risk prior to pregnancy, information management of GDM during pregnancy, and limited dropout could evidence more marked differences. Furthermore, the inexplicable results from this study indicate that more research is needed to understand both the physiology of pregnancy complications and how to prevent metabolically adverse in utero exposures. Studies with larger samples that passively collect extensive information on covariates via hospital records and physician visits and are assiduous to avoid loss-to-follow-up are required to effectively address this research question.

CHAPTER 11

HUMAN SUBJECTS

The Baystate GDM & Lipid Metabolism Study was approved by the Institutional Review Boards of the University of Massachusetts Amherst and Baystate Medical Center. All participants of the study were required to sign an informed consent form which affirmed their understanding of voluntary participation, with no impact on their medical care at Baystate.

All efforts are made to ensure that confidential information remains protected. Dataset identifiers have been removed in accordance with HIPAA law, and information is only shared with those involved in the study.

Minor discomfort and infection associated with providing repeated blood samples (rare) was offset by the benefits of subjects contributing to future research and being informed of their lipid level values. In order to boost the post-partum lipids follow-up visit, investigators offered each woman \$25 when she came to the clinic.

CHAPTER 12

PERMISSION TO ACCESS DATA

Collaborating Baystate health professionals and UMass Amherst researchers have provided authorization to access data for the Baystate GDM & Lipid Metabolism: 2007-2009 Study. Namely, Dr. Glenn Markenson, Chief of the Maternal and Fetal Medicine at Baystate Medical Center, and Dr. Lisa Chasan-Taber, dedicated researcher and Professor at UMass Amherst, have facilitated the exploration of this topic.

Both Institutional Review Boards of UMass and Baystate have approved this project in the past, providing relatively easy access to the data. Appropriate paperwork and formalities were filled out before the end of 2011 and submitted in accordance with Baystate requirements.

Figure 1: Study Design: Baystate GDM and Lipid Metabolism Study, 2007-2009

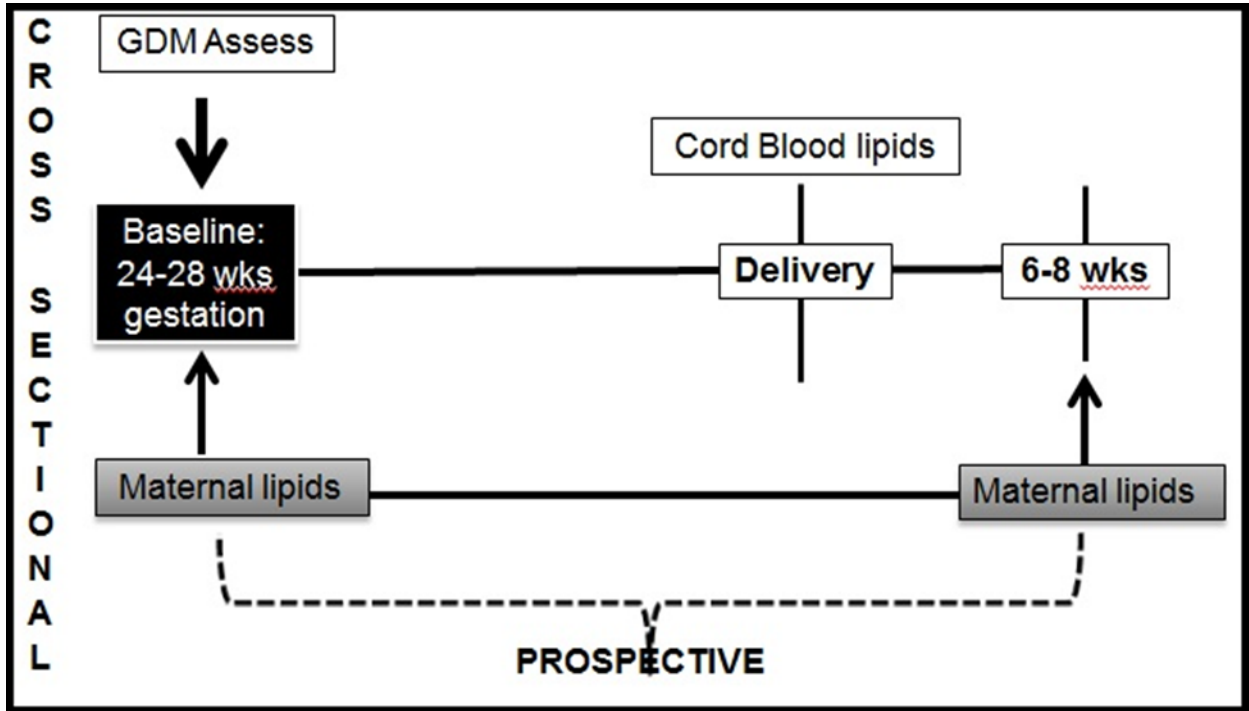


Fig.1. GDM and antenatal maternal lipids were assessed simultaneously at 24-28 weeks gestation, while postpartum lipids were assessed 6-8 weeks post-pregnancy. Cord blood lipids were obtained at delivery. The study is thus comprised of both cross-sectional and prospective components.

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