MATERNAL CHOLESTEROL LEVELS DURING PREGNANCY

By

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ABSTRACT

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BACKGROUND AND OBJECTIVES

Research suggests there may be a relationship between maternal cholesterol levels during pregnancy and both fetal growth and preterm birth. This research studied how changes in maternal cholesterol during pregnancy differ based on various maternal demographics. In addition, the relationships between changes in maternal cholesterol and fetal growth and changes in maternal cholesterol and gestational age at delivery were analyzed.

METHODS

The Archive for Research on Child Health (ARCH) database was utilized for this dissertation. Maternal cholesterol at two time points during pregnancy was obtained and changes in maternal cholesterol levels were calculated for 195 women.

RESULTS

First, second, and third trimester maternal cholesterol levels were higher in women with a pre-pregnancy body mass index less than 25 kg/m^2. No significant associations were found between changes in maternal cholesterol levels and fetal growth. Exploratory analyses found that maternal cholesterol levels at single time points during gestation were lower in pregnancies resulting in small for gestational age infants. Lastly, in women with a history of a previous preterm birth, changes in maternal cholesterol levels were found to be significantly associated with the corrected gestational age at delivery. Exploratory analyses found maternal cholesterol levels were higher in pregnancies resulting in preterm birth for all three trimesters.

CONCLUSION

Changes in maternal cholesterol levels may provide a more complete picture of cholesterol during pregnancy compared to maternal cholesterol levels at a single time point during pregnancy. This research found associations between both low and high maternal cholesterol levels and adverse birth outcomes indicating that cholesterol levels that are either too high or too low may increase risk of adverse birth outcomes.

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KEY TO SYMBOLS AND ABBREVIATIONS

%	Percent
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
1st	First
2nd	Second
AGA	Average for gestational age
ARCH	Archive for Research on Child Health
BMI	Body mass index
Cat	Categorical variable
CI	Confidence interval
Con	Continuous variable
dL	Deciliter
EDC	Expected date of confinement
GED	Graduate education degree
HDL-C	High density-lipoprotein cholesterol
in	inch
IUGR	Intrauterine growth restriction
kg	Kilogram
lbs	pounds

LGA	Large for gestational age
LDL-C	Low density-lipoprotein cholesterol
LMP	Last menstrual period
m^2	Square meter
mg	Milligram
mPTB	Medically indicated preterm birth
MSU	Michigan State University
μl	Microliter
n	Sample size
NHANES	National Health and Nutrition Examination Surveys
OR	Odds ratio
PROM	Premature rupture of membranes
SGA	Small for gestational age
sPTB	Spontaneous preterm birth
TC	Total cholesterol
vLDL-C	Very low density-lipoprotein cholesterol

CHAPTER ONE
INTRODUCTION

INTRODUCTION

A woman's body undergoes many changes during pregnancy, including changes in

maternal blood cholesterol levels. Total cholesterol (TC) and low density-lipoprotein cholesterol

(LDL-C) levels have been shown to significantly increase throughout pregnancy and peak in the

third trimester [1]. Results from a systematic literature review conducted in 2011 revealed an

average increase of 46% in TC and 60% in LDL-C from the first to the third pregnancy trimester,

whereas high density-lipoprotein cholesterol (HDL-C) levels followed a different pattern,

increasing 18% from the first to the second trimester, when they peaked [1]. If third trimester TC

and LDL-C levels were observed in the non-pregnant state, results would prompt physicians to

take therapeutic action to minimize adverse health outcomes associated with elevated cholesterol

levels, including ischemic stroke and coronary artery disease. To date, there are no guidelines for

maternal cholesterol levels during gestation.

Although increases in maternal cholesterol levels are thought to be an adaptive change

necessary for proper fetal development, there is a body of literature suggesting negative health

outcomes for the fetus associated with maternal cholesterol during gestation. For example, in one

post-mortem examination, relative to women with normocholesterolemic pregnancies, women

with pre-pregnancy or pregnancy-induced hypercholesterolemia had greater fatty streak

formation and plaque buildup identified in the arteries of 60 fetuses that were stillborn or born

premature and expired within hours of birth (p-value < 0.05) [2]. The average TC level of

normocholesterolemic women at the time of delivery was 175 mg/dL. The average TC level of

women with pre-pregnancy hypercholesterolemia was 385mg/dL and the average TC level for

women with pregnancy-induced hypercholesterolemia was 325mg/dL. In another study assessing children ages one – 13, those born to women with pregnancy-induced hypercholesterolemia were shown to have fatty lesions in their aortic arches that progressed faster than those born to normocholesterolemic women (p-value < 0.0001) [3].

On the other hand, concern has also been expressed about failure of cholesterol to rise during pregnancy, in particular in relation to fetal growth restriction and preterm delivery. A full review of the literature is available in subsequent chapters of this dissertation. Significant associations between fetal growth restriction and low maternal TC, LDL-C, and/or HDL-C at a single time point during gestation have been reported. A cohort study in South Carolina found women with low TC, TC less than 159 mg/dL, in the second trimester gave birth to smaller infants, weighing on average 147 grams less than infants born to women with normal TC levels (p-value = 0.0006) [4]. However, a study in Southwest Nigeria studied maternal cholesterol levels between 14 and 20 weeks gestation in 287 women and found that women with high second trimester TC levels were almost eight times more likely to deliver a low birthweight baby compared to their normal cholesterol counterparts (87.5% versus 10.5%, p-value = 0.019) [5].

Associations between maternal cholesterol and preterm delivery have been found in women with elevated cholesterol levels as well as in women with low cholesterol levels, suggesting a u-shaped relationship. When comparing 290 White women, a significant relationship was found between low maternal cholesterol and preterm birth (risk ratio = 0.10, 95% Confidence interval (CI): 0.01, 0.77) [6]. A second study found an association between high maternal TC and preterm birth (odds ratio = 2.0, 95% CI: 1.0, 4.2) [7]. A third study found TC levels in the lowest 10th percentile for White women were associated with a significantly increased risk of preterm delivery (odds ratio = 5.63, 95% CI: 2.58, 12.3) [4]. This study also

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found TC levels in the highest 90th percentile for Black women were associated with an increased risk of preterm delivery, although those results were not statistically significant (odds ratio = 2.6, 95% CI: 0.84, 8.0) [4].

A 2010 review of the literature largely found information on maternal cholesterol at a single time point during pregnancy as it relates to maternal post-partum health and birth outcomes [1]. Few studies have published information exploring the rates of change in maternal cholesterol levels during gestation and birth outcomes. Studying the change in maternal cholesterol is important because cholesterol levels tend to rise in women during pregnancy, including those with elevated pre-pregnancy cholesterol. Looking at cholesterol levels at a single time point during pregnancy does not take into consideration women who have elevated baseline measures and is unable to determine if a rate of change is more indicative of an adverse health outcome compared to a single value. With cholesterol measurements at two time points during pregnancy, the proposed dissertation research will focus on how the rate of change of maternal cholesterol levels, LDL-C, HDL-C, and TC, impacts fetal outcomes.

A pre-existing data archive, Archive for Research on Child Health (ARCH), contains maternal serum samples from two time points during pregnancy, a maternal questionnaire, and the birth certificate for the infant from the corresponding pregnancy. ARCH data will be utilized for this study to explore the relationship between maternal cholesterol during pregnancy and various maternal characteristics including maternal ethnicity and race, maternal age, pre-pregnancy body mass index (BMI), postpartum BMI, history of high cholesterol, and parity. Building upon the descriptive results, the association between changes in maternal cholesterol levels during pregnancy and fetal growth, as well as preterm delivery will be analyzed.

RESEARCH AIMS

Aim I

Conduct a descriptive analysis to study the changes in maternal cholesterol levels (LDL-C, HDL-C, and TC) in the ARCH study population, stratifying on maternal ethnicity, maternal race, maternal age, parity, pre-pregnancy BMI, postpartum BMI, and maternal history of clinically diagnosed high cholesterol.

Hypothesis I

Ia. Maternal age and parity will not have a significant impact on the changes observed in maternal cholesterol (LDL-C, HDL-C, and TC) during gestation.

Ib. Maternal ethnicity, race, pre-pregnancy BMI, postpartum BMI, and maternal history of clinically diagnosed high cholesterol will significantly impact the changes observed in maternal cholesterol (LDL-C, HDL-C, and TC) levels.

Aim II

Study the relationship between changes in maternal cholesterol levels (LDL-C, HDL-C, and TC) and fetal growth, both as continuous and categorical variables.

Hypothesis II

IIa. Women with rates of changes in LDL-C and/or TC in the study population's lowest quartile will give birth to infants with decreased measures of fetal growth.

IIb. Women with rates of change in LDL-C and/or TC in the study population's highest quartile will give birth to infants with increased measures of fetal growth.

IIc. No association between maternal HDL-C and fetal growth will be identified.

Aim III

Analyze the association between changes in maternal cholesterol levels (LDL-C, HDL-C, and TC) and gestational age at delivery as a continuous variable.

Hypothesis III

IIIa. Women with rates of change in LDL-C and/or TC in the study population's lowest quartile will be at an increased risk of delivering an infant with a smaller gestational age.

IIIb. Women with rates of change in LDL-C and/or TC in the study population's highest quartile will be at an increased risk of delivering an infant with a smaller gestational age.

IIIc. No association between changes in maternal HDL-C and gestational age will be identified.

ARRANGEMENT OF THE DISSERTATION

This dissertation contains five chapters, beginning with this introduction. Chapter two includes a review of the literature addressing maternal cholesterol levels during gestation. Subsequently, this dissertation is broken into three additional chapters, each pertaining to an individual research aim. Chapter three includes a description of the study design used for this dissertation and focuses on trends in maternal cholesterol levels during gestation in this population while taking various maternal demographic factors into consideration. Chapter four focuses on the relationship between changes in maternal cholesterol levels during gestation and fetal growth. Chapter five describes the relationship between changes in maternal cholesterol levels during gestation and gestational age at delivery. Each chapter is concluded with a list of corresponding references.

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CHAPTER TWO
LITERATURE REVIEW: MATERNAL CHOLESTEROL DURING PREGNANCY

INTRODUCTION

According to the National Health and Nutrition Examination Surveys (NHANES), from 1999 – 2010 the prevalence of high risk low density-lipoprotein cholesterol (LDL-C) levels in the non-pregnant adult population has stayed consistent at approximately 35% [1]. High risk levels of LDL-C are dependent on multiple risk factors and are categorized based on the number of risk factors present. Risk factors include HDL-C levels less than 60 mg/dL, family history of coronary heart disease, hypertension, cigarette use, and older age (greater than or equal to 55 years old in women and greater than or equal to 45 in men) [1]. HDL-C levels greater than or equal to 60 mg/dL are considered protective and offset one of the aforementioned risk factors [1]. For example, high risk LDL-C levels in an individual with zero or one risk factor are greater than or equal to 160 mg/dL [1]. If someone has a history of coronary heart disease and diabetes, LDL-C levels are considered high risk if they are greater than or equal to 70 mg/dL [1]. Associations between elevated LDL-C levels in the non-pregnant state and cardiovascular disease have been well studied, published, and disseminated to the general public for health education purposes [1].

In pregnancy, literature suggests the majority of women experience a rise in total cholesterol (TC), LDL-C, and high density-lipoprotein cholesterol (HDL-C). These elevated TC and LDL-C levels, which may perhaps be beneficial in pregnancy, would classify pregnant women as being at an increased risk for cardiovascular disease if observed in the non-pregnant state [2]. Aside from research purposes, maternal cholesterol levels are not routinely measured during pregnancy. The aim of this chapter is to conduct a detailed literature review focusing on

maternal cholesterol levels during gestation, the association between maternal demographics and maternal cholesterol levels, and previously published associations between maternal cholesterol levels and fetal growth and preterm birth.

CHOLESTEROL

Cholesterol is a waxy, hydrophobic lipid molecule that is a required component of cell membranes [3 – 5]. Cholesterol is essential for cell development and structure, cell signaling and communication, and is a precursor for the synthesis of bile acids and sex hormones including estrogen, progesterone, and testosterone [3 – 10]. Research suggests that 75% of cholesterol within the body is produced de novo; the remaining 25% is from dietary sources [3]. Three major lipoprotein cholesterol classes are HDL-C, LDL-C, and very low density-lipoprotein cholesterol (VLDL-C) [6]. TC is an aggregate cholesterol value that sums HDL-C, LDL-C, and VLDL-C. This dissertation will focus on TC, LDL-C, and HDL-C.

LDL-C molecules are small in size and can be deposited in the artery wall causing plaque buildup and ultimately increasing the risk of cardiovascular disease [3]. Plaque buildup is often greatest at sites of damage within the arteries [3]. The risk of artery wall damage is increased by aging, high blood pressure, diabetes, and smoking [3]. This plaque buildup can partially or fully clog arteries within the body. If arteries supplying the heart are clogged and become occluded, myocardial infarction can result. If arteries supplying the brain are clogged and become occluded, a stroke can result. HDL-C molecules are larger in size relative to LDL-C molecules and are thought to remove cholesterol from artery walls, thereby potentially reducing the risk of occlusion and cardiovascular disease [3]. Increased levels of LDL-C (greater than 160 mg/dL) and decreased levels of HDL-C (less than 40 mg/dL) have been associated with increased risk of

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coronary heart disease [3, 11]. Guidelines were developed in 1985 by the National Cholesterol Education Program for optimum cholesterol levels and levels categorized as conveying risk for adverse health outcomes (Table 2.1) [6]. These guidelines do not provide information as to whether or not they are applicable to pregnant women. The guidelines do state that the measurement of any lipid is preferably performed at the patient's baseline state, which includes not being pregnant [6]. Specific guidelines for maternal cholesterol levels during pregnancy have not yet been developed.

CHOLESTEROL DURING PREGNANCY

Sex hormones, which cholesterol is a precursor for, are vital for early fetal development, fetal growth, and maintaining the early pregnancy [4, 5, 12]. From animal and cellular tissue studies, it appears that cholesterol is essential during implantation and gestation for the fetus as it maintains the integrity and structure of cell membranes, activates key patterning proteins such as the sonic hedgehog proteins and nuclear receptors, and is a precursor for signaling lipids [4, 7, 8, 12]. Sonic hedgehog proteins play a role in development of the brain, limbs, lungs, heart, and urogenital system [4]. Nuclear receptors are important in the development of many organ systems [4]. During gestation the fetus requires substantial amounts of cholesterol and maternal cholesterol contributes substantially to fetal cholesterol by passage through the placenta [10, 12].

The fetus begins to synthesize its own cholesterol in late pregnancy [10, 13, 14]. The exact time frame when the fetus transitions from a maternal source of cholesterol to de novo cholesterol synthesis is unknown, although some research suggests this transition occurs around six months gestation [5, 14]. It appears that the fetus must make cholesterol during gestation for appropriate development. There are seven known defects in the fetus's cholesterol biosynthetic pathway that interrupt cholesterol synthesis and result in congenital malformations [8, 12]. Six of these seven

defects are rare and often fatal. The seventh defect leads to Smith-Lemli-Opitz syndrome. Infants with this syndrome do not synthesize cholesterol, yet are born with very low amounts of cholesterol in their tissues and blood, presumed to be of maternal origin [8]. Infants born with Smith-Lemli Opitz syndrome can be used to support the findings that maternal cholesterol reaches the developing fetus during gestation. It is thought that maternal cholesterol is taken up by the placenta and crosses the placental barrier to facilitate fetal development and growth [4, 7 – 10, 15]. The extent of maternal cholesterol transport across the placental barrier is likely to vary throughout pregnancy as a result of the physiological and temporal changes that occur with the placenta during pregnancy [9]. Extreme increases in maternal cholesterol during gestation, as with pregnancy induced hypercholesterolemia, have been shown to increase the risk of plaque buildup in the arteries of children and fetuses. In a Naples, Italy post-mortem study of 60 stillborn fetuses or infants born premature who had expired within hours of birth, infants born to women with hypercholesterolemia, both pre-pregnancy and pregnancy induced, had greater fatty streak formation and plaque buildup compared to infants born to normocholesterolemic women [14]. In the pre-pregnancy hypercholesterolemic group, 78% of fetal arteries had plaque buildup, and 76% of fetal arteries in the pregnancy induced hypercholesterolemic group had plaque buildup whereas only 63% of fetal arteries in the normocholesterolemic group had plaque buildup ($p < 0.05$) [14]. Also in Naples, Italy, children, ages one – 13, born to women with hypercholesterolemia were shown to have fatty lesions in their aortic arches that progressed faster than those born to normocholesterolemic women ($p < 0.0001$) [16]. The rate of lesion progression was not associated with hypercholesterolemia in the children as all 156 children studied were normocholesterolemic, regardless of if their mother was hypercholesterolemic or normocholesterolemic during pregnancy [16].

TC, LDL-C, and HDL-C increase during pregnancy. It is suggested that these changes are necessary for the appropriate development of the fetus, yet it remains unclear in the literature if and how these increased levels adversely impact or enhance maternal and fetal health. The following text provides additional details regarding the increases seen in TC, LDL-C, and HDL-C in pregnancy.

Total Cholesterol

During pregnancy, TC levels begin to rise late in the first trimester and early in the second trimester. On average, TC levels peak during the third trimester. It is not uncommon to have TC levels exceed 240 mg/dL during the final weeks of pregnancy [2]. A literature review of 21 studies, longitudinal and cross-sectional, showed an average increase of 14.5% from second to third trimester and an increase of 46% from first trimester to third trimester in TC (figure 2.1) [2, 17 – 37]. First to third trimester changes ranged across studies from a 19% increase in a 1995 longitudinal study of 35 women to a 62% increase in a 2002 cross-sectional study of 64 first trimester, 48 second trimester, and 67 third trimester women [29, 32]. A 2016 study of 137 normal weight Brazilian women reported an average rate of change for TC of 43% from first to third trimester [38]. Rates of increase differ slightly across studies and populations, but have been reported in all studies, regardless of geographic location, and maternal demographics. After pregnancy, TC levels return back to pre-pregnancy levels at a much slower rate than the rate of increase during pregnancy (figure 2.2) [2, 18, 19, 22, 23, 28, 30,32, 35, 36].

Low Density Lipoprotein Cholesterol

LDL-C follows a similar pattern as TC during pregnancy and peaks in the third trimester (figure 2.3). The average rate of increase from the first to third trimester for LDL-C is greater than the rate seen in TC. In 13 studies, the average increase for LDL-C from first to third

trimester was 59% [2, 19 – 21, 23, 27 – 29, 32 – 37]. Eight of the 13 studies reported an increase greater than 60%, the remaining five found a rate of increase less than or equal to 52%. Results did not significantly vary between longitudinal and cross-sectional studies. LDL-C levels increased, on average, 19% from second to third trimester and 35% from first to second trimester [2]. Average LDL-C levels in the last five to ten weeks of pregnancy are usually greater than 160 mg/dL [2].

Research suggests that LDL-C levels return to pre-pregnancy levels within one year postpartum. A longitudinal study following 19 women through gestation found LDL-C levels at four weeks post-partum, 153 mg/dL, to be significantly higher than LDL-C levels at 10 weeks gestation, 89 mg/dL [36]. A cross-sectional study found that LDL-C levels at six weeks post-partum in 32 women were still significantly higher (p < 0.001) than first trimester LDL-C levels in 64 women, 107.6 mg/dL compared to 84 mg/dL [32]. LDL-C levels for 11 women during the post-lactation period, here defined as 60 days after the end of the lactation period when spontaneous menses resumed, were significantly higher than first trimester levels, but were not statistically different from second trimester levels [19]. In this study, the time since delivery varied from two to six months, depending on how long a woman breastfed. Because LDL-C declines during the post-partum period, this study would have been more informative if it broke down the post-lactation period into smaller time frames. Overall, LDL-C levels begin to trend down in the post-partum period, yet additional information is needed to identify the role breastfeeding has in post-partum LDL-C levels and the post-partum time frame when LDL-C levels reach pre-pregnancy levels.

High Density Lipoprotein Cholesterol

HDL-C follows a diffrent pattern than TC and LDL-C, and peaks in the second trimester (figure 2.4). HDL-C levels begin to return to pre-pregnancy values during the final weeks of the second trimester. An 18% increase is observed, on average, from first to second trimester and an overall first to third trimester increase of 10% in 15 studies [2, 18 – 21, 23, 26 – 29, 32 – 37]. From second to third trimester, in 16 studies, there is a 7% decrease in HDL-C [2, 18 – 21, 23, 24, 26 – 29, 32 – 37]. Results for HDL-C, unlike TC and LDL-C, varied significantly between studies. Of the 15 studies reviewed, eight found an increase in HDL-C from first to third trimester between 0% – 10%, five found a rate of increase greater than 10%, and two found a decrease during this time frame [2]. Winkler et al. reported HDL-C levels to be highest in the first trimester and continually decrease throughout the duration of pregnancy for an overall decrease of 10% [27]. By contrast, three studies found HDL-C to rise continually during pregnancy and peak in the third trimester [28, 29, 36]. The changes observed in maternal HDL-C during pregnancy are smaller relative to the changes observed in LDL-C and TC levels. Given these smaller changes, some of the observed variation in study results is to be expected. Because HDL-C levels follow a different pattern, often increasing and decreasing within the same trimester, additional research is needed focusing specifically on the week of gestation rather than trimester of specimen collection and the observed discrepancies in reported trends.

MATERNAL CHARACTERISTICS AND CHOLESTEROL LEVELS

Literature suggests that during pregnancy maternal cholesterol levels increase in all populations and follow similar patterns regardless of pre-pregnancy cholesterol levels and geographic location [12, 17 – 36, 39]. However, maternal demographic factors such as race,

body mass index (BMI), and diet have been shown to be associated with varying maternal lipid levels during pregnancy.

Ethnicity and Race

In the non-pregnant population, race has little to no significant impact on cholesterol levels, as Blacks and Whites in the United States tend to have comparable cholesterol levels when all other variables are equal [6]. Most studies looking at cholesterol levels during pregnancy do not stratify on race or ethnicity, however one study of only 30 women from the United States, 15 Black and 15 White, found significantly lowers levels of TC (p-value = 0.04) and LDL-C (p-value = 0.04) in Black women when compared to White women throughout pregnancy [40]. This study did not find a significant difference in HDL-C levels between Black and White women during pregnancy [40].

A 2010 prospective community-based study, Amsterdam Born Children and their Development, focused on variation in maternal cholesterol levels during gestation by ethnicity. After adjusting for gestational age of specimen collection, analysis showed significantly lower TC levels in 235 African-Caribbean women compared to 2262 Dutch women, 192 mg/dL compared to 199 mg/dL (beta coefficient = -0.19, p-value ≤ 0.001) [41]. 54 Ghanaian women had significantly lower TC levels compared to Dutch women, 180 mg/dL compared to 199 mg/dL (beta coefficient = -0.51, p- value ≤ 0.001) as did 245 Moroccan women compared to Dutch women, 193 mg/dL compared to 199 mg/dL (beta coefficient = -0.15, p-value ≤ 0.01) [41]. TC levels in 61 women of Surinam-Hindustani ethnicity and 168 women of Turkish ethnicity compared to Dutch women were not significantly different. This data on maternal cholesterol levels in women of African ancestry compared to maternal cholesterol levels in 2262

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Dutch women adds to what little is currently known regarding maternal cholesterol levels during gestation and maternal race.

Adding to what has been found in previous studies; a study of 306 term births in New Jersey explored racial and ethnic differences in maternal cholesterol levels during the early second trimester. This study found that African American women with a term birth had higher HDL-C levels than Hispanic and non-Hispanic Caucasian women combined (50 mg/dL compared to 45 mg/dL, p-value < 0.001) [42]. TC and LDL-C levels were lower in the African American women compared to the Hispanic and non-Hispanic Caucasian women; however these findings were not statistically significant. On average, African Americans had second trimester TC levels of 167 mg/dL compared to 170 mg/dL in the Hispanic and non-Hispanic Caucasian women [42]. Average second trimester LDL-C levels in African American women were 120 mg/dL compared to 123 mg/dL in the Hispanic and non-Hispanic Caucasian women [42]. Table 2.2 summarizes the findings from these three studies.

BMI

The GROW study of 142 pregnant, affluent, White women found significantly lower rates of change in maternal TC (p-value = 0.01) and LDL-C (p-value < 0.001) levels in an overweight and obese group when compared to women in the normal weight group [39]. In the overweight and obese population, TC increased 46% from first to third trimester, while an increase of 60% was observed in the normal weight population. For LDL-C, a 50% increase was observed in the overweight and obese weight group and a 77% increase was observed in the normal weight group [39]. During the first half of pregnancy, overweight and obese women, on average, had higher absolute levels of both TC and LDL-C compared to the normal weight population. Between 20 − 24 weeks gestation, TC and LDL-C levels in the normal weight

women began to surpass cholesterol levels in the overweight and obese group. At 40 weeks gestation, normal weight women had higher TC and LDL-C levels, on average, compared to the overweight and obese weight group. No significant difference was found between BMI and HDL-C (p-value > 0.1). Despite studying 142 women during pregnancy, 58 normal weight and 84 overweight and obese, the external generalizability of the GROW study is limited. The majority of women included were college educated White women with 44% having annual household incomes greater than $80,000 per year [39].

Similar, statistically significant, results were found in a younger, more racially diverse population in Pittsburgh, Pennsylvania. 135 overweight/obese women were found to have significantly higher TC levels during the first trimester of pregnancy compared to 90 normal weight women, 161 mg/dL compared to 149 mg/dL respectively (p-value < 0.01) [43]. In addition, significantly higher first trimester LDL-C levels were found in overweight and obese women compared to normal weight women, 80 mg/dL compared to 73 mg/dL respectively (p-value < 0.01) [43]. Late second trimester TC levels in the overweight and obese group were lower than those levels in the normal weight group, 176 mg/dL compared to 184 mg/dL (p-value = 0.05). LDL-C levels in the late second trimester were also lower in the overweight and obese group compared to the normal weight group, 111 mg/dL compared to 116 mg/dL, although this difference was not statistically significant (p-value = 0.17) [43]. Normal weight women had significantly greater rates of change in TC (p-value < 0.01) and LDL-C (p-value < 0.01) levels from first to second trimester relative to the overweight and obese women [43].

The demographics of the women included in this study differed from the demographics of the women in the GROW study. 77% of women studied had a yearly income of less than $25,000 and the average number of years of education was 12 [43]. Despite two different

populations, both the GROW study and this study found overweight and obese women to have higher first trimester and lower second trimester TC and LDL-C levels compared to a normal weight group of women. Both studies also found that the rate of increase in TC and LDL-C levels in the overweight and obese was smaller than the rate of increase in TC and LDL-C for normal weight women. Unlike the GROW study, this Pennsylvania based study found HDL-C levels to be significantly lower in the overweight and obese women compared to the normal weight women in the first trimester only, 44 mg/dL compared to 48 mg/dL (p-value < 0.01) [43]. No significant differences were found in HDL-C levels in the second trimester for either weight group [43].

A third study of 137 normal weight women, 60 overweight women (BMI 25 – 29.9 kg/m^2), and 32 obese women (BMI ≥ 30 kg/m^2) from Brazil reported similar trends in TC and LDL-C as those found in women in the United States [38]. This longitudinal study had cholesterol data at three time points during gestation, adding to the understanding of how maternal cholesterol levels change during pregnancy when stratified on BMI. Women in the overweight and obese groups had higher first trimester TC levels compared to women in the normal weight group, 166 mg/dL, 168 mg/dL, and 158 mg/dL, respectively [38]. LDL-C levels were also higher in the overweight and obese groups compared to the normal weight group, 101 mg/dL, 102 mg/dL, and 94 mg/dL, respectively [38]. Third trimester TC and HDL-C levels did not significantly differ between the three weight groups. For LDL-C, the average third trimester LDL-C in the normal weight group was 139 mg/dL [38]. In the overweight and obese groups, average third trimester LDL-C levels were 135 mg/dL and 133 mg/dL [38]. The rate of change in TC from first to third trimester was smaller in overweight, 35% increase, and obese, 34% increase, women compared to the rate of increase in the normal weight group, 43% increase [38].

For LDL-C, overweight women had a 34% increase in LDL-C from first to third trimester, while obese women had a 30% increase and normal weight women had the largest increase in LDL-C at 48% [38]. Despite differing definitions of overweight and obese and characteristically variable groups of women, current research suggests changes in maternal cholesterol levels differ based on maternal pre-pregnancy BMI. Table 2.3 summarizes these findings.

Maternal Diet

Maternal diet during pregnancy has been shown to have some relationship with changes observed in maternal cholesterol levels during pregnancy. In 1981, 12 American women, average age 20 years old, were voluntarily admitted to a clinical research center for the second and third trimesters of pregnancy. These women were fed closely monitored metabolic diets, with either 600 mg/day of cholesterol or 0 mg/day of cholesterol, for periods of four to nine weeks [44]. Each of the 12 women underwent two periods of high cholesterol diets and two periods of cholesterol-free diets. It was found that by completely eliminating cholesterol from a pregnancy, diet can significantly reduce the rates of increase in cholesterol [44]. A 12% decrease (p-value <0.005) in maternal TC levels was observed from the first high cholesterol diet (207 mg/dL) to the first cholesterol free diet (183 mg/dL) [44]. A 19% increase was observed as women switched from the first cholesterol free diet (187 mg/dL) to the second high cholesterol diet (223 mg/dL) (p-value < 0.001) and a 8% decrease was observed in TC levels as women switched from the second high cholesterol diet (238 mg/dL) to the second cholesterol free diet (218 mg/dL) (p-value < 0.05) [44]. The results of this study, which did not control for gestational age at specimen collection, suggest maternal diet during gestation plays a significant role in the changes, both increases and decreases, in maternal cholesterol. Although the population used to

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study the dietary effects on changes in maternal cholesterol was young and non-diverse, the results of this study should be further explored.

In a Norwegian study of 290 White women, women were randomly assigned to follow their usual diet or to consume a diet low in dietary cholesterol and saturated fats. Women consuming a diet low in cholesterol during pregnancy were found to have lower TC, LDL-C, and HDL-C, levels at three time points during pregnancy (n=127) relative to women who consumed a normal diet (n=142) [24]. When looking at rates of change in TC from the second trimester to 36 weeks gestation, women with the cholesterol lowering diet had a 21.5% increase in TC levels and those in the control group had a 25.4% increase (mean difference= 3.9%, 95% Confidence Interval (CI) = 0.4%, 7.3%, p-value = 0.03) [24]. LDL-C levels increased by 28% in the intervention group and 34% in the control group (mean difference= 6.3%, 95% CI= 0.4%, 12.3%, p-value = 0.04) [24]. Although statistically significant, the overall effects of the dietary intervention on maternal cholesterol levels were small.

Both the American and Norwegian studies, with different mechanisms to control and monitor cholesterol intake during pregnancy, found women who consume fewer grams of cholesterol on a daily basis during pregnancy have a reduced rate of cholesterol increase. The Norwegian study also noted a reduced rate of preterm delivery in the subset of women who consumed fewer grams of cholesterol, a finding to be elaborated upon in a subsequent section of this review.

A third study of 199 women from Rio de Janeiro, Brazil looked at the relationship between pre-pregnancy dietary patterns and maternal cholesterol levels at three time points during pregnancy [45]. The women in this study were from families with a low-income and low-education level. This study found that women who had high adherence to a pre-pregnancy

dietary pattern of vegetables and dairy had higher levels of third trimester HDL-C, 57.1 mg/dL, compared to women with low adherence to the vegetable and dairy pattern, 52.4 mg/dL (p-value = 0.026) [45]. There was no significant relationship found between maternal cholesterol levels and those women in the fast food and candies pattern as well as in those women in the beans, bread, and fat pattern [45]. Table 2.4 summarizes the findings from the three studies.

Maternal Age

Maternal age does not seem to significantly influence cholesterol rates of change during pregnancy. Although no published literature was found looking at maternal cholesterol levels during gestation stratified on maternal age, a single studying looking specifically at maternal cholesterol during pregnancy in women over the age of 35 was found [46]. 53 pregnant women, all over the age of 35, had first and second trimester TC (168 mg/dL and 193 mg/dL), LDL-C (89 mg/dL and 99 mg/dL), and HDL-C (59 mg/dL and 59 mg/dL) levels comparable to levels found in younger women from other research studies [2, 46]. TC levels increased 15% from first to second trimester, LDL-C levels increased 11% from first to second trimester, and HDL-C levels did not change from first to second trimester. Additional research is needed looking at maternal cholesterol levels during pregnancy stratified on maternal age.

Parity

It is unclear if parity has an impact on rates of change in maternal cholesterol levels observed during pregnancy. During non-pregnant periods, TC levels have generally been found to be lower in nulliparous women than multiparous women, although this finding is not consistent [47 – 48]. If parity had long lasting effects on maternal cholesterol levels, we would expect to see a positive association between parity and health conditions associated with cholesterol levels (for example cardiovascular disease). Literature shows conflicting results regarding a relationship between parity and maternal cardiovascular disease [48 – 50]. A 1987 study that followed

women aged 30 years – 55 years for six years, from 1976 through 1982, suggested nulliparous women had a slightly higher, non-significant, risk of cardiovascular disease (rate ratio = 1.2, 95% CI= 0.8, 1.8), compared to parous women during this time frame [50]. In 3,828 British women between 60 and 79 years of age who reported having at least two live births, a 30% increased risk of coronary heart disease was found when adjusting for current age (OR = 1.3, 95% CI= 1.17, 1.44) [49]. Additionally, the Rotterdam Study of women from the Netherlands also found a positive association between parity and risk of cardiovascular disease. In the Rotterdam study, parous women had a 36% greater risk for cardiovascular disease relative to nulliparous women (95% CI= 1.09, 1.71) [48]. Women with a parity greater than or equal to four were found to have a 64% increased risk of cardiovascular disease relative to nulliparous women (95% CI= 1.19, 2.27) [48]. Research also suggests the number of children a woman delivers is associated with lower levels of HDL-C later in life [48 – 49]. For each live birth, HDL-C levels later in life have been shown to decrease by 0.8 mg/dL (p-value = 0.001), but no statistically significant association was identified for LDL-C (p-value = 0.37) [49]. TC levels later in life have also been found to have an inverse association with parity greater than two (p-value <0.001), although additional research within more racially and geographically diverse populations is needed to support these findings [48]. These results also need to be interpreted with caution as the outcome of interest is being measured decades after the exposure of interest. In addition, maternal covariates such as socioeconomic status, age at menarche, and age at menopause, which are all associated with both parity and risk of cardiovascular disease should be controlled for when studying the relationship between parity and maternal cholesterol levels [49].

It is unclear if a woman's changing cholesterol levels during gestation will follow a similar pattern for subsequent pregnancies or if the pattern is unique to each pregnancy. No literature was found on this topic. Although a challenge to follow women through multiple pregnancies, this information would be valuable especially when comparing multiple pregnancies with varying fetal outcomes.

Maternal Hypercholesterolemia

Women with familial hypercholesterolemia experience an increase in cholesterol during pregnancy at rates similar to women with normal cholesterol levels prior to pregnancy. Literature that stratifies the data based on familial hypercholesterolemia suggests that both groups, those with hypercholesterolemia and those without, on average, experience similar rates of increase in TC from second to third trimester, approximately $20\% - 30\%$ [44, 51]. One study showed the average third trimester TC level in a group of 19 women with familial hypercholesterolemia was 449 mg/dL, significantly greater ($p < 0.05$) than their second trimester TC levels, which averaged 352 mg/dL [51]. Despite these women having high cholesterol levels prior to pregnancy, their cholesterol levels continue to rise throughout gestation. This rise in maternal cholesterol, even in women with elevated pre-pregnancy cholesterol levels, may suggest that studying maternal cholesterol levels at a single time point during pregnancy does not accurately capture the body's biological response to pregnancy. Looking at the change in maternal cholesterol may provide a better reflection of the role of maternal cholesterol levels during pregnancy.

MATERNAL CHOLESTEROL LEVELS AND FETAL GROWTH

Fetal growth restriction is a multifactorial birth outcome that quantifies how a fetus is growing while in utero. Growth restricted infants have increased risk of intrauterine demise,

neonatal morbidity and mortality, cognitive delay in childhood, and adulthood diseases such as diabetes, coronary artery disease and stroke [52]. Causes of fetal growth restriction have been grouped into three main categories, placental insufficiencies, maternal insufficiencies, and fetal insufficiencies. Placental insufficiency, poor placental perfusion, is the most common pathology associated with fetal growth restriction [52]. Research suggests that maternal insufficiencies that may impact fetal growth include pre-gestational diabetes, renal insufficiency, and pre-eclampsia. Fetal insufficiencies that may impact fetal growth include genetic and structural disorders, such as trisomy 13, congenital heart disease, and gastroschisis [52].

Different methods can be used to calculate fetal growth and various maternal and fetal characteristics can be adjusted for. In some instances, researchers will use the terms small for gestational age, fetal growth restriction, and intrauterine growth restriction (IUGR) interchangeably, although there are differences between the three measurements. Small for gestational age is a classification used to categorize fetuses who have failed to achieve normal weight [52]. Fetal growth restriction is a measure of fetal growth, calculated using birth weight and gestational age at birth. Other fetal characteristics such as sex, race, and length can be adjusted for when calculating fetal growth. There is a body of research investigating how best to measure fetal growth restriction and what variables to adjust for [53 – 55]. IUGR is the term used when placental insufficiencies are thought to be involved in reduced fetal growth. These placental insufficiencies may not be present in all instances of fetal growth restriction [5, 56]. The most widely used measure of fetal growth in the United States is the relationship of birth weight to gestational age at birth [52]. Literature suggests that the fetal demand for cholesterol is positively associated with the rate of growth in the fetus [56 – 57]. Perhaps as fetal demand for cholesterol increases, maternal cholesterol levels increase at least until the fetus begins to

produce its own cholesterol. Fetal growth with respect to maternal cholesterol levels during pregnancy has been studied although results are inconclusive. Given this suggested association, this dissertation will explore the associations between maternal cholesterol during gestation and fetal growth.

Current literature suggests that women with low TC and/or LDL-C during the pregnancy are at an increased risk of delivering an infant who is small for their gestational age [56 – 59]. A retrospective cohort study in South Carolina measured maternal TC between 13 weeks and 23 weeks of gestation and defined low maternal TC as being below the 10th percentile relative to the study population [58]. In this study, low maternal TC equated to less than 159 mg/dL [58]. The time frame in which women's TC was measured limits this study as maternal cholesterol is expected to increase during this time frame and comparing a woman's TC during the 13th week of pregnancy to a woman's TC during the 23rd week of pregnancy is not a valid comparison. With that, term infants (37 weeks gestation – 41 weeks gestation) born to mothers with low TC were found to weigh 147 grams less than those term infants born to mothers with higher cholesterol (p-value = 0.0006) [58]. White women with TC levels in the lowest third percentile, less than 138 mg/dL, were at a greater risk of having an infant with a birthweight in the lowest 10th percentile for gestational age (odds ratio 3.42, 95% CI= 0.84, 13.9) compared to women with TC levels in the middle reference range (159 mg/dL – 261 mg/dL) [58]. This result should be further studied as only 37 women in this study population had TC levels in the lowest third percentile and of those 37 only five had a small for gestational age birth. In Germany, a study found low maternal TC at birth to be associated with IUGR [56]. For this study, according to American Congress of Obstetricians and Gynecologists guidelines, IUGR was defined as a fetus having an estimated fetal weight below the 10th percentile and one of the following four

criterion also had to be met: (1) deceleration of fetal growth velocity during the last four weeks gestation, (2) elevated resistance index in umbilical artery Doppler sonography above the 95th percentile or absent or reversed end-diastolic blood flow, (3) fetal asymmetry, or (4) oligohydramnios [56]. 97 women in the control group had an average TC level at birth of 271 mg/dL while 36 women in the IUGR group had an average TC level at birth of 231 mg/dL (p-value < 0.0001) [56]. This study also found a significant association between low maternal LDL-C in the IUGR group (119 mg/dL) compared to the control group (157 mg/dL) (p-value < 0.0001) [56]. A small case-control study of 16 women, eight cases and eight controls, found women with IUGR pregnancies to have third trimester TC levels of 191 mg/dL (range 130 mg/dL – 275 mg/dL) compared to third trimester TC levels of 289 mg/dL (range 222 mg/dL – 327 mg/dL) (p-value < 0.01) in women with fetuses having fetal growth in the normal range [59]. LDL-C levels were also significantly lower in the IUGR group (95 mg/dL, range 37 mg/dL – 139 mg/dL) relative to the control group (164 mg/dL, range 130 mg/dL – 217 mg/dL) (p-value < 0.01) [59]. Several other studies found no significant association between maternal cholesterol levels during pregnancy and fetal growth, including IUGR [25, 29, 60 – 61]. Despite multiple studies suggesting lower maternal cholesterol in growth restricted pregnancies, each study measures fetal growth differently and has a unique definition of what low cholesterol is and has measured maternal cholesterol levels at different weeks during gestation. Table 2.5 summarizes these findings.

Chapter four of this dissertation will provide additional evidence regarding the relationship between fetal growth, measured by birth weight, gestational age at birth, and sex of the fetus, and maternal cholesterol levels during pregnancy. Analyzing maternal cholesterol over time, rather than at a single time point during pregnancy, will add to the current literature.

Additionally, the generalizability of the study population for this dissertation is much more robust when compared to current literature.

MATERNAL CHOLESTEROL LEVELS AND GESTATIONAL AGE AT DELIVERY

Preterm birth, defined as a live birth less than 37 weeks gestation, is one of the leading causes of morbidity and mortality in neonates [62]. In 2015, preterm birth occurred in 10% of all births in the United States [63]. Rates of preterm birth are disproportionately higher in Black women. Black women are 50% more likely to deliver a preterm infant compared to White women [64].

Preterm birth is a complex, multifactorial medical condition. The etiology of preterm birth is still unknown. Maternal vascular disturbances and inflammation have been cites as two possible causes [65]. In pregnancy, hyperlipidemia is an instigator of inflammation [65]. Elevated maternal cholesterol levels may increase risk of preterm birth. To date, the association between maternal cholesterol levels during gestation and preterm birth has been studied and the results are inconclusive.

A cholesterol lowering diet was studied in 290 pregnant Norwegian women [24]. In this study, women in the dietary intervention group consumed a diet low in cholesterol and had a preterm birth rate of 0.7% (n=1), compared to women in the control group with a preterm birth rate of 7.4% (n=11) (relative risk 0.10, 95% CI= 0.01, 0.77) [24]. The length of gestation increased 3.9 days in the intervention group [24]. In addition to significantly less cholesterol consumption in the intervention group compared to the control group, the diet for women in the intervention group significantly differed from the control diet in total energy, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, protein, carbohydrates, sugar, vitamin C, alpha-tocopherol, magnesium, calcium, and vitamin D. Because this study looked at a low cholesterol

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diet and not specifically at the association between maternal cholesterol levels and preterm birth, the authors notes that it is unknown if decreased cholesterol levels reduced the risk of preterm birth or if improved diet reduced the risk of preterm birth.

A South Carolina study of a cohort of 1,058 women referred to a genetic center for routine second trimester screening found a "U" shaped relationship between second trimester TC levels and the incidence of preterm birth [58]. White women with TC in the lowest 10th percentile, TC less than 159 mg/dL, had a greater incidence of preterm birth compared to women with TC in the 10th – 90th percentile (21% compared to 5%, odds ratio 5.63, 95% CI= 2.58, 12.3, p-value < 0.0001) [58]. Low TC levels in Black women had more of a protective effect on preterm birth rates (odds ratio 0.81, 95% CI= 0.18, 3.74, p-value = 0.79), although this finding was not significant [58]. In Black women with TC levels in the 90th percentile, TC greater than 261 mg/dL, the rate of preterm birth was two and a half times greater relative to women with TC in the 10th – 90th percentile, 13.2% compared to 5.2% (odds ratio 2.60, 95% CI= 0.84, 8.00, p-value = 0.10) [58]. These findings within Black women, although not statistically significant and within a selective population, highlight the importance of including maternal race as an important covariate when researching preterm birth. A multicenter study of 1010 women (49% Black) found a relationship between maternal pre-pregnancy TC levels and preterm birth independent of maternal race, after adjusting for race, parity, BMI, physical activity at baseline, maternal age at selected birth, ever having gestational hypertension or gestational diabetes, and time between cholesterol measurement and the birth being studied [66]. Women with pre-pregnancy TC levels in the lowest quartile, TC less than 156 mg/dL, were at an increased risk for preterm birth between 34 and 37 weeks gestation (adjusted odds ratio 1.86, 95% CI= 1.10, 3.15) and preterm birth less than 34 weeks gestation (adjusted odds ratio 3.04, 95% CI= 1.35, 6.81)

compared to women with pre-pregnancy TC levels between 156 mg/dL and 171 mg/dL [66]. Crude analyses had the same significant findings with pre-pregnancy TC levels in the lowest quartile compared to those with TC levels between 156 mg/dL and 171 mg/dL [66]. In addition, after excluding women with hypertension in pregnancy, women with pre-pregnancy TC levels either higher or lower than the referent group, TC levels between 156 mg/dL and 171 mg/dL, were at increased risk for preterm birth less than 34 weeks gestation [66]. This study, although looking at a large, diverse population, looked at pre-pregnancy maternal cholesterol levels that were drawn up to six years prior to the pregnancy outcome being studied and did not capture the changes occurring to maternal cholesterol during pregnancy. This study suggests pre-pregnancy maternal cholesterol levels may be a potentially modifiable risk factor for preterm birth, although additional research is needed to support this finding. A nested case-control study of 207 women with preterm birth and 444 term controls suggested that higher second trimester levels of maternal HDL-C provided a protective effect against preterm birth in the control arm (mean HDL-C = 70 mg/dL) compared to the case arm (mean HDL-C = 62 mg/dL) (odds ratio 0.5, 95% CI: 0.3, 0.8) [67]. A second case-control study of 183 cases and 376 controls, within a racially diverse population, found significantly higher maternal HDL-C levels in women with preterm birth (49 mg/dL) compared to women with a term birth (47 mg/dL), p-value < 0.001 [42]. This significant finding adjusted for maternal age, pre-pregnancy BMI, parity, cigarette smoking, and ethnicity (Black vs. Hispanic and non-Hispanic White). When breaking HDL-C levels into quartiles, women with HDL-C levels in the highest quartile, greater than 54 mg/dL, relative to the lowest quartile were at an increased risk of preterm birth (odds ratio 1.91, 95% CI= 1.15, 3.20) [42]. This study found no association between maternal LDL-C and TC with preterm birth.

No significant interaction between maternal ethnicity (Black vs. Hispanic and non-Hispanic White) and preterm birth were found for LDL-C, HDL-C, or TC [42].

In Ghana, a random sample of 320 women from a larger prospective cohort study had cholesterol measurements at 20 weeks gestation and 36 weeks gestation [68]. This study looked at the relationship between maternal cholesterol levels and length of gestation, since only 11 women from the sampled population delivered preterm. All women in this study received some type of dietary supplement starting at enrollment and continuing through delivery, lipid-based nutrient supplement, iron and folic acid capsule, or a multiple micronutrient capsule. Cholesterol levels at 20 weeks gestation were not associated with length of gestation [68]. Women with HDL-C levels in the lowest 10th percentile at 36 weeks gestation were found to have significantly shorter pregnancies compared to women with HDL-C levels in the 10th – 90th percentile (mean difference= -6.2 days, 95% CI= -10.1 days, -2.3 days) [68]. After adjusting for gestational age at enrollment, baseline BMI, age, parity, infant gender, season at enrollment, and time since last meal, the relationship between low HDL-C and shorter gestation remained (adjusted mean difference = -5.9 days, 95% CI= -10.7 days, -1.1 days) [68]. LDL-C levels at 20 weeks gestation were not significantly associated with length of gestation. At 36 weeks, however, women with LDL-C levels in the lowest 10th percentile had pregnancies that were 4.9 days longer than women with LDL-C levels in the 10th – 90th percentile [68]. This finding was statistically significant only after adjusting for gestational age at enrollment, baseline BMI, age, parity, infant gender, season at enrollment, and time since last meal (mean difference = 4.9, 95% CI= 0.02 days, 9.8 days) [68]. Supplement group was also included in all adjusted models, although this study found no significant difference in maternal cholesterol levels between supplement groups. No significant associations between length of gestation and TC were

identified [68]. Additional research looking at length of gestation and maternal cholesterol levels should be completed in a population without any supplemental interventions as the biological effects of the dietary supplements on maternal cholesterol levels and preterm delivery are not well described. In addition, the cholesterol levels for women in this population, particularly cholesterol levels used to define the lowest 10th percentiles, were significantly lower than what has been found in other studies. At 20 weeks gestation, TC levels less than 102.3 mg/dL were included in the lowest 10th percentile and at 36 weeks gestation the cut off was 112.1 mg/dL [68]. The percent change in TC levels was 14.5% which is similar to what has been summarized elsewhere in this chapter. For LDL-C, levels under 30.9 mg/dL were included in the lowest 10th percentile at both 20 and 36 weeks gestation. LDL-C levels only increased 7%, on average, in this study population [68]. This increase is quite lower than what has been previously described at 19%. For HDL-C, levels had to be less than 30.9 mg/dL at 20 weeks gestation and less than 27.1 mg/dL at 36 weeks gestation to be included in the lowest 10th percentile. In this study population, HDL-C levels increased 17% from second to third trimester [68]. On average, current literature shows a 7% decrease from second to third trimester for HDL-C levels. As 100% of women in this study were African/Black living in Ghana, this study highlights the importance of controlling for maternal race and possibly even geographic location, developing compared to developed country, when studying the relationship between maternal cholesterol and birth outcomes.

A case-control study of 90 preterm births (cases) and 199 term births (controls) from Pennsylvania measured maternal cholesterol levels at two time points during pregnancy [69]. The first time point was before 15 weeks gestation (mean 8.4 weeks) and the second specimen was collected after 26 weeks gestation. In the case group, the average gestational age for the

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second specimen collection was 33.4 weeks. In the control group the average gestation age for the second specimen collection was 39.8 weeks. A subset of women, 32 cases and 89 controls, had a third specimen collected at 18.3 weeks gestation, on average. This study found no difference in mean HDL-C and LDL-C before 15 weeks and preterm birth [69]. This result controlled for race, CMI, and gestational age at sampling. TC levels before 15 weeks gestation were modestly higher in women who delivered before 34 weeks gestation compared to term births, after controlling for gestational age at sampling, BMI, and race (203.3 mg/dL compared to 188 mg/dL, p-value= 0.04) [69]. When stratified on maternal BMI, overweight women (BMI greater than or equal to 25 kg/m^2) who delivered prior to 34 weeks gestation had significantly elevated TC and LDL-C levels in early pregnancy comparted to those who delivered term [69]. This study also found women with early pregnancy TC greater than 230 mg/dL were 2.8 times (95% CI= 1.0, 7.9) more likely to deliver before 34 weeks and 2.0 times (95% CI= 1.0, 3.9) more likely to deliver between 34 – 37 weeks compared to those with TC less than or equal to 230 mg/dL after adjusting for race, BMI, education, and family history of hypertensive disorder [69]. In the subset of women with three cholesterol measurements, this study found no difference in the rate of change for LDL-C, HDL-C, or TC for preterm delivery status [69]. This study highlights the importance of stratifying analysis of maternal cholesterol and preterm birth on maternal BMI as their results differed across BMI groups. This study is one of the first studies looking at how the rate of change in maternal cholesterol is associated with preterm birth [69].

A Michigan study of 1,309 women looked at the association between maternal cholesterol levels in the second trimester and two types of preterm birth, medically indicated and spontaneous preterm birth [70]. After adjusting for maternal race, parity, and gestational age at time of blood draw, this study found an increased risk of spontaneous preterm birth in women

with TC levels in the highest 30th percentile compared to women with TC levels in the 10th – 70th percentile (adjusted odds ratio = 1.51, 95% CI= 1.06, 2.15) [70]. Among medically indicated preterm births, it was low TC, HDL-C, and/or LDL-C levels that increased the risk of preterm birth (TC: odds ratio 2.04, 95% CI= 1.12, 3.72, p-value <0.05; HDL-C: odds ratio 1.89, 95% CI= 1.04, 3.42, p-value <0.05; LDL-C: odds ratio 1.96, 95% CI= 1.09, 3.54, p-value <0.05) [70]. This study provided the average cholesterol levels for TC, LDL-C, and HDL-C; however, did not provide the cholesterol levels used for cut-off values for the 10th percentile and the 70th percentile. This missing information is a limitation to this study and the ability to compare this study to others in the literature. An Amsterdam based study found no association between maternal TC levels and preterm birth [71].

In addition to studies that reported some type of association between maternal cholesterol levels at a single time during pregnancy and preterm birth, a 2013 study looked at multiple maternal cholesterol levels during pregnancy in 2,699 Iowa women [72]. The primary goal of this large study was to develop a predictive model for preterm birth. In this population, the average first trimester TC for term infants was 173.8 mg/dL compared to 177.9 mg/dL for preterm infants (p-value = 0.07) [72]. The average difference between first and second trimester TC levels was 22.4 mg/dL and 19.3 mg/dL for term and preterm births, respectively (p-value = 0.02) [71]. The final predictive model included ten covariates, including first trimester TC levels and change in TC from first to second trimester as significant predictors for preterm birth [72]. Maternal education, pre-pregnancy diabetes, previous preterm birth, previous live birth, first trimester BMI < 18.5 kg/m^2, first trimester BMI > 40 kg/m^2, alpha-fetoprotein levels, and inhibin A levels were also included in the final model [72]. In the final model, first trimester TC had a beta coefficient of 0.005, an odds ratio of 1.17, a 95% CI= 1.01, 1.36, and a p-value of 0.03

[72]. In the final model, change in maternal TC from first to second trimester had a beta coefficient of -0.008, an odds ratio of 0.87, a 95% CI= 0.74, 1.01, and a p-value of 0.07 [72]. LDL-C and HDL-C were determined not significant predictors of preterm birth and information on their levels within the study population were not provided. In addition to developing a predictive model, an unadjusted analysis was completed looked at the association between maternal cholesterol and preterm birth. Maternal cholesterol levels were broken into quartiles and neither the highest nor the lowest quartiles of maternal cholesterol (TC, LDL-C, or HDL-C) were significantly associated with preterm birth [72]. One limitation of this study is the lack of diversity within the study population. The study population strongly represented the Iowa demographics and consisted of almost 90% non-Hispanic, White women. Given this limitation, the results from this study, along with all other presented results, highlight the need for additional research on this topic. Table 2.6 summarizes these findings.

Most studies looking at cholesterol and preterm birth only have data at a single time period during pregnancy, predominantly in the second trimester. For those studies that have maternal cholesterol at multiple time points during pregnancy, rates of change are not always analyzed in relation to preterm birth. Cholesterol at a single time point during pregnancy, although valuable, fails to take into consideration maternal characteristics and fails to adjust for differences in rates of change. This dissertation will analyze the relationship between preterm birth and changes in maternal cholesterol levels while adjusting for maternal characteristics, including but not limited to maternal race.

SUMMARY

In pregnancy many essential changes are occurring to accommodate new life. Changes in maternal cholesterol occur in nearly all healthy pregnancies regardless of pre-pregnancy cholesterol levels. These changes have been well documented in diverse populations. For this dissertation, no literature was found that suggested maternal cholesterol levels do not change during gestation. Research from animal models suggests these changes are required for appropriate fetal development in humans. Research in human models suggests adverse maternal and fetal outcomes associated with cholesterol levels that are either too high or too low.

A review of the literature found TC and LDL-C follow similar patterns of change, peaking in the third trimester. Third trimester TC levels and LDL-C levels can reach averages of 240 mg/dL and 160 mg/dL, respectively, and individuals may have levels greater than 350 mg/dL and 250 mg/dL, respectively [39, 71]. HDL-C peaks during the second trimester and begins to return to pre-pregnancy levels in the third trimester. Second trimester HDL-C levels can reach 70 mg/dL. Table 2.7 summarizes the average rates of change for TC, LDL-C, and HDL-C [2]. When looking at maternal cholesterol levels stratified by race, the findings differ in each study. In one study, Black women had significantly lower LDL-C and TC levels compared to White women, and no difference in HDL-C levels [40]. Another study found HDL-C levels to be significantly higher in Blacks compared to Whites and no significant difference in TC and LDL-C levels [42]. Overweight and obese women had lower rates of increase in LDL-C and TC compared to normal weight women. In the first trimester, LDL-C and TC levels were lower in the normal weight population. In the third trimester, LDL-C and TC levels were higher in the normal weight population relative to the overweight and obese groups. Consuming a diet low in cholesterol during pregnancy reduced changes in HDL-C, LDL-C, and TC slightly. Research is limited on the effects of age, parity, and hyperlipidemia on cholesterol levels during gestation.

The risk of fetal growth restriction was found to be higher in women with low LDL-C and/or low TC. No association between HDL-C and fetal growth restriction was found. For preterm birth, results on the association between maternal cholesterol levels and delivering a preterm infant had quite a bit of variability. Some studies found relationships between preterm birth and low cholesterol levels, some found an association with high cholesterol levels, some found "U' shaped relationships, and some found no associations at all.

Studies that solely focus on maternal cholesterol levels at a single time point during gestation may provide useful information with regards to relative cholesterol levels and pregnancy outcomes but may also miss valuable information associated with the rate of change. Analyzing cholesterol at a single time point during pregnancy does not take into consideration the complete picture and fails to consider women who either have increased rates of change or women whose cholesterol fails to increase. Only taking into consideration maternal cholesterol levels at a single time point during gestation assumes similar rates of change in a study population as well as similar pre-pregnancy cholesterol levels. Both are assumptions that current literature refutes.

There are many limitations in the current literature that should be addressed. Maternal cholesterol levels, despite research to suggest its significant role in fetal development and growth are not routinely monitored during pregnancy. The lack of monitoring during pregnancy reduces the number of studies researching maternal cholesterol at multiple time points during gestation and the link between cholesterol and fetal growth and preterm birth. Current literature and standards fail to establish guidelines and/or recommendations for maternal cholesterol levels during pregnancy. There are no clear clinical guidelines on measuring cholesterol during pregnancy and if treatment of these elevated cholesterol levels is necessary. Guidelines on what

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type of change in maternal cholesterol levels is expected and beneficial for a pregnancy should be developed to encourage clinician's to monitor these levels during pregnancy and in the post-partum period. In current research, definitions of high and low maternal cholesterol levels are relative to the population being studied. This variation can introduce complexities when attempting to compare results across studies.

Descriptive data looking at various maternal characteristics and the relationship these characteristics have with maternal cholesterol during pregnancy are lacking in the literature. The relationship between maternal race and changes in maternal cholesterol is needed when looking at fetal outcomes whose incidence differs by race. For example research suggests that both maternal race and maternal cholesterol levels are associated with preterm delivery. Teasing apart this relationship may provide additional information on the maternal factors influencing risk of preterm delivery. Parity is another maternal characteristic whose relationship with maternal cholesterol levels is under studied. Few studies suggest a positive correlation between parity and cardiovascular disease, but additional updated research adjusting for maternal cholesterol levels would contribute to this field. This additional information would help researchers gain a better understanding as to what trends in maternal cholesterol levels should be observed in the various cohorts of women. Developing trends and expectations in changes in maternal cholesterol levels will help researchers identify those women with abnormal cholesterol profiles during pregnancy.

Currently, no literature was found looking at maternal cholesterol levels across multiple pregnancies. Information on how maternal cholesterol profiles change across pregnancies, especially across pregnancies with different fetal outcomes, would greatly contribute to this field. Having a women act as her on control would adjust for any individual genetic and biological factors, as long as these confounders do not change with time.

This dissertation will address some of the gaps in the descriptive analysis as well as studying the rate of change rather than maternal cholesterol at a single time point. Subsequently, data from this study can be used to help develop guidelines/recommendations for optimal levels of maternal cholesterol during pregnancy.

APPENDICES

APPENDIX A

Tables

Table 2.1: 1985 cholesterol guidelines developed by the National Cholesterol Education Program* [6]

TOTAL CHOLESTEROL	
< 200 mg/dL	Desirable
200-239 mg/dL	Borderline High Risk
≥ 240 mg/dL	High Risk
LDL CHOLESTEROL	
< 100 mg/dL	Optimal
100-129 mg/dL	Near optimal/above optimal
130-159 mg/dL	Borderline High Risk
160-189 mg/dL	High Risk
≥ 190 mg/dL	Very High Risk
HDL CHOLESTEROL	
< 40 mg/dL	Low
≥ 60 mg/dL	High

*Pregnancy status is not taken into consideration for these recommendations

Table 2.2: Summary of literature on maternal cholesterol levels during pregnancy by race and ethnicity

Author Last Name (year) [Ref]	Race/Ethnicity	Gestational age for Cholesterol levels	HDL-C	LDL-C	TC
Patrick (2004) [40]	Black (B) White (ref)	9 weeks gestation 18 weeks gestation 28 weeks gestation 39 weeks gestation	No significant difference	Significantly lower in B (p-value 0.04) compared to ref at all time points during gestation	Significantly lower in B (p-value 0.04) compared to ref at all time points during gestation
Schreuder (2010) [41]	African-Caribbean (AC) Ghanaian (G) Moroccan (M) Surinam-Hindustani (SH) Turkish (T) Dutch (ref)	Range 11.9 – 14.3 weeks gestation	Not studied	Not studied	Significantly lower in AC (p-value ≤ 0.001) compared to ref Significantly lower in G (p-value ≤ 0.001) compared to ref Significantly lower in M (p-value ≤ 0.01) compared to ref No significant difference in SH or T compared to ref
Chen (2017) [42]	Black (B) Hispanic/non-Hispanic White (ref)	Average 14.2 weeks gestation	Significantly higher in B (p-value < 0.001) compared to ref	No significant difference	No significant difference

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Table 2.3: Summary of literature on maternal cholesterol levels during pregnancy by BMI

Author Last Name (year) [Ref]	BMI	Gestational age for Cholesterol levels	HDL-C	LDL-C	TC
Farias (2016) [38]	Overweight (Ov) Obese (Ob) Normal weight (ref)	5 – 12 weeks gestation 20 – 26 weeks gestation 30 – 36 weeks gestation	HDL-C significantly lower in Ob compared to ref group for all 3 trimesters	Lower rate of change in Ov and Ob compared to ref 1st trimester LDL-C highest in the Ob group, second highest in Ov group, and lowest in ref group 2nd trimester LDL-C lowest in Ob group and highest in ref group 3rd trimester LDL-C lowest in Ob group and highest in ref group	Lower rate of change in Ov and Ob compared to ref 1st trimester TC highest in the Ob group, second highest in Ov group, and lowest in ref group 2nd trimester TC lowest in Ov group and highest in ref group 3rd trimester no significant differences between groups
Vahratian (2010) [39]	Overweight/ Obese (O) Normal weight (ref)	6 – 9 weeks gestation 10 – 14 weeks gestation 16 – 20 weeks gestation 22 – 26 weeks gestation	No significance difference	Lower rate of change in O (p-value < 0.001) compared to ref 1st trimester LDL-C higher in O compared to ref	Lower rate of change in O (p-value = 0.01) compared to ref 1st trimester LDL-C higher in O compared to ref

Table 2.3 (cont'd)

		32 – 36 weeks gestation		3rd trimester LDL-C lower in O compared to ref	3rd trimester LDL-C lower in O compared to ref
Scifres (2013) [43]	Overweight/ Obese (O) Normal weight (ref)	< 13 weeks gestation 24 – 28 weeks gestation	No significant difference in rate of change between groups	Lower rate of change in O (p-value < 0.01) compared to ref	Lower rate of change in O (p-value < 0.01) compared to ref
			1st trimester HDL-C lower in O (p-value < 0.01) compared to ref	1st trimester LDL-C higher in O compared to ref	1st trimester TC higher in O compared to ref
			2nd trimester HDL-C no significant difference between groups	2nd trimester no significant difference between groups	2nd trimester TC lower in O compared to ref

HDL-C: high density-lipoprotein cholesterol; LDL-C: low density-lipoprotein cholesterol; TC: total cholesterol

Table 2.4: Summary of literature on maternal cholesterol levels during pregnancy by diet

Author Last Name (year) [Ref]	Dietary Intervention	Gestational age for Cholesterol levels	HDL-C	LDL-C	TC
McMurry (1981) [44]	Institutionalized with monitored diet. Cycle 1: Normal → Low cholesterol diet Cycle 2: Low cholesterol → Normal diet Cycle 3: Normal diet → Low cholesterol diet	2nd and 3rd trimester- testing dependent on dietary interventions not gestational age	Not studied	Not studied	Cycle1: 12% decrease in TC, p-value < 0.005 Cycle 2: 19% increase in TC, p-value < 0.001 Cycle 3: 8% decrease in TC, p-value < 0.05
Khoury (2005) [24]	Randomized cohort with assigned diet - Normal diet (control) - Diet low in dietary cholesterol and saturated fat	Baseline (17 - 18 weeks gestation) 24 weeks gestation 30 weeks gestation 36 weeks gestation	No significant difference between cohort groups across gestation	LDL-C significantly lower in intervention group starting at 24 weeks gestation 28% increase from baseline to 36 weeks gestation in intervention group 34% increase from baseline to 36 weeks gestation in control group	TC significantly lower in intervention group starting at 24 weeks gestation 21.5% increase from baseline to 36 weeks gestation in intervention group 25.4% increase from baseline to 36 weeks gestation in control group
Eshriqui (2017) [45]	Pre-pregnancy dietary patterns	5 – 13 weeks gestation	Higher adherence to the vegetables and	No statistical differences in LDL-C	No statistical differences in TC

Table 2.4 (cont'd)

-Fast food and candies -Vegetables and dairy -Beans, bread, and fat	20 – 26 weeks gestation 30 – 36 weeks gestation	dairy pattern had higher HDL-C during 3rd trimester compared to those with low adherence in this group.	levels between adherence levels within each of the dietary groups	levels between adherence levels within each of the dietary groups

HDL-C: high density-lipoprotein cholesterol; LDL-C: low density-lipoprotein cholesterol; TC: total cholesterol

Table 2.5: Summary of literature on maternal cholesterol levels during pregnancy and fetal growth restriction

Author Last Name (year) [Ref]		Fetal Growth	Gestational age for Cholesterol levels	HDL-C	LDL-C	TC
Pecks (2012) [56]	Intrauterine Growth Restriction (IUGR) Small for gestational age (SGA) No growth restriction (ref)	IUGR defined in accordance with American College of Obstetrics and Gynecology SGA defined by antenatal and neonatal weight below the 10th percentile and confirmed intrauterine growth along this percentile for more than four weeks and otherwise normal sonographic findings	24 weeks Prior to onset of active labor	No significant difference between IUGR and ref group or SGA and ref group	LDL-C lower in IUGR group compared to ref (p-value < 0.0001) No significant difference in LDL-C between SGA and ref group	TC level at birth significantly lower in IUGR group (p-value < 0.0001) No significant difference in TC between SGA and ref group
Wadsack (2007) [57]	Intrauterine Growth Restriction (IUGR) Average for gestational age (ref)	IUGR defined by ultrasound measurements of abdominal circumference together with birthweight below the 10th percentile	At the time of delivery	No significant difference between IUGR and ref group	LDL-C lower in the IUGR group (p-value = 0.05) compared to ref	Not studied

Table 2.5 (cont'd)

		with abnormal fetal pulsatility index				
Edison (2007) [58]	Low maternal serum TC below the 10th percentile (LMSC 10) Low maternal serum TC below the 3rd percentile (LMSC 3) Maternal serum TC between 10th – 90th percentile (ref)	IUGR defined by weight and length both below the 10th percentile Low birthweight defined by weight below the 10th percentile for gestational age	13 – 23 weeks gestation (mean 17.6 weeks gestation)	Not studied	Not studied	No significant difference in IUGR rates between LMSC and ref groups Low birthweight rates higher in white women with LMSC 3 (p-value = 0.09) compared to ref group
Sattar (1999) [59]	Intrauterine Growth Restriction (IUGR) Average for gestational age (ref)	IUGR defined by estimated fetal weight less than the 5th percentile for gestation with associated decreased liquor volume (oligohydramnios)	27 – 37 weeks gestation (mean 34 weeks gestation) for IUGR group 32 – 37 weeks gestation (mean 35 weeks gestation) for ref group	No significant difference between IUGR and ref group	LDL-C lower in the IUGR group (p-value < 0.01) compared to ref	TC lower in IUGR group (p-value < 0.01) compared to ref

HDL-C: high density-lipoprotein cholesterol; LDL-C: low density-lipoprotein cholesterol; TC: total cholesterol

Table 2.6: Summary of literature on maternal cholesterol levels during pregnancy and gestational age at delivery

Author Last Name (year) [Ref]		Preterm birth	Gestational age for Cholesterol levels	HDL-C	LDL-C	TC
Khoury (2005) [24]	Intervention, low cholesterol diet (Int) Normal diet (ref)	< 37 weeks gestation *Rates of preterm birth were significantly lower in the Int group compared to ref group (relative risk 0.10, 95% confidence interval 0.01, 0.77)	Baseline (17 - 18 weeks gestation) 24 weeks gestation 30 weeks gestation 36 weeks gestation	HDL-C levels lower in Int group (p-value = 0.02) compared to ref	LDL-C levels lower in Int group (p-value = 0.009) compared to ref	TC levels lower in Int group (p-value = 0.001) compared to ref
Edison (2007) [58]	Low maternal serum TC below the 10th percentile (LMSC 10)	< 37 weeks gestation	13 – 23 weeks gestation (mean 17.6 weeks gestation)	Not studied	Not studied	White women with LMSC 10 had significantly higher rates of preterm birth (p-value < 0.0001) compared to ref

Table 2.6 (cont'd)

	High maternal serum TC above the 90th percentile (LMSC 90) Maternal serum TC between 10th – 90th percentile (ref)					White women with LMSC 90 had significantly higher rates of preterm birth (p-value < 0.015) compared to ref Black women with LMSC 90 had higher rates of preterm birth (p-value = 0.10)
Catov (2010) [66]	Maternal serum quartiles- Q1, Q2 (ref), Q3, Q4	Early preterm birth, < 34 weeks gestation (ePTB) Preterm birth, 34 – < 37 weeks gestation (PTB)	Pre-pregnancy cholesterol, average six years prior to pregnancy being studied	No significant difference in ePTB or PTB rates between Q1/Q3/Q4 HDL-C levels and ref group	No significant difference in ePTB or PTB rates between Q1/Q3/Q4 LDL-C levels and ref group	Women with TC in Q1 had higher rates of ePTB and PTB compared to ref group
Kramer (2009) [67]	Spontaneous preterm birth (cases) Term birth (control)	Spontaneous preterm birth < 37 weeks gestation (SPTB)	24 – 26 weeks gestation	HDL-C levels significantly lower (p-value < 0.001) in cases compared to controls	No significant difference in LDL-C levels between cases and controls	No significant difference in TC levels between cases and controls

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Table 2.6 (cont'd)

Chen (2017) [42]	Spontaneous preterm birth (cases) Term birth (control)	Spontaneous preterm birth < 37 weeks gestation (SPTB)	Average 14.2 weeks gestation	HDL-C levels significantly higher (p-value < 0.001) in cases compared to controls	No significant difference in LDL-C levels between cases and controls	No significant difference in TC levels between cases and controls
Oaks (2016) [68]	Maternal cholesterol: < 10th percentile 10th − 90th percentile (ref) > 90th percentile	Gestational age, by days, as a continuous variable	Baseline, ≤ 20 weeks gestation 36 weeks gestation	Baseline HDL-C levels not significantly associated with gestational age at birth Pregnancy significantly shorter (p-value = 0.002) in women with HDL-C at 36 weeks in the < 10th percentile compared to ref	Baseline LDL-C levels not significantly associated with gestational age at birth Pregnancy significantly longer (p-value = 0.05) in women with LDL-C at 36 weeks in the < 10th percentile compared to ref (significant finding only in adjusted model)	Baseline TC levels not significantly associated with gestational age at birth TC at 36 weeks gestation not significantly associated with gestational age at birth
Catov (2007) [69]	Preterm birth (cases) Term birth (control)	Preterm birth, 34 − < 37 weeks gestation	Before 15 weeks gestation (mean 8.4 weeks gestation)	No difference in HDL-C before 15 weeks gestation and preterm birth	No difference in LDL-C before 15 weeks gestation and preterm birth	TC levels before 15 weeks gestation higher in < 34 weeks gestation births compared to term

Table 2.6 (cont'd)

	Overweight (BMI ≥ 25 kg/m^2)	< 34 weeks gestation	Between 16 and 21 weeks gestation (mean 18.3 weeks gestation) After 26 weeks gestation (mean for preterm: 33.4 weeks gestation. Mean for term: 39.8 weeks gestation)	No difference in rate of change in HDL-C for preterm births	LDL-C levels before 15 weeks gestation higher in overweight women who delivered < 34 weeks gestation compared to overweight women who delivered term No difference in rate of change in LDL-C for preterm births	births (p-value = 0.04) TC levels before 15 weeks gestation higher in overweight women who delivered < 34 weeks gestation compared to overweight women who delivered term No difference in rate of change in TC for preterm births
Mudd (2012) [70]	Maternal cholesterol: < 10th percentile 10th – 70th percentile (ref) > 70th percentile	Preterm birth, < 37 weeks gestation Spontaneous preterm birth (SPTB) Medically indicated preterm birth (mPTB)	15 – 27 weeks gestation (mean 22.4 weeks)	Odds of mPTB significantly increased (p-value < 0.05) in HDL-C < 10th percentile compared to ref	Odds of mPTB significantly increased (p-value < 0.05) in LDL-C < 10th percentile compared to ref	TC levels higher in SPTB group (p-value < 0.05) compared to term births Odd s of SPTB significantly increased (p-value < 0.05) in TC >70th percentile compared to ref (adjusted model only)

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Table 2.6 (cont'd)

						Odds of mPTB significantly increased (p-value < 0.05) in TC $<$ 10th percentile compared to ref
Alleman (2013) [72]	Maternal cholesterol: Q1 Q2-Q3 (ref) Q4	Spontaneous preterm birth, < 37 weeks gestation	First trimester Second trimester	No significant difference in HDL-C levels between Q1 and ref No significant difference in HDL-C levels between Q4 and ref	No significant difference in LDL-C levels between Q1 and ref No significant difference in LDL-C levels between Q4 and ref	No significant difference in TC levels between Q1 and ref No significant difference in TC levels between Q4 and ref

HDL-C: high density-lipoprotein cholesterol; LDL-C: low density-lipoprotein cholesterol; TC: total cholesterol

Table 2.7: Summary of literature on TC, LDL-C, and HDL-C average rates of change [2]

TOTAL CHOLESTEROL	
First to second trimester	**28% increase**
Second to third trimester	**16% increase**
First to third trimester	**46% increase**
LDL CHOLESTEROL	
First to second trimester	**35% increase**
Second to third trimester	**19% increase**
First to third trimester	**60% increase**
HDL CHOLESTEROL	
First to second trimester	**18% increase**
Second to third trimester	**7% decrease**
First to third trimester	**10% increase**

HDL-C: high density-lipoprotein cholesterol; LDL-C: low density-lipoprotein cholesterol; TC: total cholesterol

APPENDIX B

Figures

Figure 2.1: Average TC levels during pregnancy in 21 studies stratified by the type of sample used for cholesterol assays [2, 17 – 37]
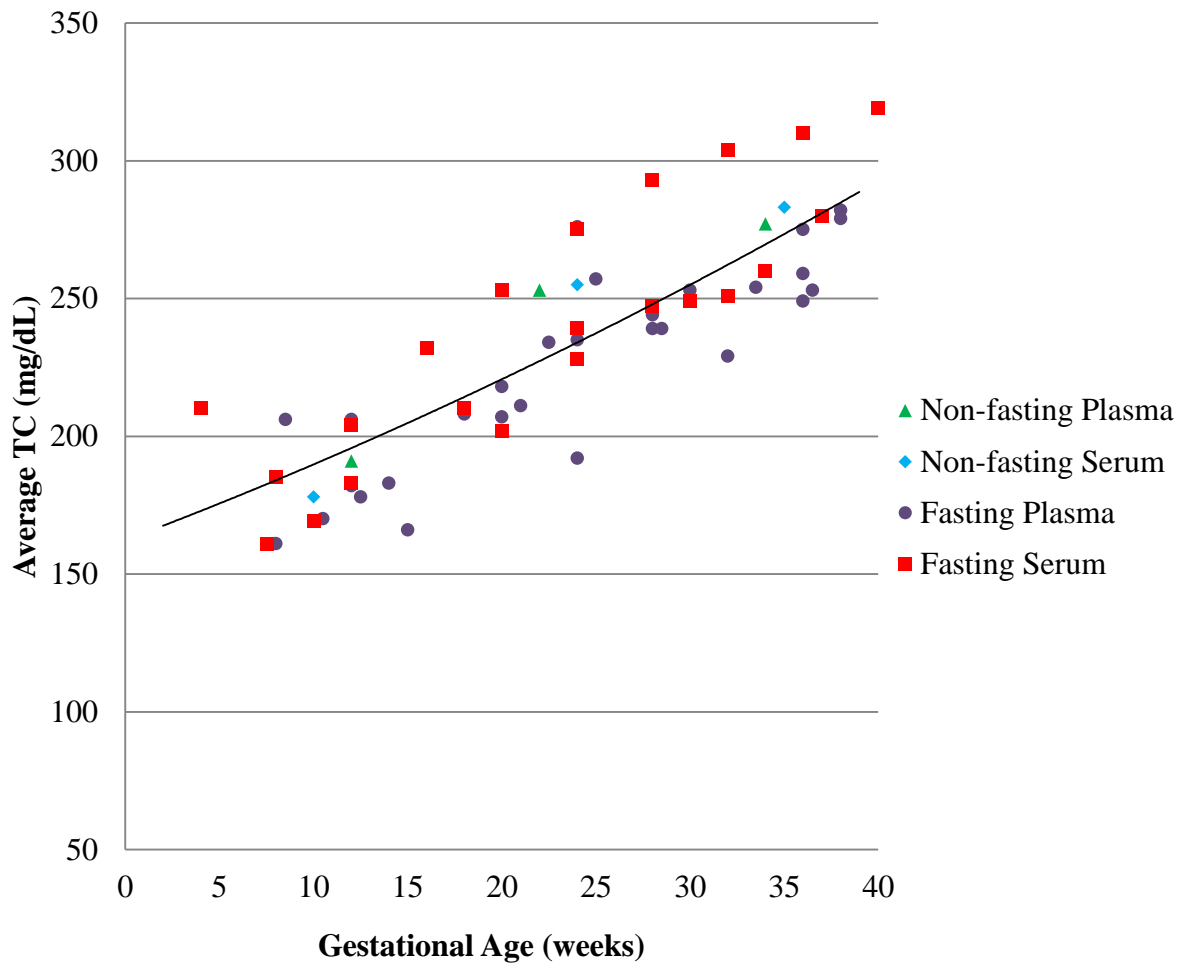
TC: total cholesterol

Figure 2.2: Average TC levels during pregnancy and the postpartum period from nine studies [2, 18 – 19, 22 – 23, 28, 30, 32, 35 – 36]
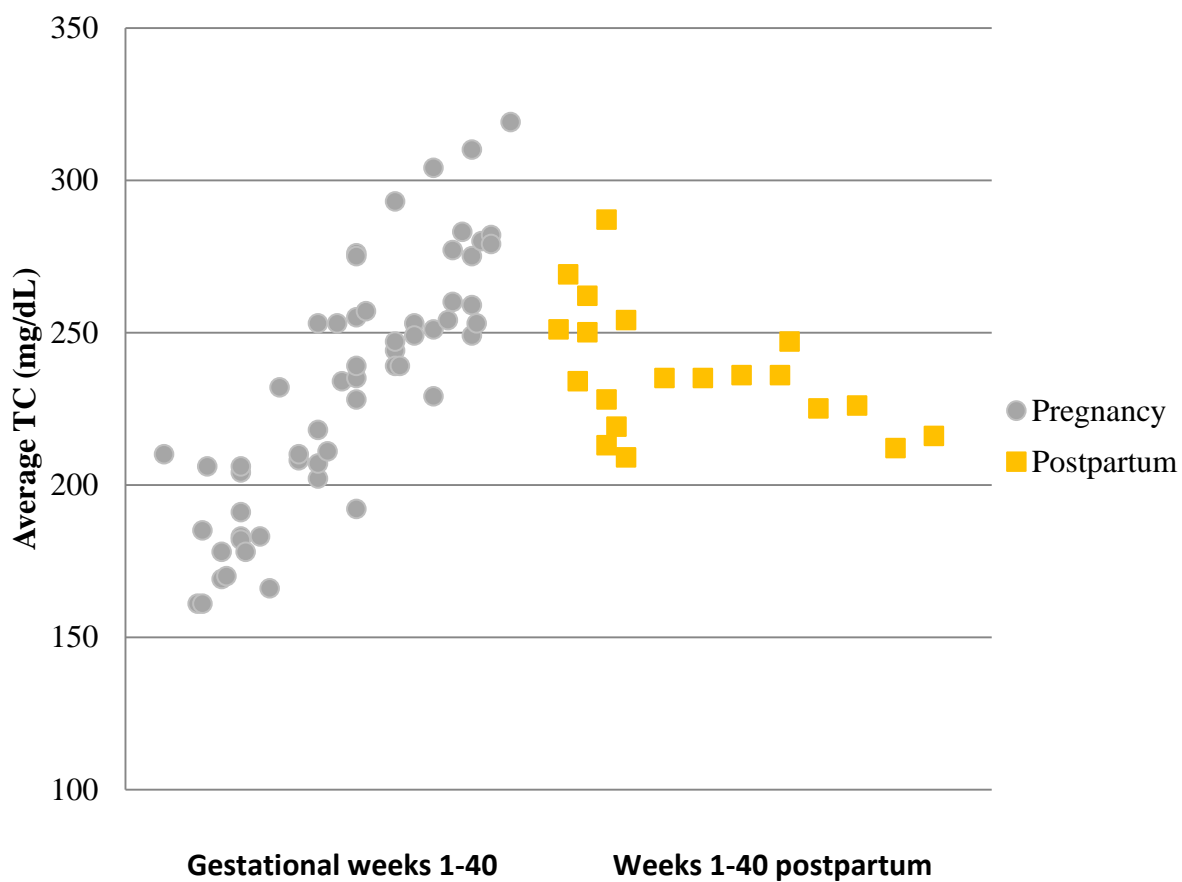
TC: total cholesterol

Figure 2.3: Average LDL-C levels during pregnancy in 14 studies stratified by the type of sample used for cholesterol assays [2, 19 – 21, 23 – 24, 27 – 29, 32 – 37]
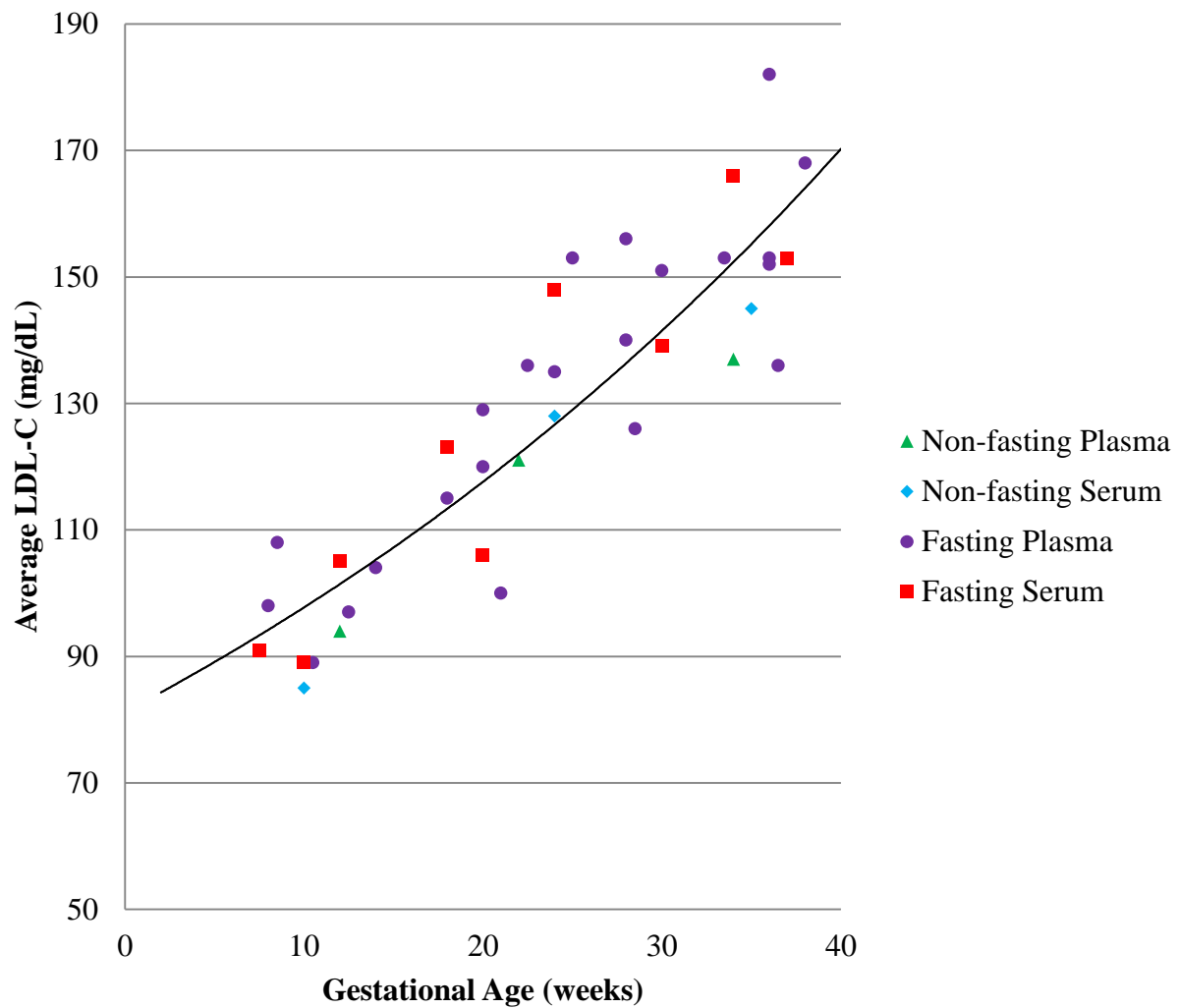
LDL-C: low density-lipoprotein cholesterol

Figure 2.4: Average HDL-C levels during pregnancy in 16 studies stratified by the type of sample used for cholesterol assays [2, 18 – 21, 23, 24, 26 – 29, 32 – 37]
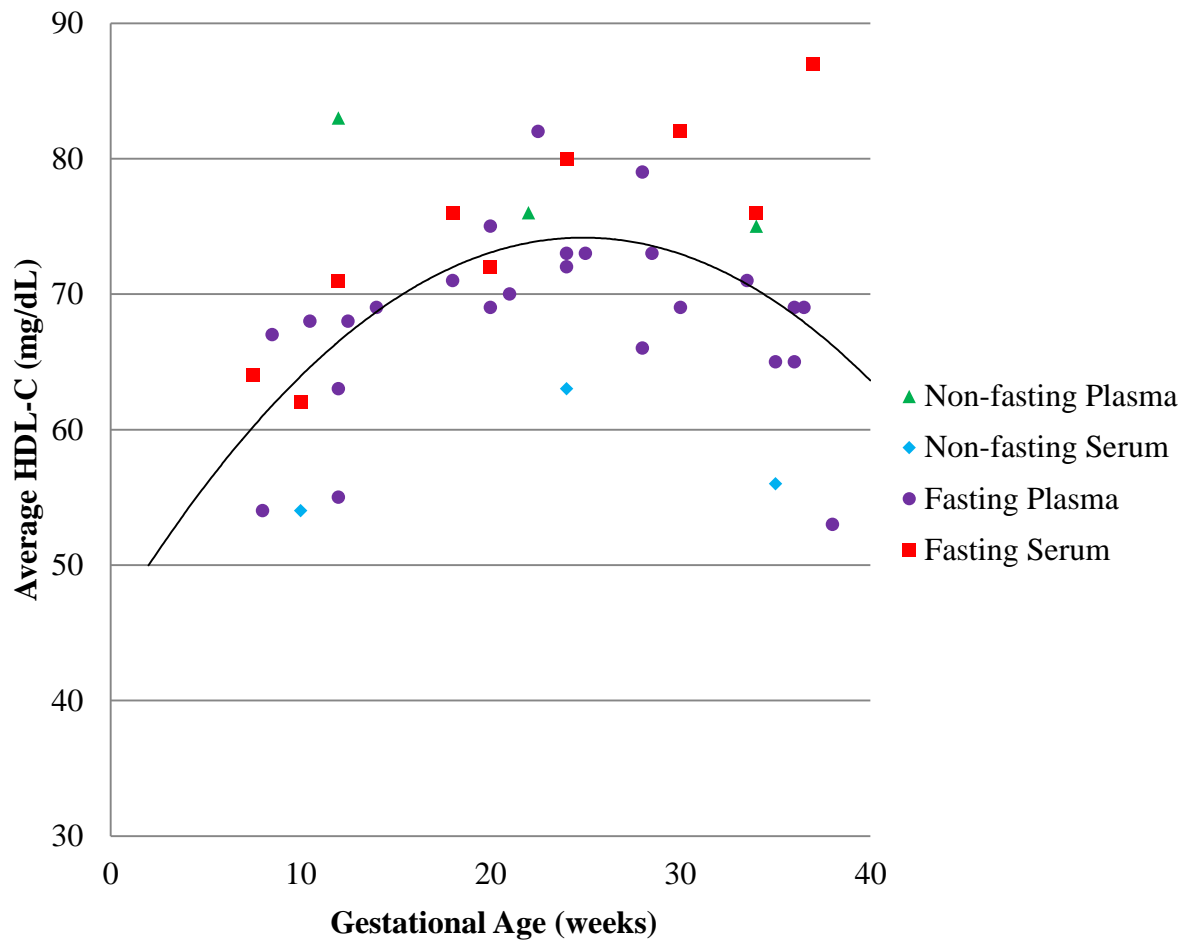
HDL-C: high density-lipoprotein cholesterol

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CHAPTER THREE
ARCHIVE FOR RESEARCH ON CHILD HEALTH

INTRODUCTION

Studying pregnancy longitudinally from as early as possible until delivery can provide valuable information on how events in early pregnancy impact fetal and maternal outcomes. Maternal information and biological markers collected during pregnancy can be used to provide insight into processes that take place during gestation. Maternal blood cholesterol fractions, including high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and total cholesterol (TC), significantly increase during gestation, as described in chapter two of this dissertation. HDL-C, which peaks in the second trimester, on average increases by 18% at peak (chapter 2: table 2.7 and figure 2.4). LDL-C and TC both peak in the third trimester and, on average, increase by 60% and 46%, respectively (chapter 2: Table 2.7, figure 2.3, and figure 2.1). As reviewed in chapter 2, changes in maternal cholesterol are a biological response to pregnancy, although the specific advantages provided by this rise are poorly understood. Maternal cholesterol has been shown to cross the placental barrier and has been implicated in the formation of cell membranes, hormone synthesis, and fetal development [1 − 6]. Negative consequences potentially associated with maternal cholesterol levels and changes in pregnancy that are either high or low outliers within specific study populations are still uncertain, and the subject of investigations [7 − 8].

The aim of this chapter is to study the changes in maternal cholesterol levels, HDL-C, LDL-C, TC, in a sample of women from the Archive for Research on Child Health (ARCH) study. These changes will be stratified on various maternal characteristics, including maternal

ethnicity and race, maternal age, parity, pre-pregnancy body mass index (BMI), postpartum BMI, and maternal history of clinically diagnosed high cholesterol.

DATA SOURCE

ARCH is a partnership between the Michigan State University (MSU) College of Human Medicine, College of Engineering, and College of Communications. The purpose of this archive was to create a rich cohort of medical and biological information from mothers and babies to better understand pregnancy. ARCH began in March 2008 at MSU's Women's Health Care Department of Obstetrics and Gynecology (MSU clinic) in Lansing, Michigan. In October 2008, the project branched off to the Sparrow Obstetrics and Gynecology Women's Center (Residency clinic) in Lansing, Michigan. Finally, in December 2010, ARCH expanded to the Ingham County Health Department in Lansing, Michigan. The cohort stopped enrolling new participants in December 2016. English speaking women at least 18 years of age and receiving prenatal care at one of the three participating clinics were invited to participate. All participants signed informed consents verifying their agreement to participate.

Women were enrolled during their first routine prenatal doctor's appointment at one of the three participating clinics by an ARCH volunteer. At enrollment, ARCH participants completed a self-reported questionnaire (figure 3.1a, 3.1b) and provided consent for ARCH to obtain and store biological specimens from pregnancy and delivery. Specimens included two maternal blood specimens and three urine specimens during pregnancy, placental tissue (including parenchyma, membrane and umbilical cord specimens) collected at delivery, and permission to access the state archive of leftover blood after newborn genetic screening collected shortly after birth. The use of biological specimens that are routinely collected at prenatal

doctor's appointments eliminates the need for additional procedures and lab visits for participants. Staff at the participating clinics helped facilitate specimen collection for the blood and urine specimens.

In addition to a questionnaire and biological specimens, participants also consented for ARCH to gain access to the birth certificate of the corresponding pregnancy, pregnancy related medical records, and medical records of the child for the first five years of life. The ARCH study office also conducted phone interviews with participants one month post-partum, and annually thereafter until the child was at least five years of age.

ARCH is a valuable database with biological specimens, maternal self-reported information, and state records. All data collected for ARCH is managed through MSU and available for researchers to utilize for retrospective research as needed. ARCH is approved by the institutional review boards at Michigan State University, Sparrow Hospital, and the Michigan Department of Health and Human Services.

METHODS

Study Population

Active ARCH participants with a singleton pregnancy and an expected date of confinement (EDC) on or before December 31, 2013 were considered for this retrospective cohort study. All participants were classified as active unless the participant asked to be withdrawn from the study. Participants were allowed to enroll in ARCH multiple times for each unique pregnancy if they met inclusion criteria. Active participants with archived serum samples at two time points during pregnancy are the main focus of this dissertation. The first serum specimen was to be collected at the time of enrollment and was non-fasting. The second

specimen was to be collected at the same time as the glucose tolerance testing during the 26th –

28th week of gestation, which is recommended by the American Congress of Obstetricians and

Gynecologists for all pregnancies. If the second specimen was collected in conjunction with the

glucose tolerance test, the participant would be fasting. If the serum was not collected at the

same time as the glucose tolerance test, the specimen would be from a non-fasting participant.

Cholesterol testing was performed on available serum samples. In the data used in this

dissertation, no women had more than one pregnancy included. Data for this research was

obtained from enrollment questionnaires and birth certificates. The institutional review board of

Michigan State University approved the study for this dissertation.

Specimen Testing

All ARCH serum specimens were sent to the Department of Pediatrics and Human

Development at MSU and stored at -80° C. Prior to being stored, serum specimens were divided

into 500 µl aliquots. Dividing serum samples into smaller aliquots allows the lab to only provide

researchers with the amount of serum needed for their hypotheses and more importantly

eliminates the freeze-thaw cycle to retrieve specimens for projects utilizing serum. For

cholesterol testing, aliquots from the first and second serum samples were thawed, refrigerated,

and transported to the chemical laboratory at Sparrow Hospital in Lansing, Michigan for lipid

analysis. Participants with only one serum sample or no serum samples did not have cholesterol

testing completed. TC profiles were measured using a single enzymatic reaction and

spectrophotometric methods. Testing methods for TC were restricted to levels between 25 mg/dL

and 700 mg/dL. HDL-C was measured using a two-step enzymatic reaction. HDL-C levels were

valid from 2 mg/dL to 200 mg/dL. LDL-C levels were calculated in the lab using the Friedwald

equation, formula 3.1 [9].

Formula 3.1:

$$Total\ Cholesterol - [(High\ Density\ Lipoprotein - Cholesterol) + \left(\frac{Triglycerides}{5}\right)]$$

Triglyceride levels greater than 400 mg/dL produced an inaccurate LDL-C measurement [9]. All

of the cholesterol testing procedures abided by the National Cholesterol Education Program

performance criteria.

Formula 3.2 is the formula used to calculate BMI for this dissertation.

Formula 3.2:

$$BMI = \left(\frac{Weight\ (lbs)}{Height\ (in)}\right)^2 \times 703$$

Data Analysis

Maternal cholesterol levels were stratified on various maternal characteristics. Maternal

ethnicity and race, maternal age, and pre-pregnancy BMI were available from the enrollment

questionnaire. Parity and postpartum BMI were available from the corresponding birth

certificate. Pearson Chi-Square tests were completed to determine if first and second cholesterol

levels, measured as continuous variables, significantly differed between maternal demographics

of interest. It is hypothesized that maternal age and parity will not have a significant impact on

the changes seen in maternal cholesterol (HDL-C, LDL-C, TC) during gestation. Maternal

ethnicity and race, pre-pregnancy BMI, postpartum BMI, and maternal history of clinically

diagnosed high cholesterol will significantly impact the changes observed in maternal (HDL-C,

LDL-C, TC) levels.

RESULTS

694 women with an EDC on or before December 31, 2013 were enrolled in the ARCH project. 36 participants elected to withdraw from the study and are excluded from all analyses. Table 3.1 highlights the diversity of the ARCH population stratified by the clinic of enrollment. The average gestational age at enrollment for ARCH participants was 13.4 weeks. Of the 658 active ARCH participants, 202 unique participants had two serum samples analyzed for cholesterol data. Two women were pregnant with twins and one woman had her first and second serum specimens with the same collection date, likely a result of error at the time of specimen labeling and data entry. These three women have been excluded from analyses looking at maternal cholesterol levels. Of the remaining 199 women, four women did not have a corresponding birth certificate. One of these four women delivered a stillborn baby. These four women will be excluded from analyses. The final sample size for this analysis was 195 (figure 3.2).

None of the women included in this dissertation had TC or HDL-C levels outside of the aforementioned testing ranges. Five women had triglyceride levels greater than the testing upper limit of 400 mg/dL for either their first or second serum specimen. One participant had a second trimester LDL-C value of negative 7 mg/dL. This result was likely due to either a testing error or an error in reporting the result. This participant with a negative LDL-C value and the five participants with triglyceride levels greater than the upper testing limit were excluded from LDL-C analyses. 189 of the 195 study participants were included in LDL-C analyses.

Since ARCH collected all specimens during routine prenatal care visits, the gestational age at the time of specimen collection varied across women. For this dissertation, the first trimester is defined as the first 13 weeks of gestation. The second trimester is defined as 14 – 26

weeks gestation. The third trimester is defined as 27 weeks gestation through birth. Using the physician estimated gestational age at delivery from the birth certificate to calculate the gestational age when a woman's serum specimens were collected, 138 women had their first serum specimen collected in the first trimester. The range of timing for specimen collection in the first trimester was four weeks gestation to 13 weeks gestation. 55 women had their first specimen collected during the second trimester. The gestational age range of timing for the first specimen being collected in the second trimester was 14 weeks gestation through 26 weeks gestation. Two women had their first serum sample collected in the third trimester, 27 weeks gestation and 33 weeks gestation. For the second specimen, 57 women had a second trimester serum specimen, 13 – 26 weeks gestation. The remaining 138 women had their second specimen collected during the third trimester, 27 – 37 weeks gestation. The average number of weeks between the first and second serum specimen was 15.6 weeks.

The demographics of ARCH participants with cholesterol data at two time points during pregnancy, did not significantly differ from the demographics of those ARCH participants who did not have two cholesterol measurements. Although no statistically significant differences in demographics were identified between the two groups, indices of social advantage (higher levels of education, greater household income, and married living with the father of the baby) were more common in women with two serum samples compared to those without two serum samples. Table 3.2 summarizes demographics of ARCH study participants with two serum samples.

Figure 3.3 is a box plot highlighting the ranges in maternal cholesterol levels by trimester as well as the median for each trimester. HDL-C levels, on average, increased 14% from first to second trimester, 1.4% from second to third trimester, and 15% from first to third trimester.

LDL-C levels, on average, increased 23% from first to second trimester, 24% from second to third trimester, and 52% from first to third trimester. On average, TC levels increased 25% from first to second trimester, 15% from second to third trimester, and 44% from first to third trimester.

Figures 3.4a – 3.6a plot each cholesterol value by gestational age at collection, independent of the paired cholesterol value. Figure 3.4a has 390 HDL-C data points. Figure 3.5a has 378 LDL-C data points. Figure 3.6a has 390 TC data points. During the first trimester, HDL-C, LDL-C, and TC levels ranged from 28 – 101 mg/dL, 37 – 159 mg/dL, and 105 – 286 mg/dL respectively. Second trimester ranges for HDL-C, LDL-C, and TC levels were 39 – 103 mg/dL, 23 – 203 mg/dL, and 120 – 326 mg/dL, respectively. Third trimester HDL-C levels ranged from 36 – 114 mg/dL, LDL-C levels ranged from 50 – 282 mg/dL, and TC levels ranged from 124 – 383 mg/dL.

Figures 3.4b – 3.6b link the first and second maternal cholesterol values for each study subject for HDL-C, LDL-C, and TC. Looking specifically at paired data points and not adjusting for gestational age at specimen collection, HDL-C levels increased 12% on average, LDL-C levels increased 40%, and TC levels increased 34%. If we consider gestational age at time of specimen collection, paired HDL-C samples increased 16% from first to second trimester, 1% from second to third trimester, and 14% from first to third trimester. Paired LDL-C samples increased 38% from first to second trimester, 18% from second to third trimester, and 52% from first to third trimester. Paired TC samples increased 35% from first to second trimester, 15% from second to third trimester, and 44% from first to third trimester.

In the 195 women with cholesterol data for two time points during pregnancy, HDL-C, LDL-C, and TC values were stratified by maternal race, maternal ethnicity, maternal age,

maternal pre-pregnancy BMI and postpartum BMI, and parity. Information on maternal history

of high cholesterol was minimal; two women reported a positive history, so this variable was

excluded. Prior to looking at maternal cholesterol levels by race, race was collapsed from seven

different categories into four. Women who selected a race of American Indian, Alaska Native,

Native Hawaiian, Pacific Islander, Asian, or Multiracial were grouped into a racial category of

other, n=18. Women that left the question of race blank were classified as unknown, n=14. The

four race categories were White, Black, Other, Unknown.

Women with a race of Other had lower first trimester HDL-C levels compared to the

other race categories, although the difference was not statistically significant (p-value = 0.45).

Second and third trimester HDL-C levels were similar regardless of race (figure 3.7a). Black

women and women with an unknown race had similar first trimester LDL-C levels and lower

second trimester LDL-C levels relative to White women and women in the race group of Other.

Women with unknown race had the highest average LDL-C levels in the third trimester, with

White women having the second highest levels (figure 3.7b). First, second, and third trimester

LDL-C levels did not significantly differ by race. TC levels were comparable for all racial

groups for first and second trimesters. In the third trimester, women with unknown race had the

highest average TC levels and White women had the second highest levels (figure 3.7c), these

differences were trending towards significance but were not statistically significant in this

population (p-value = 0.06).

First and third trimester HDL-C levels, when stratified by ethnicity, were lowest in the

non-Hispanic/Latino group. In the second trimester, non-Hispanic/Latino women had higher

HDL-C levels than the Hispanic/Latino group (figure 3.8a). First, second, and third trimester

HDL-C levels did not statistically differ between ethnic groups. LDL-C levels were comparable

for Hispanic/Latino and non-Hispanic/Latino women for all three trimesters (figure 3.8b). Figure 3.8c shows higher TC levels the first, second, and third trimester for Hispanic/Latino women compared to non-Hispanic/Latino women, although not statistically significant.

For maternal age, there was one participant over the age of 40 who was included in the 31 years – 40 years age group. HDL-C levels followed similar patters regardless of age in the first, second, and third trimester (figure 3.9a). Women greater than 31 years of age tended to have slightly higher third trimester LDL-C levels and TC levels, although there was no significant difference between age groups (figure 3.9b – 3.9c). First and second trimester LDL-C values were not statistically different between age groups. First and second trimester TC values were also not statistically different between age groups.

Only two women in the included study population had a pre-pregnancy BMI less than 18.5kg/m^2 in the second trimester. Given this small sample size, pre-pregnancy BMI was reduced to two categories (BMI less than 25kg/m^2, BMI greater or equal to than 25kg/m^2) from the original four categories. Cholesterol levels for women whose pre-pregnancy BMI levels were greater than or equal to 25kg/m^2 were consistently lower throughout pregnancy compared to women with pre-pregnancy BMI levels less than 25kg/m^2. Figures 3.10a – 3.10c show the relationships between maternal cholesterol and the regrouped pre-pregnancy BMI. For HDL-C, in the first trimester the average cholesterol level for women with a pre-pregnancy BMI less than 25 was 60 mg/dL and was 53 mg/dL in women with a pre-pregnancy BMI greater than or equal to 25 (p-value = 0.001). In the second trimester, HDL-C levels were not significantly different between pre-pregnancy BMI groups. In the third trimester women with a pre-pregnancy BMI less than 25 had an average HDL-C level of 69 mg/dL, which was significantly higher than

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HDL-C levels in women with a pre-pregnancy BMI greater than or equal to 25, 62 mg/dL, p-value = 0.01. Second trimester LDL-C levels were significantly different between the two pre-pregnancy BMI groups, p-value = 0.03. In women with a pre-pregnancy BMI less than 25, their average second trimester LDL-C level was 109 mg/dL. For women with a pre-pregnancy BMI greater than or equal to 25, average second trimester LDL-C levels were 96 mg/dL. First and third trimester LDL-C levels were not statistically different between the two pre-pregnancy BMI groups. For TC, there was no statistical difference between the two pre-pregnancy BMI groups in the first trimester. In the second trimester, women with a pre-pregnancy BMI less than 25 had an average TC of 210 mg/dL compared to an average TC of 192 mg/dL in women with pre-pregnancy BMI greater than or equal to 25, p-value = 0.009. In the third trimester, women with a BMI less than 25 had an average TC of 240. Women with a pre-pregnancy BMI greater than or equal to 25 had average third trimester TC levels of 223 mg/dL. The difference between these two groups was statistically significant, p-value = 0.03.

To look at the relationship between maternal cholesterol levels and postpartum BMI, pre-pregnancy BMI was controlled for. There were zero women included in this study with a postpartum BMI level less than 18.5. First, second, and third trimester HDL-C levels did not differ between postpartum BMI levels (figure 3.11a). Women with a postpartum BMI greater than or equal to 30 had consistently lower levels of LDL-C and TC (figure 3.11b – 3.11c). First and third trimester LDL-C levels did not statistically differ between postpartum BMI levels. Controlling for pre-pregnancy BMI, second trimester LDL-C levels were statistically different between postpartum BMI levels, p- value = 0.03. First, second, and third trimester TC levels did not differ between postpartum BMI levels.

For this research, to measure parity, we chose to look at whether or not the pregnancy being studied was the participant's first pregnancy. When looking at the relationship between maternal cholesterol levels and whether or not this was the participants first pregnancy, we controlled for maternal race and maternal age, as both were significantly different between those whose it was their first pregnancy and for those whose it was not. For women who were in their first pregnancy, first, second, and third trimester HDL-C levels trended lower compared to women who were not pregnant for the first time, although not statistically significant (figure 3.12a). LDL-C and TC values were higher throughout pregnancy for women during their first pregnancy compared to those not pregnant for the first time, although these findings were not statistically significant (figure 3.12b – 3.12c).

55 women experienced a decrease in HDL-C, LDL-C, and/or TC levels from the first to second specimen. Table 3.3 shows the number of study participants with decreases in cholesterol levels by the trimester the two specimens were collected in. 44 women experienced a decrease in at least HDL-C. As described in chapter two, HDL-C levels tend to peak in the second trimester and then decrease. This second trimester peak was also observed in this study population (figure 3.4a). Given this second trimester peak, we would expect to see a decrease in HDL-C levels in those women with paired second and third trimester serum specimens and possibly even in those with paired first and third trimester specimens. Of the 44 women with decreases in HDL-C, 40 of these women had an expected decrease in HDL-C. Four women had unexpected decreases in only HDL-C levels. LDL-C and TC were expected to increase throughout gestation peaking in the third trimester and decreasing in the postpartum period. Seven women had unexpected decreases in only LDL-C levels. Two women had unexpected decreases in only TC levels. Three women had unexpected decreases in both HDL-C and LDL-C levels. Two women had

unexpected decreases in LDL-C and TC levels. Six women had unexpected decreases in HDL-C, LDL-C, and TC levels. A total of 24 women had unexpected decreases in their HDL-C, LDL-C, and/or TC levels during pregnancy. Among these 24 women, the average decrease in HDL-C levels was 8% from first to second trimester. The average decrease in LDL-C levels was 16% from first to second trimester. TC levels decreased 6% on average from first to second trimester. For the purpose of comparing these women to the other women in this study, this group of 24 women will be referred to as group C and the remaining 171 women will be referred to as group D. Table 3.4 compares group C to group D. The age distribution, when looked at as a categorical variable, in the 24 women in group C was younger (p-value = 0.03) than that of the women in group D. 8% of women in group C were older than 30 years of age compared to 19% of women in group D. The average maternal pre-pregnancy BMI was significantly higher, 30.7, in group C compared to group D, 27.4, p-value = 0.05. Specific birth outcomes of interest to this dissertation, birthweight, gestational age at birth, and sex of the baby did not differ between groups C and D. For the women whose LDL-C levels decreased but their TC levels increased, n=10, there were no significant differences in birth outcomes.

For the one ARCH participant with two serum specimens who delivered a stillborn baby, we thought it was important to look at her cholesterol data and how it compared to the cholesterol data of the other 195 women. This participant had her first serum specimen collected during the seventh week of gestation. Her second specimen was collected during the 27th week of gestation. Her first trimester cholesterol levels, HDL-C, LDL-C, and TC, all fell within the range observed in the larger group, 56 mg/dL, 101 mg/dL, and 175 mg/dL, respectively. Her third trimester cholesterol levels, HDL-C, LDL-C, and TC, also fell within the range observed in the larger group, 63 mg/dL, 121 mg/dL, and 228 mg/dL, respectively. The rate of change for her

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HDL-C was 12.5%, LDL-C was 19.8%, and TC was 30%, again, all within the range of the larger population.

DISCUSSION

Research suggests that maternal cholesterol may play an important role in pregnancy and fetal development. Although thought to be necessary in pregnancy, a relationship between high maternal cholesterol during pregnancy and atherosclerosis in the offspring has been suggested [10]. This finding, along with others discussed in chapter two, highlight the importance of studying maternal cholesterol level during gestation.

In a previous 2010 literature review, it was concluded that maternal cholesterol during pregnancy, from first to third trimester, increased by 10% in HDL-C, 60% in LDL-C, and 46% in TC, on average, and a rise in maternal cholesterol levels was observed in 70% of pregnancies [11]. Cholesterol data for ARCH participants parallel the change described in literature with LDL-C having the greatest rate of change and HDL-C the least. When looking at each individual cholesterol values independent of each other, unpaired, there were 390 HDL-C values, 378 LDL-C values, and 390 TC values. Average cholesterol levels were calculated for each trimester and then a rate of change was calculated using each of the averages. Unpaired HDL-C, LDL-C and TC levels rose 15%, 52%, and 44%, respectively, from first to third trimester. 72% of women in this ARCH study population had a rise in their cholesterol levels.

Adding to the current body of literature, this study has cholesterol data from all three trimesters and has two HDL-C and two TC samples for 195 women, and two LDL-C samples for 189 women. These paired samples were used to look at the changes in maternal cholesterol across two time points in pregnancy. The rate of change was calculated for each individual participant and then averaged. On average, HDL-C increased 14%, LDL-C increased 52%, and

TC increased 44%   from first to third trimester. These paired findings parallel the unpaired findings in that LDL-C increases the most and HDL-C increases the least. This result shows that when looking at aggregate data and average cholesterol levels, data from studies with maternal cholesterol values measured once during pregnancy, but at different gestational ages for each study subject, can be used in place of longitudinal studies with maternal cholesterol at multiple time points of pregnancy. Longitudinal data has an advantage in that research can study the specific relationships between multiple cholesterol values at various time points during pregnancy.

Using the ARCH population, significant relationships were identified between specific maternal demographics and HDL-C, LDL-C, and TC. As hypothesized, first, second, and third trimester maternal cholesterol levels did not differ by maternal age, or whether or not this was the participant's first pregnancy. Although it was hypothesized that cholesterol levels would significantly differ by maternal ethnicity, this was not identified in the ARCH population. TC levels between race groups trended towards being statistically different, but results were not significant in this population. HDL-C and LDL-C levels did not statistically differ by race. As hypothesized, HDL-C levels were statistically different between maternal pre-pregnancy BMI levels but only in the first and third trimesters. In the second trimester, LDL-C levels were statistically different in women categorized by maternal pre-pregnancy BMI levels. For TC, TC levels were significantly different between maternal pre-pregnancy BMI levels in the second and third trimesters. Larger sample sizes with greater diversity in pre-pregnancy BMI may capture statistically significant differences in maternal cholesterol levels across all three trimesters. LDL-C levels statistically differed between postpartum BMI levels but only in the second trimester. These differences, which have been observed elsewhere in the literature, may be a result of

metabolic dysregulations associated with maternal obesity [12 – 13]. Additional research is needed to further explore the significant differences in maternal cholesterol levels in over-weight and obese women.

Research consistently shows changes in maternal cholesterol levels during pregnancy. The literature has gaps when looking at how these changes impact maternal and fetal outcomes, and stratifying maternal cholesterol levels on various maternal characteristics and demographics. This information may be helpful in better understanding maternal and fetal outcomes. This research, which utilized a rich database, expands the knowledge on three of the four identified gaps. The ARCH project is one initiative in the research community that can be utilized to gain a better understanding of what occurs during pregnancy. Although this dissertation only studied women with an EDC on or before December 31, 2013, ARCH enrolled study participants until December 2016. At the time enrollment ceased, ARCH enrolled 968 women and had data on 871 infants and children. The average gestational age at enrollment of the larger population was identical to the average gestational age at enrollment for the study population of this dissertation, 13.4 weeks. 28 women enrolled in the ARCH project for more than one pregnancy, allowing research to look at pregnancy and outcomes in the same participant across different pregnancies. The sample size and diversity of this population allows for results to be generalizable to a larger population. Another strength of ARCH is the range of maternal serum specimens spans early in the first trimester through late in the third trimester, four weeks gestation through 37 weeks gestation. The range of these serum specimens would allow researchers to study changes in biological markers across pregnancy rather than at single time points during gestation. The additional maternal data, demographics and information on birth outcomes enhances this

database and allows for new research and findings to be added to the growing literature on associations between events during pregnancy and maternal and fetal health.

Despite the strengths of using ARCH for this dissertation, this cohort does have a few limitations that must be acknowledged. The ARCH population is a convenience sample and not a random sample from a population. Women who did not obtain prenatal care nor had minimal care were most likely missed and not enrolled. These high risk women may add valuable information to studying biological markers and fetal outcomes. The majority of women in ARCH did not graduate college (79%) and had an annual household income less than $25,000 (65%). As with high risk women adding valuable information, it has been suggested that women with low socioeconomic status have different maternal and fetal outcomes compared to women with higher levels of socioeconomic status. This was not explored in this chapter of the dissertation, but should be taken into consideration for future research. Although the ARCH population is diverse, maternal races other than Black or White are underrepresented in this population. Results should be interpreted with caution for women in the other race category. Lastly, only 195 out of the 658 eligible women for this dissertation (30%) had two serum samples. This highlights some of the challenges of collecting specimens at routine visits and utilizing clinic staff. Future research may want to focus on collecting serum samples in all study subjects rather than just a small subset.

APPENDICES

APPENDIX A

Tables

Table 3.1: Characteristics of active ARCH participants with EDC on or before December 31, 2013 stratified by enrollment location

	MSU Clinic N (%)	Residency Clinic N (%)	Health Dept N (%)	Unknown Clinic N (%)	Total N (%)
NUMBER OF ENROLLED PARTICIPANTS	230	336	91	1	658
MATERNAL AGE AT EDC					
AVERAGE MATERNAL AGE AT EDC (YEARS)	27	24	25	28	25
MEDIAN MATERNAL AGE AT EDC (YEARS)	27	23	24	28	24
18 - 24 years	82 (35.7)	207 (61.6)	48 (52.8)	0 (0)	337 (51.2)
25 - 30 years	85 (37.0)	96 (28.6)	28 (30.8)	1 (100.0)	210 (31.9)
31 - 40 years	61 (26.5)	33 (9.8)	14 (15.4)	0 (0)	108 (16.4)
> 40 years	2 (0.9)	0 (0)	1 (1.1)	0 (0)	3 (0.5)
Missing Data	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MATERNAL RACE					
American Indian or Alaska Native	1 (0.4)	3 (0.9)	2 (2.2)	0 (0)	6 (0.9)
Black or African American	39 (17.0)	80 (23.8)	28 (30.8)	0 (0)	147 (22.3)
Native Hawaiian or Pacific Islander	0 (0)	1 (0.4)	0 (0)	0 (0)	1 (0.2)
Asian	16 (7.0)	3 (0.9)	3 (3.3)	0 (0)	22 (3.3)
White	154 (67.0)	192 (57.1)	43 (47.3)	1 (100.0)	390 (59.3)
Multiracial	6 (2.6)	29 (8.6)	10 (11.0)	0 (0)	45 (6.8)
Missing Data	14 (6.1)	28 (8.3)	5 (5.5)	0 (0)	47 (7.1)
MATERNAL ETHNICITY					
Hispanic or Latino	26 (11.3)	52 (15.5)	13 (14.3)	0 (0)	91 (13.8)
Not Hispanic or Latino	196 (85.2)	275 (81.8)	77 (84.6)	0 (0)	548 (83.3)
Missing Data	8 (3.5)	9 (2.7)	1 (1.1)	1 (100.0)	19 (2.9)
MATERNAL EDUCATION					

Table 3.1 (cont'd)

Did not finish high school	24 (10.4)	58 (17.3)	16 (17.6)	0 (0)	98 (14.9)
High school graduate or GED	44 (19.1)	124 (36.9)	36 (39.6)	0 (0)	204 (31.0)
Some College	77 (33.5)	112 (33.3)	30 (33)	0 (0)	219 (33.3)
College graduate or more	83 (36.1)	34 (10.1)	8 (8.8)	0 (0)	125 (19)
Missing Data	2 (0.9)	8 (2.4)	1 (1.1)	1 (100)	12 (1.8)
HOUSEHOLD INCOME					
Under $25,000	111 (48.3)	234 (69.6)	79 (86.8)	1 (100)	425 (64.6)
$25,000 to $49,999	49 (21.3)	71 (21.1)	10 (11)	0 (0)	130 (19.8)
$50,000 to $74,999	28 (12.2)	11 (3.3)	0 (0)	0 (0)	39 (5.9)
$75,000 or above	33 (14.3)	3 (0.9)	0 (0)	0 (0)	36 (5.5)
Missing Data	9 (3.9)	17 (5.1)	2 (2.2)	0 (0)	28 (4.3)
MARITAL STATUS					
Married, living with the baby's father	94 (40.9)	65 (19.3)	9 (9.9)	0 (0)	168 (25.5)
Married	22 (9.6)	17 (5.1)	5 (5.5)	0 (0)	44 (6.7)
Unmarried, living with the baby's father	63 (27.4)	132 (39.3)	34 (37.4)	0 (0)	229 (34.8)
Unmarried	50 (21.7)	121 (36)	43 (47.3)	1 (100)	215 (32.7)
Missing Data	1 (0.4)	1 (0.3)	0 (0)	0 (0)	2 (0.3)

ARCH: Archive for Research on Child Health; EDC: expected date of confinement

Table 3.2: Characteristics of active ARCH participants with EDC on or before December 31, 2013 with two serum samples and a corresponding birth certificate compared to ARCH participants with no cholesterol data

	Participants with two serum samples N (%)	Participants without two serum samples* N (%)	P-value**
NUMBER OF ENROLLED PARTICIPANTS	**195**	**463**	
MATERNAL AGE AT EDC			0.50
AVERAGE MATERNAL AGE AT EDC	25 years	25 years	
MEDIAN MATERNAL AGE AT EDC	25 years	24 years	
18-24 years	91 (46.7)	246 (53.1)	
25-30 years	69 (35.4)	141 (30.5)	
31-40 years	34 (17.4)	74 (16)	
>40 years	1 (0.5)	2 (0.4)	
Missing Data	0 (0)	0 (0)	
MATERNAL RACE			0.40
American Indian or Alaska Native	1 (0.5)	5 (1.1)	
Black or African American	37 (19.0)	110 (23.8)	
Native Hawaiian or Pacific Islander	0 (0)	1 (0.2)	
Asian	6 (3.1)	16 (3.5)	
White	127 (65.1)	263 (56.8)	
Multiracial	10 (5.1)	35 (7.6)	
Missing Data	14 (7.2)	33 (7.1)	
MATERNAL ETHNICITY			0.66
Hispanic or Latino	26 (13.3)	65 (14.6)	
Not Hispanic or Latino	169 (86.7)	379 (81.9)	
Missing	0 (0)	19 (4.1)	
MATERNAL EDUCATION			0.13
Did not finish high school	25 (12.8)	73 (15.8)	

Table 3.2 (cont'd)

High school graduate or GED	61 (31.3)	143 (30.9)	
Some College	58 (29.7)	161 (34.8)	
College graduate or more	47 (24.1)	78 (16.8)	
Missing Data	4 (2.1)	8 (1.7)	
HOUSEHOLD INCOME			0.51
Under $25,000	130 (66.7)	295 (63.7)	
$25,000 to $49,999	34 (17.4)	96 (20.7)	
$50,000 to $74,999	15 (7.7)	24 (5.2)	
$75,000 or above	11 (5.6)	25 (5.4)	
Missing Data	5 (2.6)	23 (5)	
MARITAL STATUS			0.19
Married, living with the baby's father	60 (30.8)	108 (23.3)	
Married	10 (5.1)	34 (7.3)	
Unmarried, living with the baby's father	62 (31.8)	167 (36.1)	
Unmarried	63 (32.3)	152 (32.8)	
Missing Data	0 (0)	2 (0.4)	
MATERNAL PRE-PREGNANCY BMI			0.51
AVERAGE PRE-PREGNANCY BMI (KG/M2)	27.8	27.1	
MEDIAN PRE-PREGNANCY BMI(KG/M2)	25.8	25.4	
< 18.5	9 (4.6)	21 (4.5)	
18.5 - < 25	75 (38.5)	188 (40.6)	
25 - < 30	47 (24.1)	124 (26.8)	
≥ 30	60 (30.8)	115 (24.8)	
Missing Data	4 (2.1)	15 (3.2)	
PRE-PREGNANCY HIGH CHOLESTEROL			0.80
Yes	2 (1.0)	5 (1.1)	
No	126 (64.6)	255 (55.1)	

Table 3.2 (cont'd)

Missing Data	67 (34.4)	203 (43.8)	
PLANNED PREGNANCY			0.63
Yes	75 (38.5)	168 (36.3)	
No	120 (61.5)	293 (63.3)	
Missing Data	0 (0)	2 (0.4)	

*This group includes women with a twin pregnancy (n=2), two serum samples with the same collection date (n=1), and missing birth certificate data (n=4)

**Pearson Chi-Square test p-value

ARCH: Archive for Research on Child Health; EDC: expected date of confinement

Table 3.3: Number of study participants with observed decreases in cholesterol levels between the first and second specimen

	First - Second trimester	First - Third trimester	Second - Second trimester	Second - Third trimester	Third - Third trimester
Decreases in HDL-C only (n=35)	4*	11	4	16	0
Decreases in LDL-C only (n=7)	1*	4*	0	2*	0
Decreases in TC only (n=2)	0	1*	0	1*	0
Decreases in HDL-C and LDL-C (n=3)	0	1*	0	2*	0
Decreases in HDL-C and TC (n=0)	-	-	-	-	-
Decreases in LDL-C and TC (n=2)	0	0	0	2*	0
Decreases in HDL-C, LDL-C, and TC (n=6)	1*	2*	0	2*	1*
Total (n=55)	6	19	4	25	1

*Indicates unexpected decreased in maternal cholesterol (n=24). These 24 women are referred to as group C in future analysis.

HDL-C: high density-lipoprotein cholesterol; LDL-C: low density-lipoprotein cholesterol; TC: total cholesterol

Table 3.4: Characteristics of active ARCH participants with EDC on or before December 31, 2013 and a corresponding birth certificate (n=195) stratified by those with unexpected decreases in maternal cholesterol

	Group C (unexpected decrease in cholesterol) N (%)	Group D (cholesterol changes as expected) N (%)	P-value**
NUMBER OF ENROLLED PARTICIPANTS	**24**	**171**	
MATERNAL AGE AT EDC			
AVERAGE MATERNAL AGE AT EDC	25 years	25 years	0.55
MEDIAN MATERNAL AGE AT EDC	24 years	25 years	
18 - 24 years	12 (50.0)	79 (46.2)	**0.03***
25 - 30 years	10 (41.7)	59 (34.5)	
31 - 40 years	1 (4.2)	33 (19.3)	
> 40 years	1 (4.2)	0 (0)	
Missing Data	0 (0)	0 (0)	
MATERNAL RACE			0.60
American Indian or Alaska Native	0 (0)	1 (0.6)	
Black or African American	7 (29.2)	30 (17.5)	
Native Hawaiian or Pacific Islander	0 (0)	0 (0)	
Asian	0 (0)	6 (3.5)	
White	15 (62.5)	112 (65.5)	
Multiracial	1 (4.2)	9 (5.3)	
Missing Data	1 (4.2)	13 (7.6)	
MATERNAL ETHNICITY			0.54
Hispanic or Latino	2 (8.3)	24 (14.0)	
Not Hispanic or Latino	22 (91.7)	147 (86.0)	
Missing Data	0 (0)	0 (0)	
MATERNAL EDUCATION			0.82

Table 3.4 (cont'd)

Did not finish high school	4 (16.7)	21 (12.3)	
High school graduate or GED	9 (37.5)	52 (30.4)	
Some College	6 (25.0)	52 (30.4)	
College graduate or more	5 (20.8)	42 (24.6)	
Missing Data	0 (0)	4 (2.3)	
HOUSEHOLD INCOME			0.56
Under $25,000	18 (75.0)	112 (65.5)	
$25,000 to $49,999	4 (16.7)	30 (17.5)	
$50,000 to $74,999	1 (4.2)	14 (8.2)	
$75,000 or above	0 (0)	11 (6.4)	
Missing Data	1 (4.2)	4 (2.3)	
MARITAL STATUS			0.78
Married, living with the baby's father	7 (29.2)	53 (31.0)	
Married	2 (8.3)	8 (4.7)	
Unmarried, living with the baby's father	6 (25.0)	56 (32.7)	
Unmarried	9 (37.5)	54 (31.6)	
Missing Data	0 (0)	0 (0)	
MATERNAL PRE-PREGNANCY BMI			
AVERAGE PRE-PREGNANCY BMI	30.7	27.4	**0.05***
MEDIAN PRE-PREGNANCY BMI	30.6	25.8	
< 18.5	0 (0)	9 (5.3)	0.13
$18.5 - < 25$	8 (33.3)	67 (39.2)	
$25 - < 30$	4 (16.7)	46 (26.9)	
≥ 30	12 (50.0)	49 (28.7)	
Missing Data	0 (0)	0 (0)	
PRE-PREGNANCY HIGH CHOLESTEROL			0.24
Yes	1 (4.2)	1 (0.6)	

Table 3.4 (cont'd)

No	15 (62.5)	111 (64.9)	
Missing Data	8 (33.3)	59 (34.5)	
PLANNED PREGNANCY				0.82
Yes	10 (41.7)	65 (38.0)	
No	14 (58.3)	106 (62.0)	
Missing Data	0 (0)	0 (0)	
SEX OF BABY				0.82
Male	11 (45.8)	86 (50.3)	
Female	13 (54.2)	85 (49.7)	
Missing Data	0 (0)	0 (0)	
BIRTHWEIGHT OF BABY				0.94
AVERAGE BIRTHWEIGHT	3356 grams	3392 grams	
MEDIAN BIRTHWEIGHT	3280 grams	3402 grams	
Missing Data	0 (0)	0 (0)	
GESTATIONAL AGE OF BABY AT BIRTH				
AVERAGE GESTATIONAL AGE	38 weeks	39 weeks	0.24
MEDIAN GESTATIONAL AGE	39 weeks	39 weeks	
Pre-term (< 37 weeks gestation)	1 (4.2)	7 (4.1)	0.79
Term (37-41 weeks gestation)	22 (91.7)	146 (85.4)	
Post-term (> 41 weeks gestation)	1 (4.2)	18 (10.5)	
Missing Data	0 (0)	(0)	

*p-value ≤0.05
**Pearson Chi-Square test p-value
ARCH: Archive for Research on Child Health; EDC: expected date of confinement

APPENDIX B

Figures

Figure 3.1a: ARCH maternal questionnaire, page 1

ARCH's Self-Recorded Maternal Questionnaire

1. Name:

First   M   Last

2. Your date of birth:
/ /

3. Your social security number:
- -

4. Baby's due date:
/ /

5. Your ethnic category:
Hispanic or Latino ☐ Yes ☐ No

6. Your racial category (check all that apply):
☐ American Indian or Alaska Native
☐ Black or African American
☐ Native Hawaiian or Pacific Islander
☐ Asian
☐ White

7. Highest level of education you have completed?
☐ Did not finish high school
☐ High school graduate or GED
☐ Some college
☐ College graduate or more

8. What is your current marital status?
☐ Married, living with baby's father
☐ Married
☐ Unmarried, living with baby's father
☐ Unmarried

9. What is your annual household income?
☐ Under $25,000
☐ $25,000 to $49,999
☐ $50,000 to $74,999
☐ $75,000 or above

10. Do you own...
a home? ☐ Yes ☐ No
a car? ☐ Yes ☐ No
any stock or bonds? ☐ Yes ☐ No

11. How tall are you without shoes?
___ feet ___ inches

12. Weight just before pregnancy:
___ pounds

13. Was this pregnancy planned?
☐ Yes ☐ No

14. Before this pregnancy were you told by a healthcare provider that you had any of the following conditions?

Periodontal disease or infections of gums ☐ Yes   Depression ☐ Yes

Any other psychiatric conditions ☐ Yes *(please specify)* _____

Seizure disorder or epilepsy ☐ Yes

Any other neurological conditions ☐ Yes *(please specify)* _____

High Cholesterol ☐ Yes *(if yes)* were you taking cholesterol lowering medication? ☐ Yes

15. Have any of the following blood relatives ever been diagnosed with any of the following conditions:

Autism	☐ my parent	☐ my brother/sister	☐ my grandparent	☐ my child	☐ other relative
Mental Retardation	☐ my parent	☐ my brother/sister	☐ my grandparent	☐ my child	☐ other relative
Cerebral Palsy	☐ my parent	☐ my brother/sister	☐ my grandparent	☐ my child	☐ other relative
Severe child disability	☐ my parent	☐ my brother/sister	☐ my grandparent	☐ my child	☐ other relative
Other childhood disability *(please specify)*	☐ my parent	☐ my brother/sister	☐ my grandparent	☐ my child	☐ other relative

16. Your Phone Number:
- -

Your Email Address:

17. Alternate Contact Name:

Alternate Contact Phone Number:

18. Your Maiden Name:

19. Your Mother's Full Maiden Name:

20. Your Father's Full Name:

ARCH: Archive for Research on Child Health

Figure 3.1b: ARCH maternal questionnaire, page 2

These next sets of questions are about physical activity, exercise, and sports that you take part in during your free time. If you have any questions or if you are not sure if the activity you partake in is moderate or vigorous, please ask and we can help you.

We will first ask you about moderate activities. A moderate activity is one that causes a small increase in your breathing or heart rate. Some examples of moderate activities are brisk walking, bicycling, dancing and yoga.

21- During the past month, did you do any <u>moderate</u> activities for more than 10 minutes that caused a small increase in your breathing and heart rate?
☐ Yes ☐ No ☐ Don't Know/Refuse
21a- If yes, how many days a week do you usually do these moderate activities?
_____ Days per week ☐ Do not exercise at least 10min a week ☐ Don't know/Not sure
21b How much time do you usually spend doing these moderate activities in one day?
(Please fill in the blanks) _____ Hours and _____ Minutes

Now we will ask you about vigorous activities. A vigorous activity is one that causes an increase in your breathing or heart rate. or heart rate. Some examples of vigorous activities are running, jogging and aerobics.

22- During the past month, did you do any vigorous activities for more than 10 minutes that caused a large increase in your breathing and caused you to sweat?
☐ Yes ☐ No ☐ Don't Know/Refuse
21a- If yes, how many days a week do you usually do these vigorous activities?
_____ Days per week ☐ Do not exercise at least 10min a week ☐ Don't know/Not sure
21b How much time do you usually spend doing these vigorous activities in one day?
(Please fill in the blanks) _____ Hours and _____ Minutes

These next sets of questions are about physical activity, exercise at work

23- Do you currently work at least 30 hours a week?
☐ Yes ☐ No ☐ Don't Know/Refuse
23a- If yes, how do you spend most of your time at work on a typical day? Are you mostly sitting, standing, walking, or doing physical labor?   Please rank these activities from 1 to 4 in order of most (1) to least (4) time spent during a normal work day below. If there are any activities you do not normally do at work, please put a 0 next to it.
Please rank the following work activities using this scale: 1   2   3   4   0   I do the most   I do the least   I never do
Sitting: _____   Standing: _____   Walking: _____   Physical Labor (like lifting/moving things): _____

ARCH: Archive for Research on Child Health

Figure 3.2: Algorithm for ARCH participants to be included in the sample population
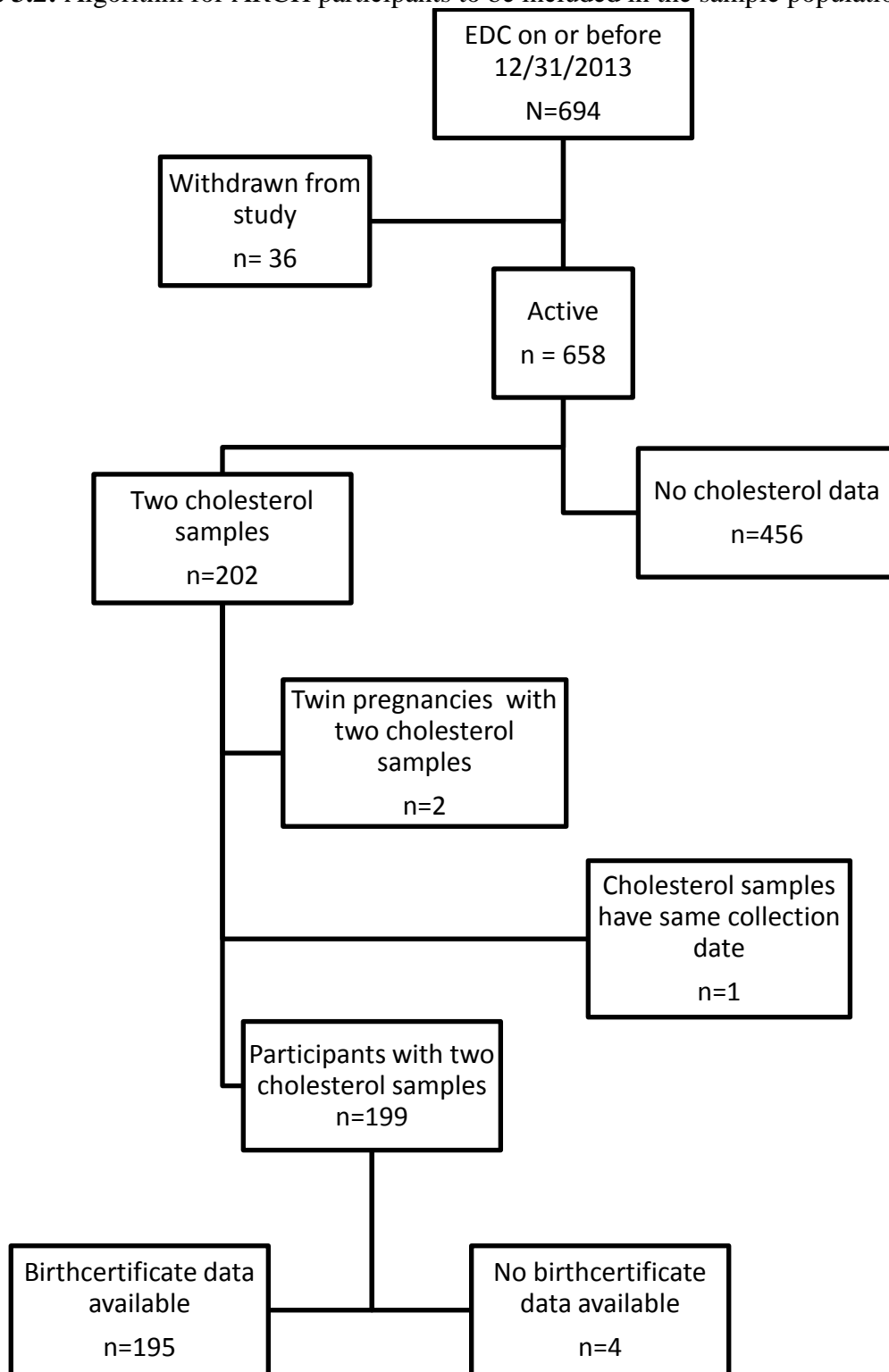
ARCH: Archive for Research on Child Health; EDC: expected date of confinement

Figure 3.3: Box plot of cholesterol values, for first, second, and third trimesters for HDL-C, LDL-C, and TC
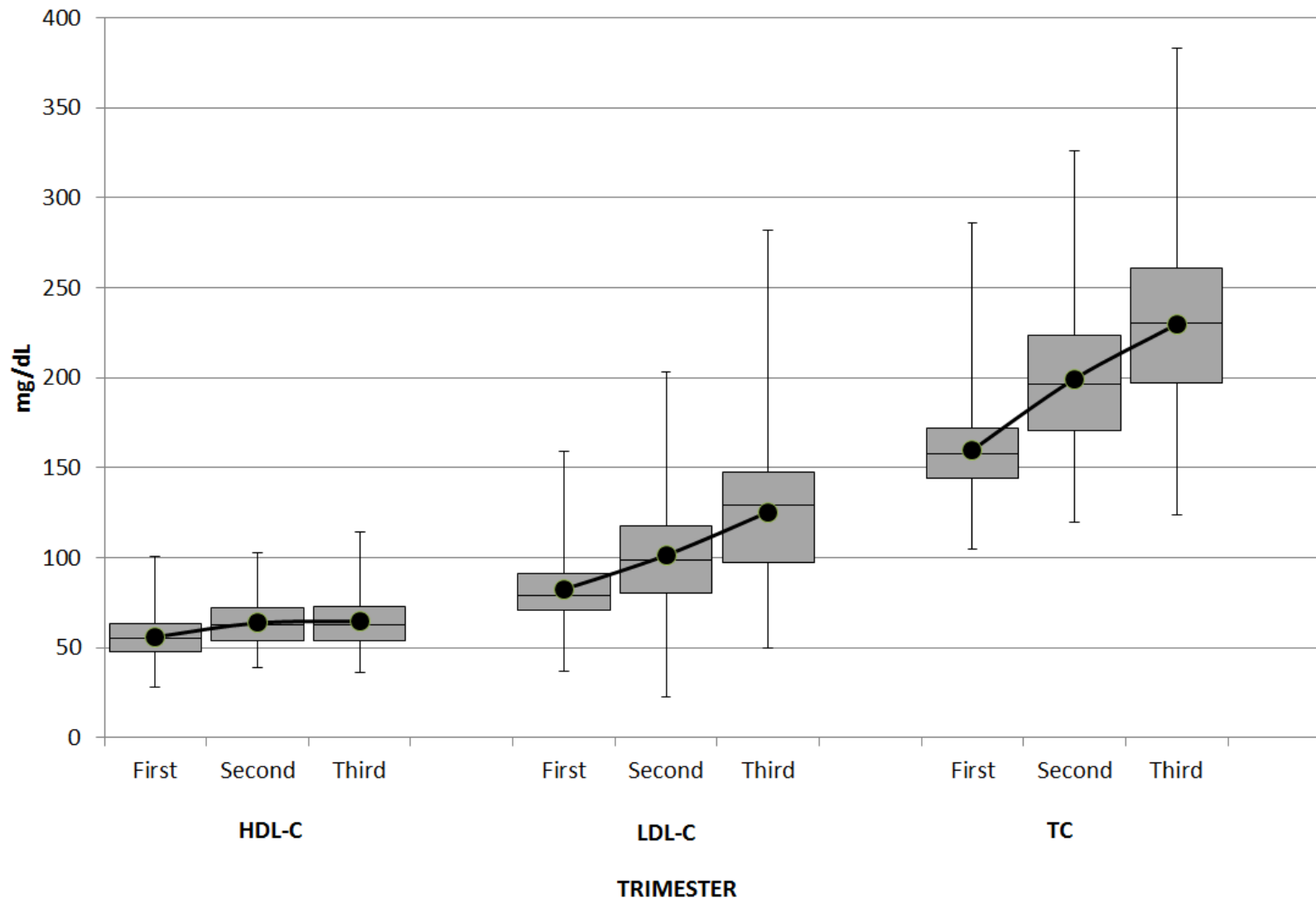
Graphic shows minimum cholesterol level, second quartile, median value, third quartile, and maximum cholesterol value.
HDL-C: high density-lipoprotein cholesterol; LDL-C: low density-lipoprotein cholesterol; TC: total cholesterol

Figure 3.4a: Unpaired HDL-C (mg/dL) levels by gestational week of specimen collection. (n=390 HDL-C data points)
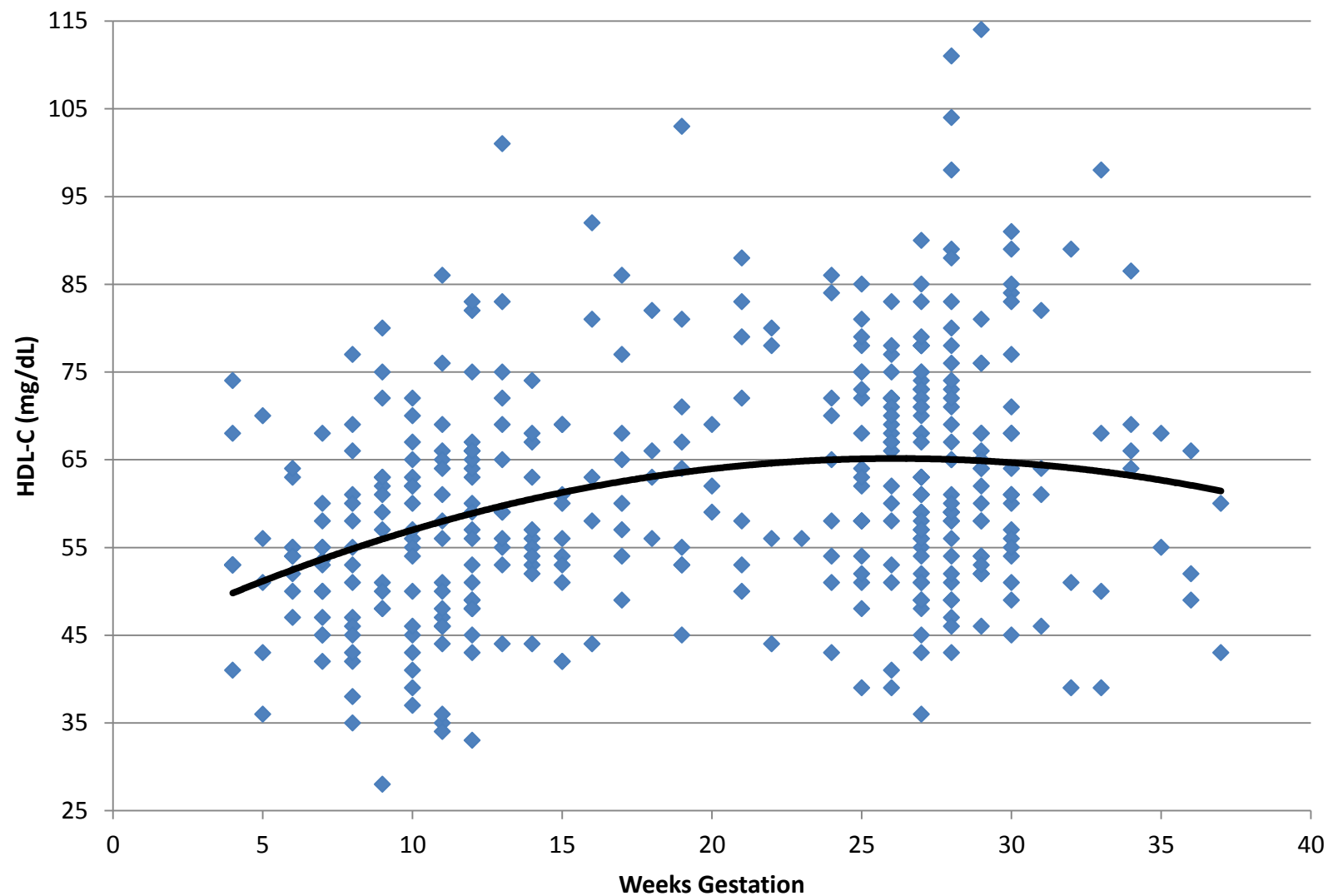
HDL-C: high density-lipoprotein cholesterol

Figure 3.4b: Paired HDL-C levels for each subject by gestational week of specimen collection
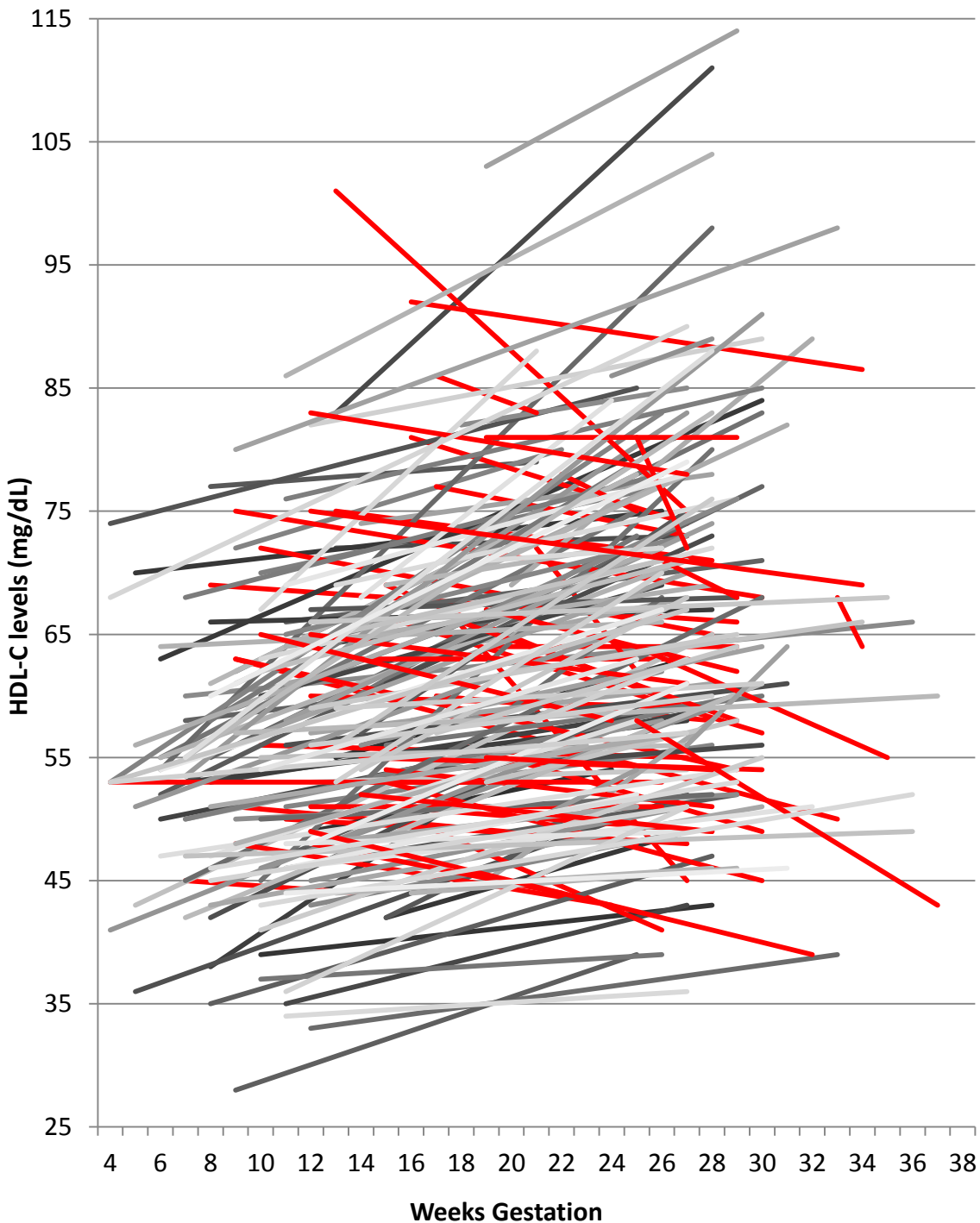
Pairs colored in grey scale indicate an increase in HDL-C levels from the first to second specimen (n=151). Grey scale used to help with visualization. Pairs colored red indicate a decrease in HDL-C levels from the first to second specimen (n=44).

HDL-C: high density-lipoprotein cholesterol

Figure 3.5a: Unpaired LDL-C (mg/dL) levels by gestational week of specimen collection. (n= 378 LDL-C data points)
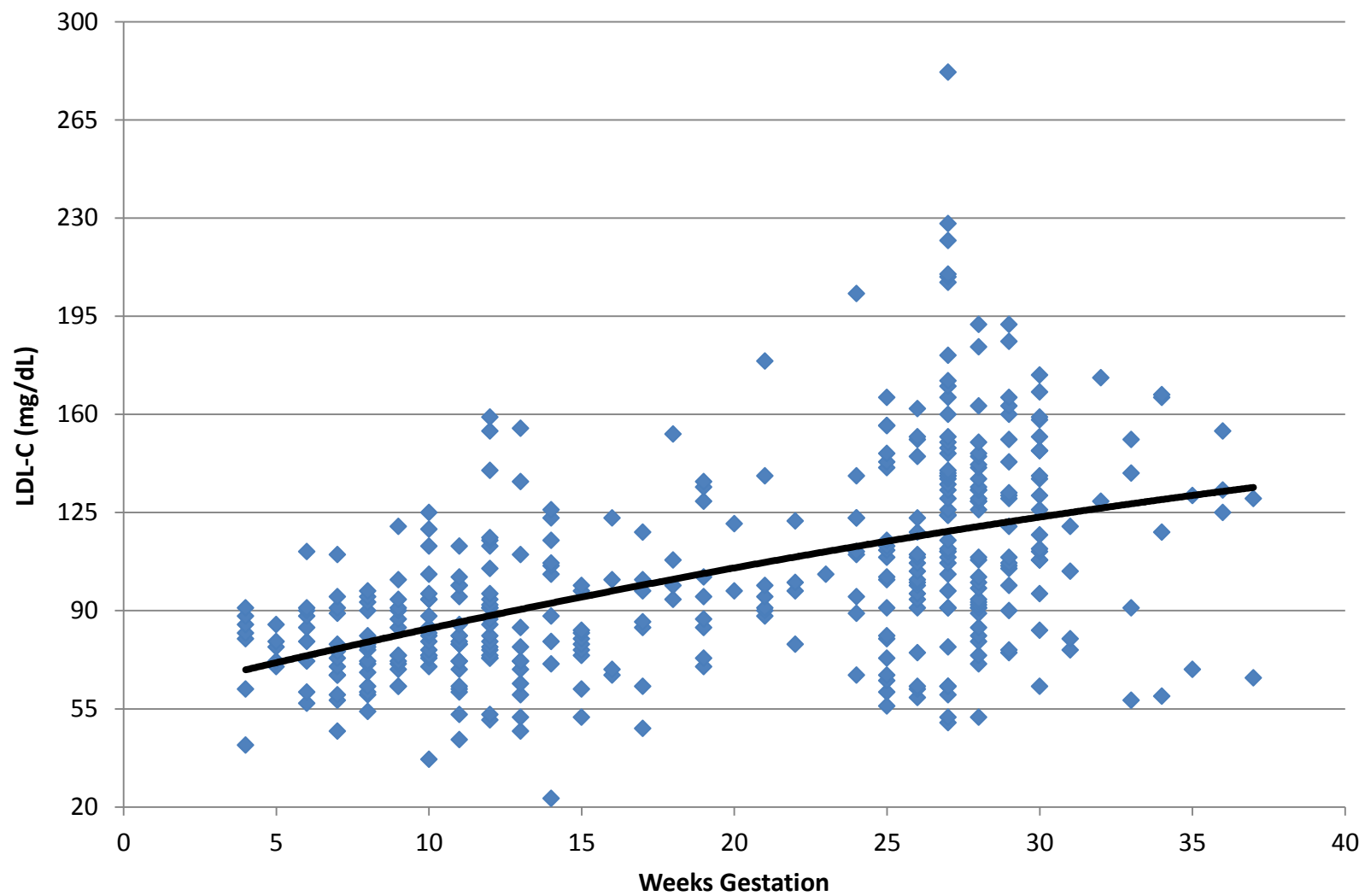
LDL-C: low density-lipoprotein cholesterol

Figure 3.5b: Paired LDL-C levels for each subject by gestational week of specimen collection
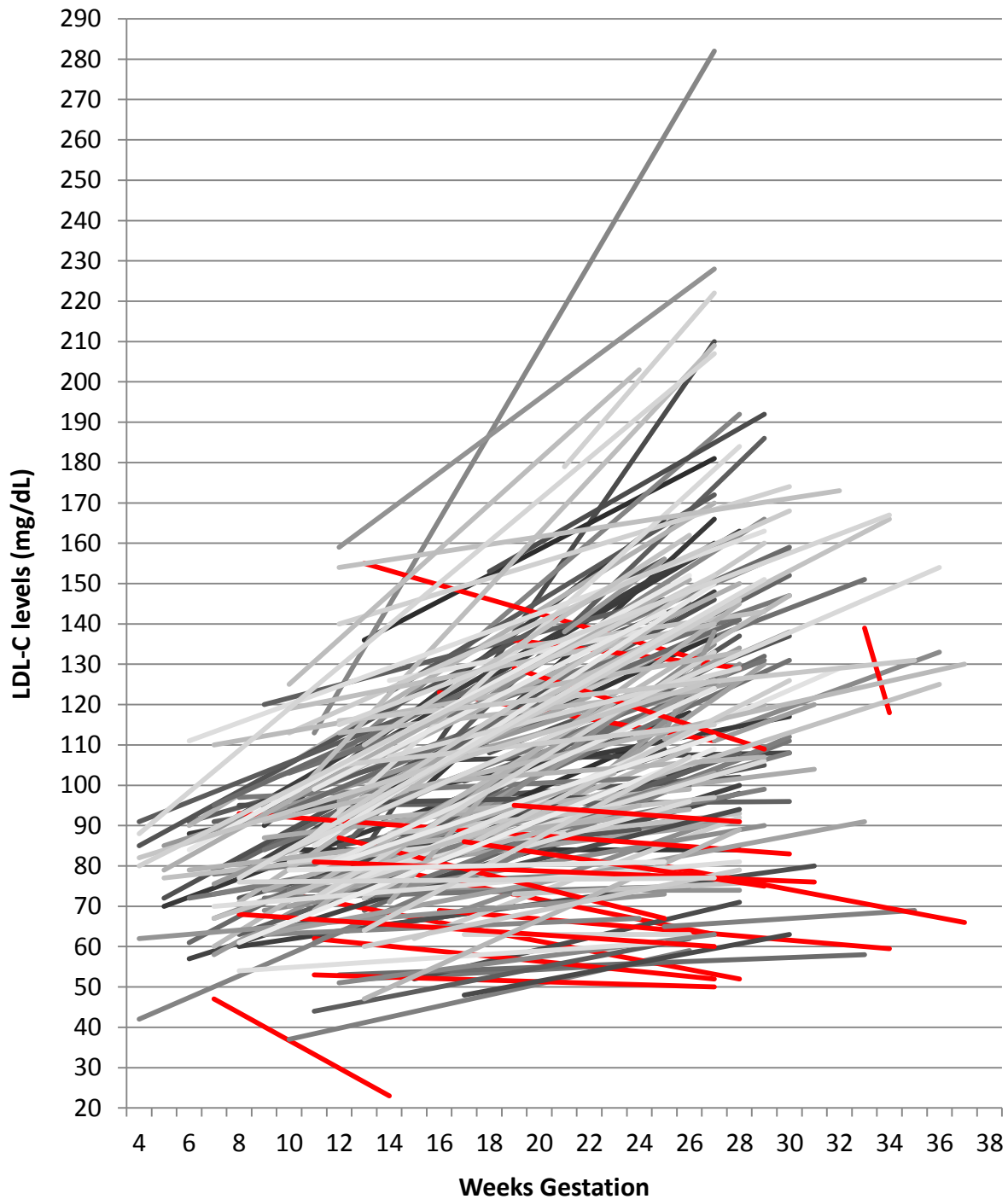
Pairs colored in grey scale indicate an increase in LDL-C levels from the first to second specimen (n=171). Grey scale used to help with visualization. Pairs colored red indicate a decrease in LDL-C levels from the first to second specimen (n=18).
LDL-C: low density-lipoprotein cholesterol

Figure 3.6a: Unpaired TC (mg/dL) levels by gestational week of specimen collection. (n= 390 TC data points)
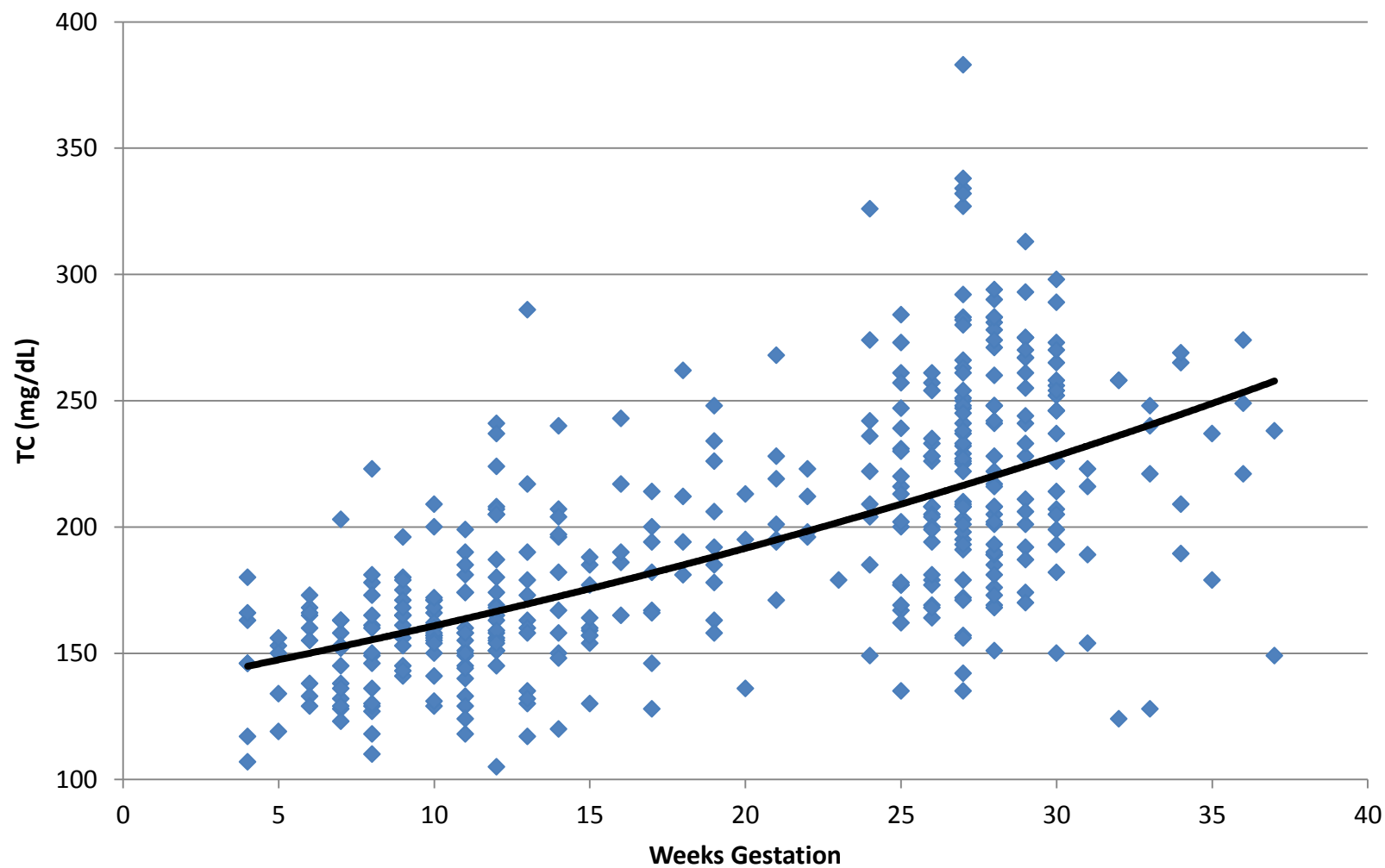
TC: total cholesterol

Figure 3.6b: Paired TC levels for each subject by gestational week of specimen collection
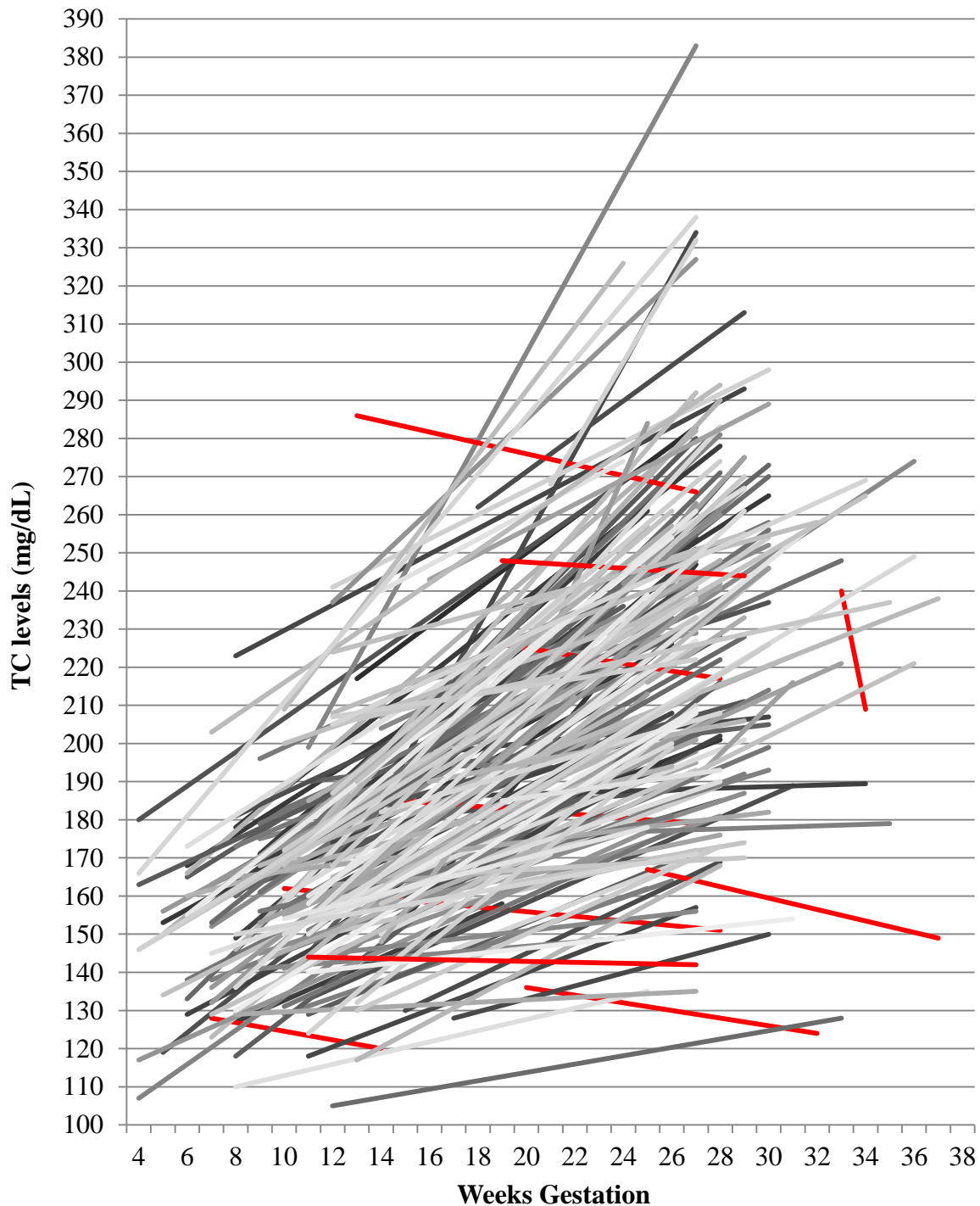
Pairs colored in grey scale indicate an increase in TC levels from the first to second specimen (n=185). Grey scale used to help with visualization. Pairs colored red indicate a decrease in TC levels from the first to second specimen (n=10).

TC: total cholesterol

Figure 3.7a: Average HDL-C levels (mg/dL) in sample population by trimester stratified by maternal race

	1st Trimester	2nd Trimester	3rd Trimester
Black	58	68	67
White	56	63	64
Other	50	66	64
Unknown	58	61	62
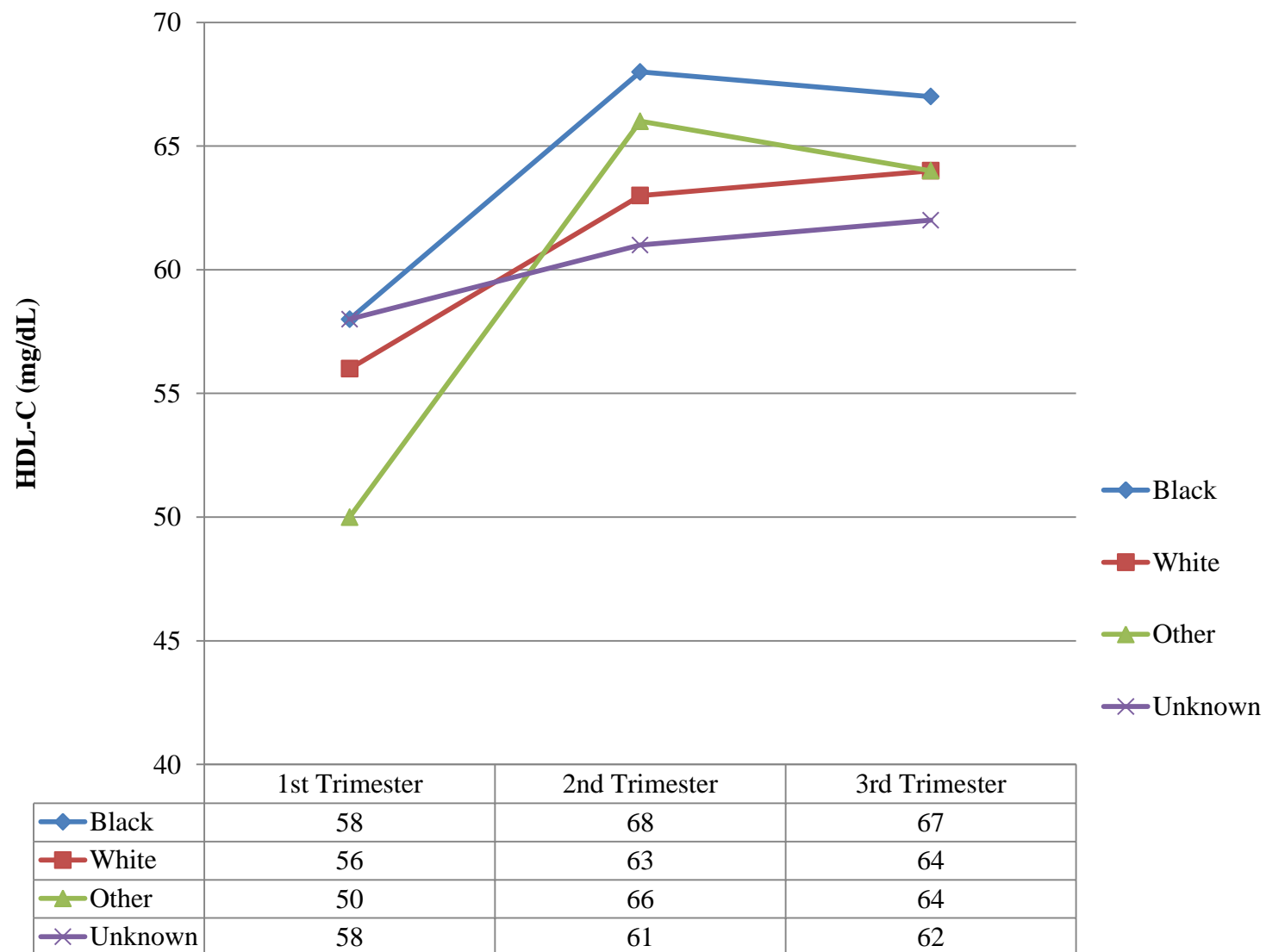
HDL-C: high density-lipoprotein cholesterol

Figure 3.7b: Average LDL-C levels (mg/dL) in sample population by trimester stratified by maternal race

	1st Trimester	2nd Trimester	3rd Trimester
Black	81	89	114
White	83	105	129
Other	78	105	115
Unknown	83	89	143
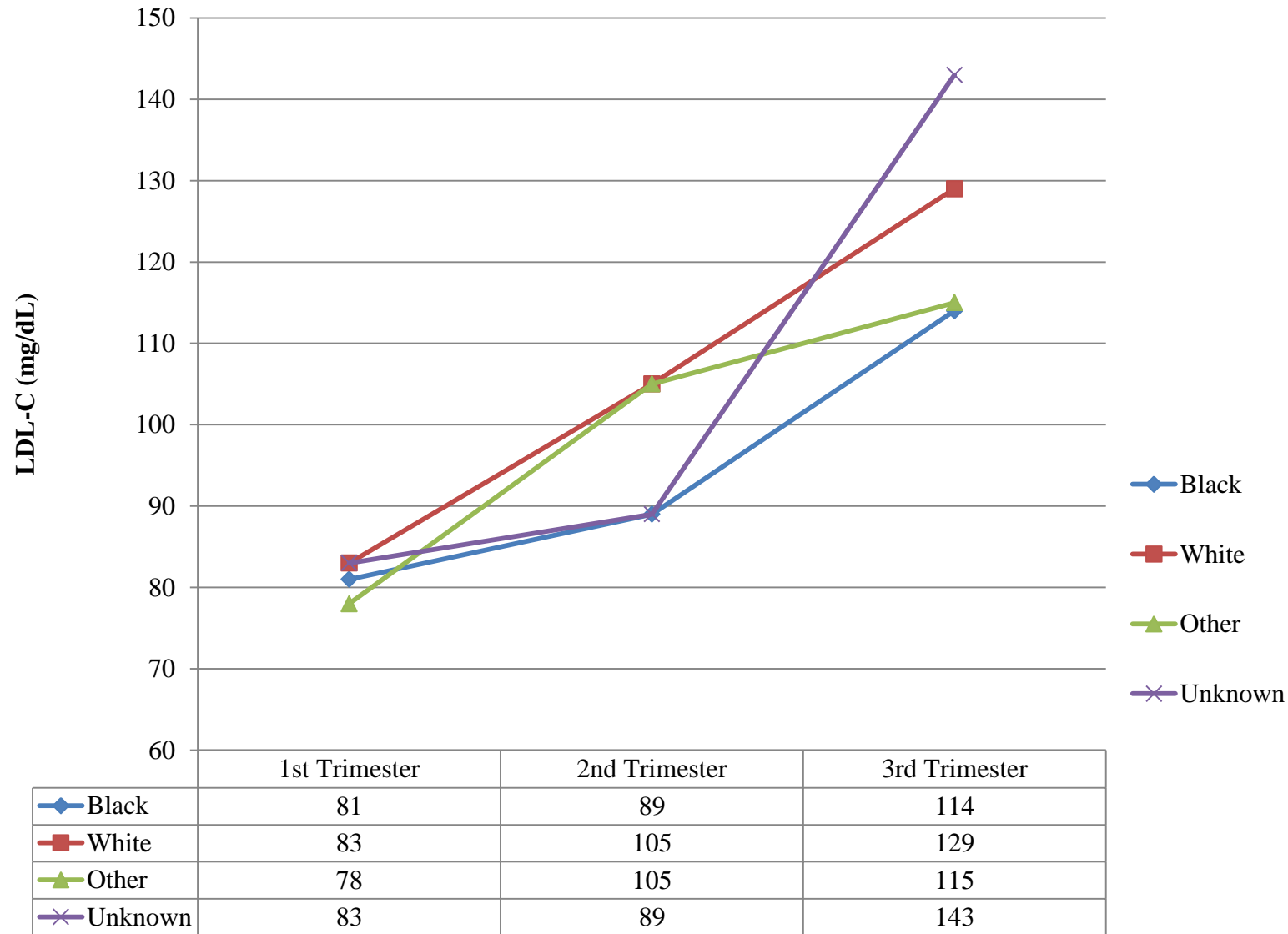
LDL-C: low density-lipoprotein cholesterol

Figure 3.7c: Average TC levels (mg/dL) in sample population by trimester stratified by maternal race

	1st Trimester	2nd Trimester	3rd Trimester
Black	154	185	214
White	162	204	235
Other	150	202	218
Unknown	165	186	249
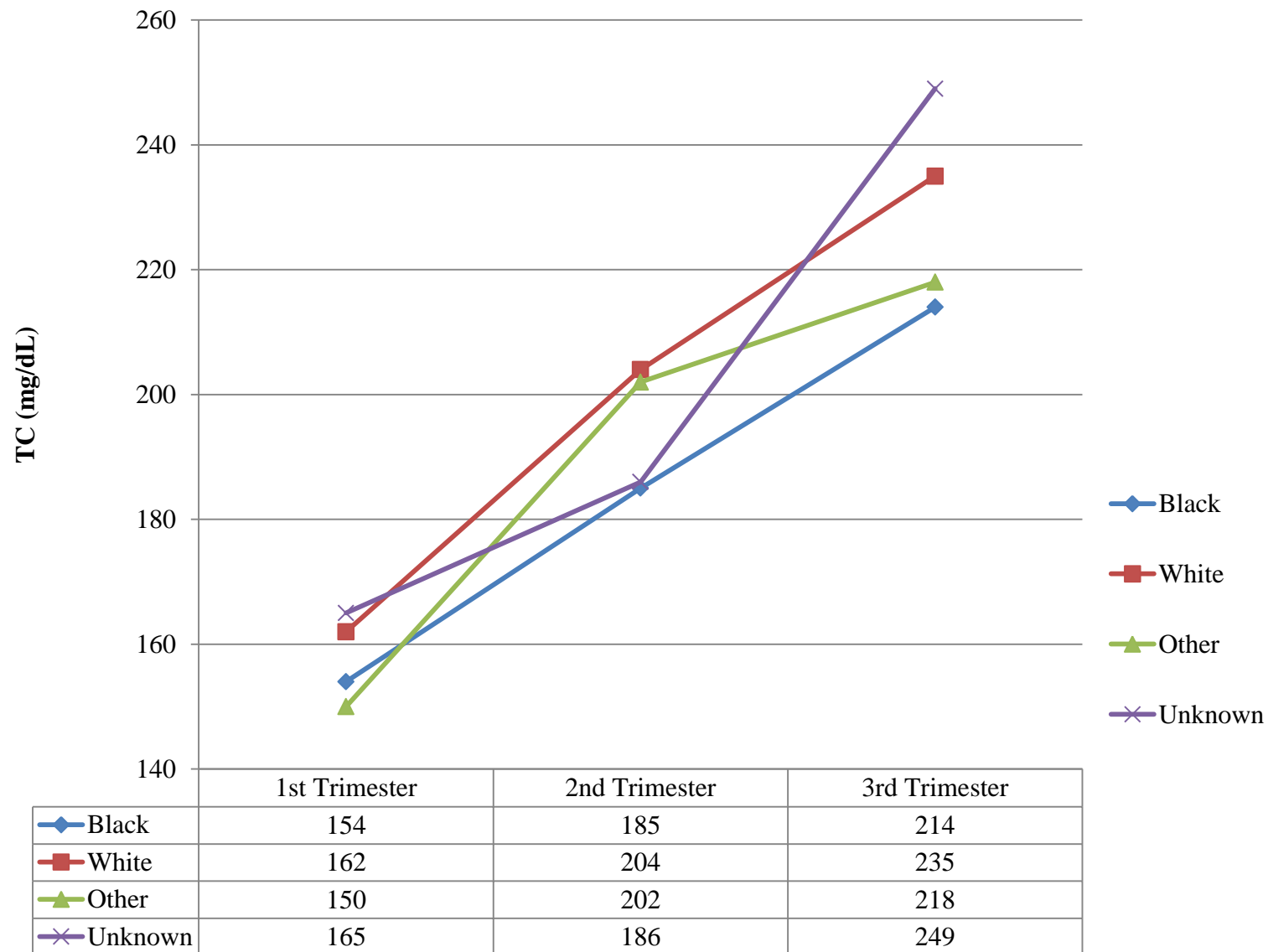
TC: total cholesterol

Figure 3.8a: Average HDL-C (mg/dL) levels in sample population by trimester stratified by maternal ethnicity

	1st Trimester	2nd Trimester	3rd Trimester
Non-Hispanic/Latino	56	64	64
Hispanic/Latino	58	62	70
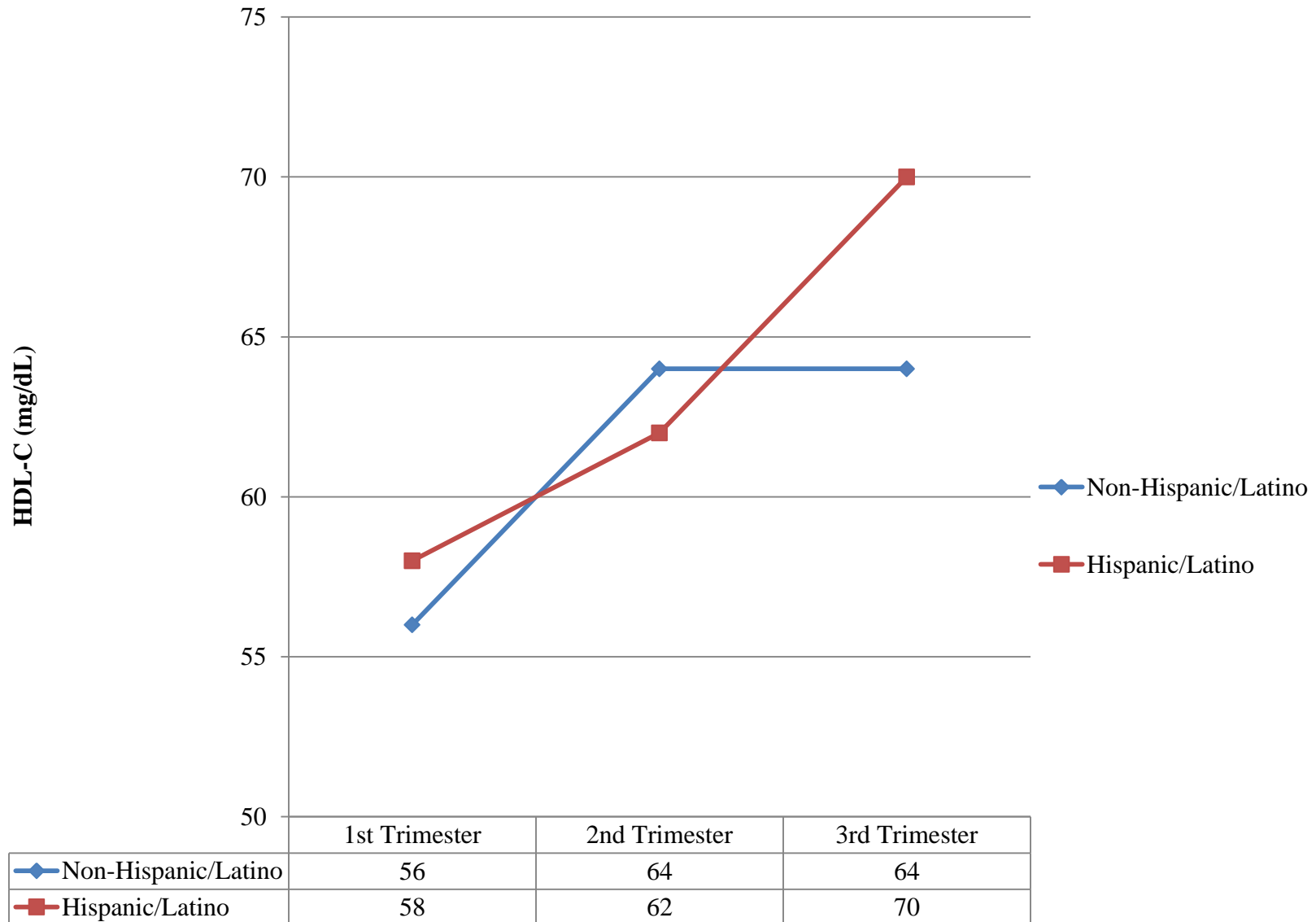
HDL-C: high density-lipoprotein cholesterol

Figure 3.8b: Average LDL-C (mg/dL) levels in sample population by trimester stratified by maternal ethnicity

	1st Trimester	2nd Trimester	3rd Trimester
Non-Hispanic/Latino	83	101	125
Hispanic/Latino	80	104	127
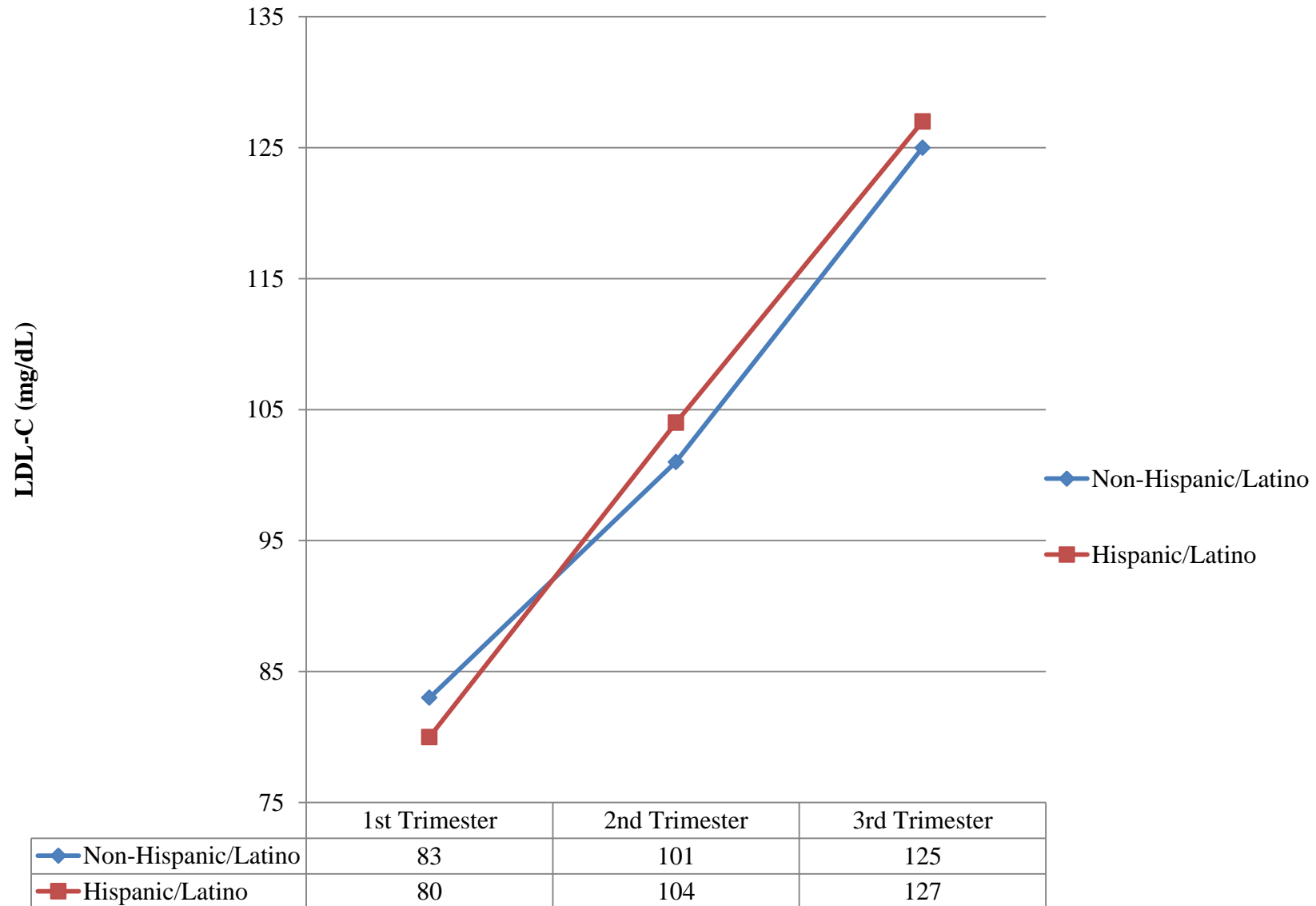
LDL-C: low density-lipoprotein cholesterol

Figure 3.8c: Average TC (mg/dL) levels in sample population by trimester stratified by maternal ethnicity

	1st Trimester	2nd Trimester	3rd Trimester
Non-Hispanic/Latino	160	199	229
Hispanic/Latino	162	204	237
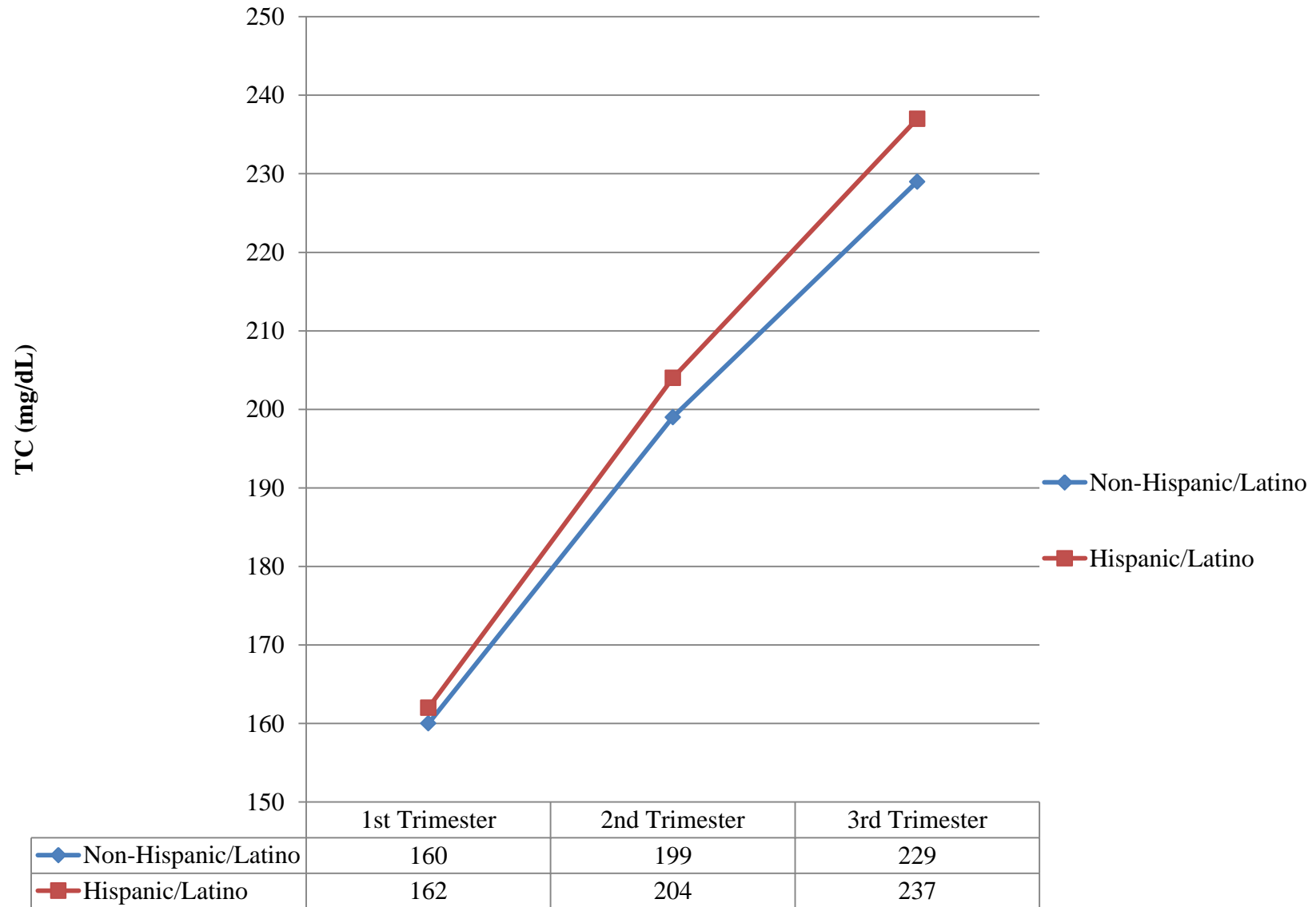
TC: total cholesterol

Figure 3.9a: Average HDL-C (mg/dL) levels in sample population by trimester stratified by maternal age

	1st Trimester	2nd Trimester	3rd Trimester
18-24 years old	55	61	64
24-30 years old	56	67	65
31+ years old	58	66	66
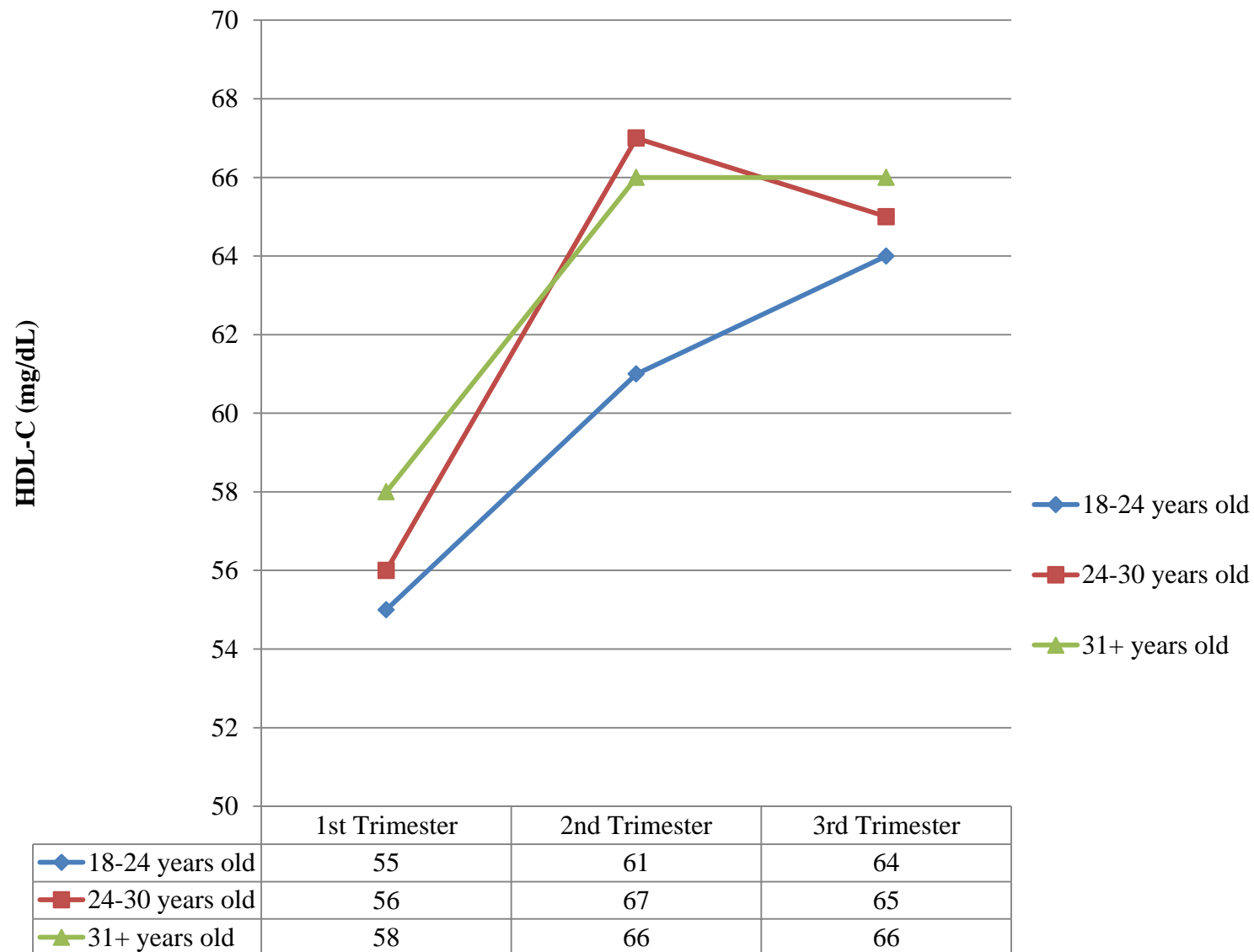
HDL-C: high density-lipoprotein cholesterol

Figure 3.9b: Average LDL-C (mg/dL) levels by trimester stratified by maternal age

	1st Trimester	2nd Trimester	3rd Trimester
18-24 years old	86	104	126
24-30 years old	79	95	118
31+ years old	84	105	143
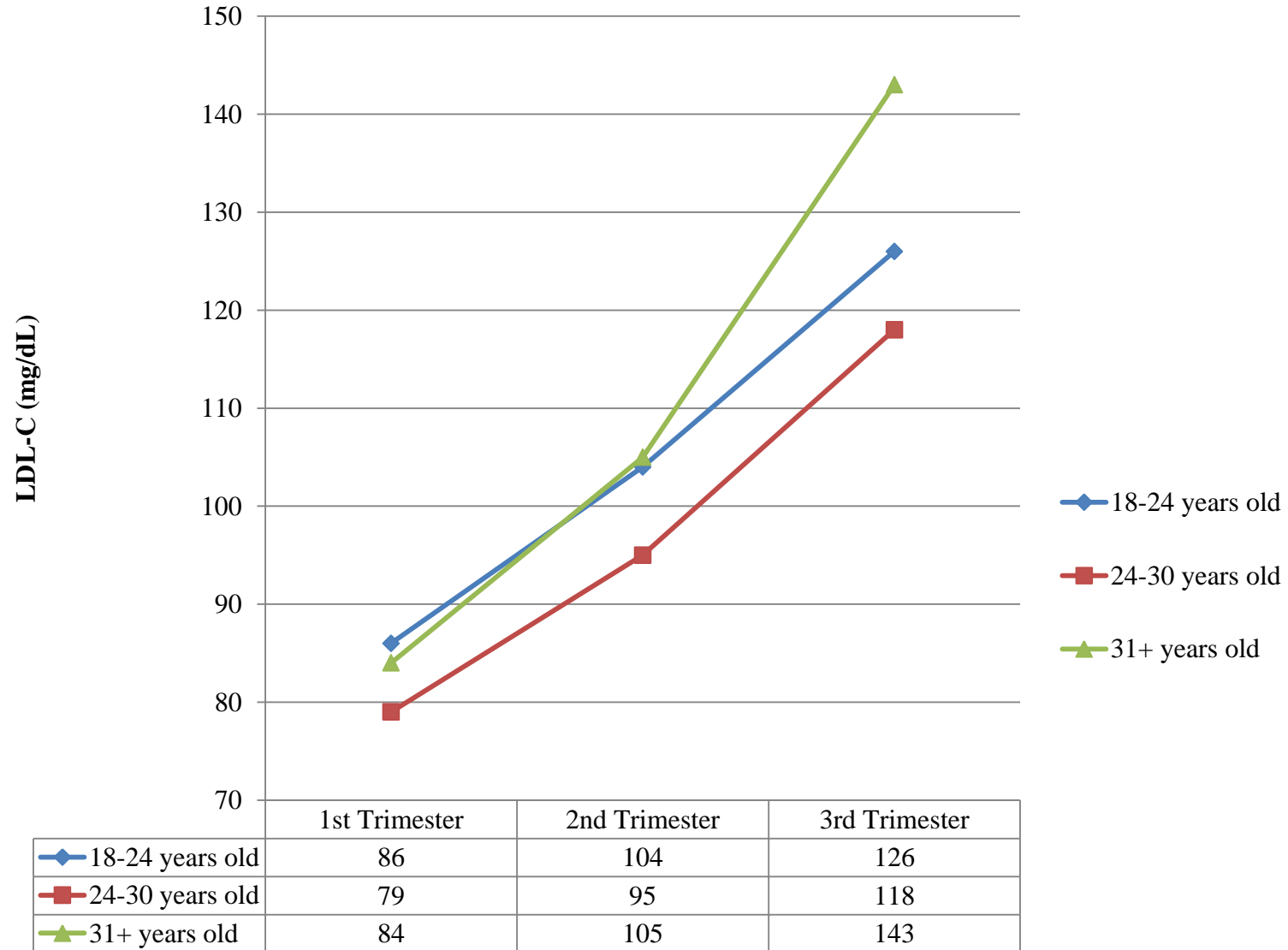
LDL-C: low density-lipoprotein cholesterol

Figure 3.9c: Average TC (mg/dL) levels in sample population by trimester stratified by maternal age

	1st Trimester	2nd Trimester	3rd Trimester
18-24 years old	161	199	226
24-30 years old	158	197	226
31+ years old	161	203	252
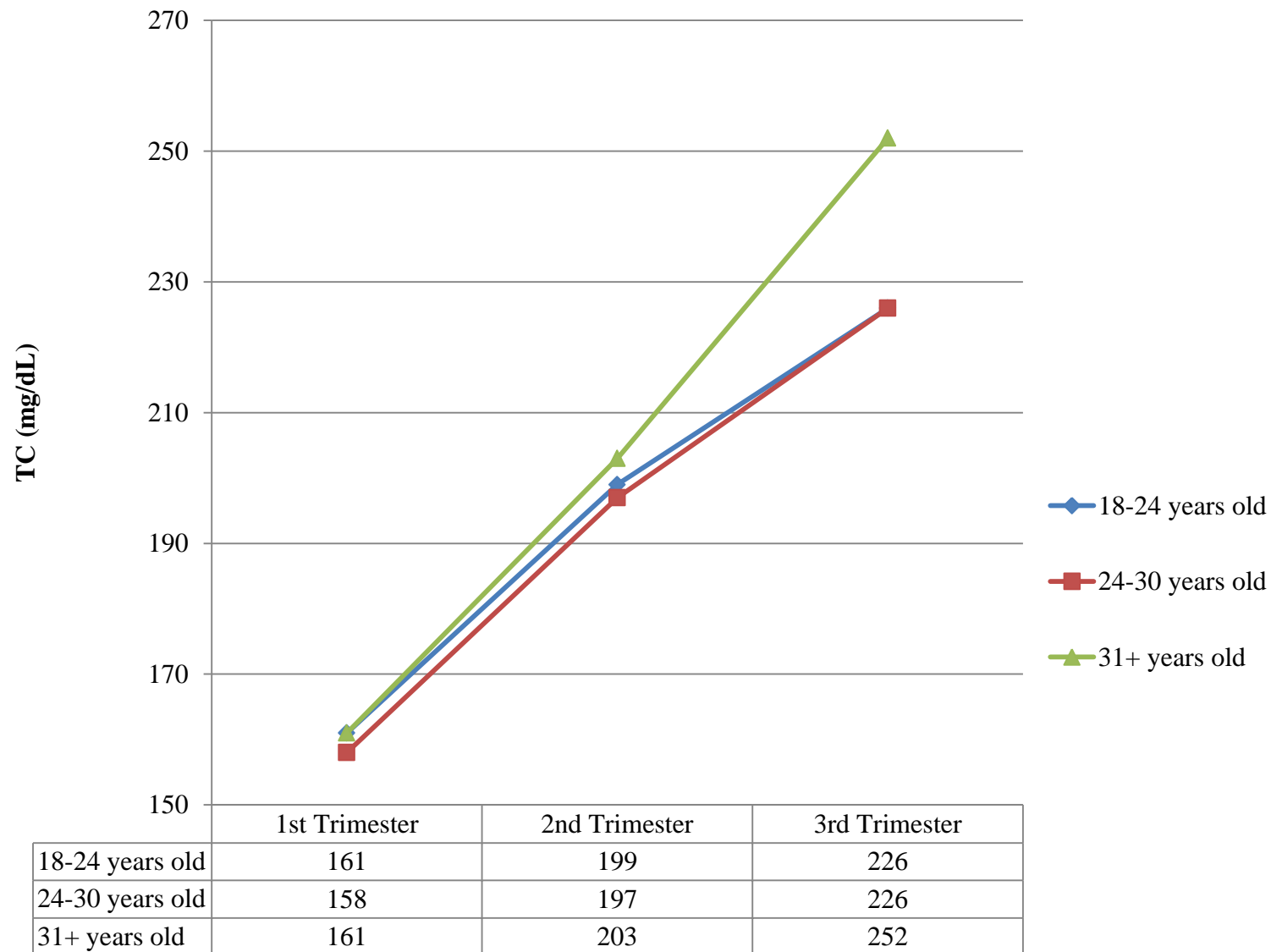
TC: total cholesterol

Figure 3.10a: Average HDL-C (mg/dL) levels in sample population by trimester stratified by maternal pre-pregnancy BMI

	1st Trimester*	2nd Trimester	3rd Trimester**
< 25	60	65	69
>= 25	53	63	62
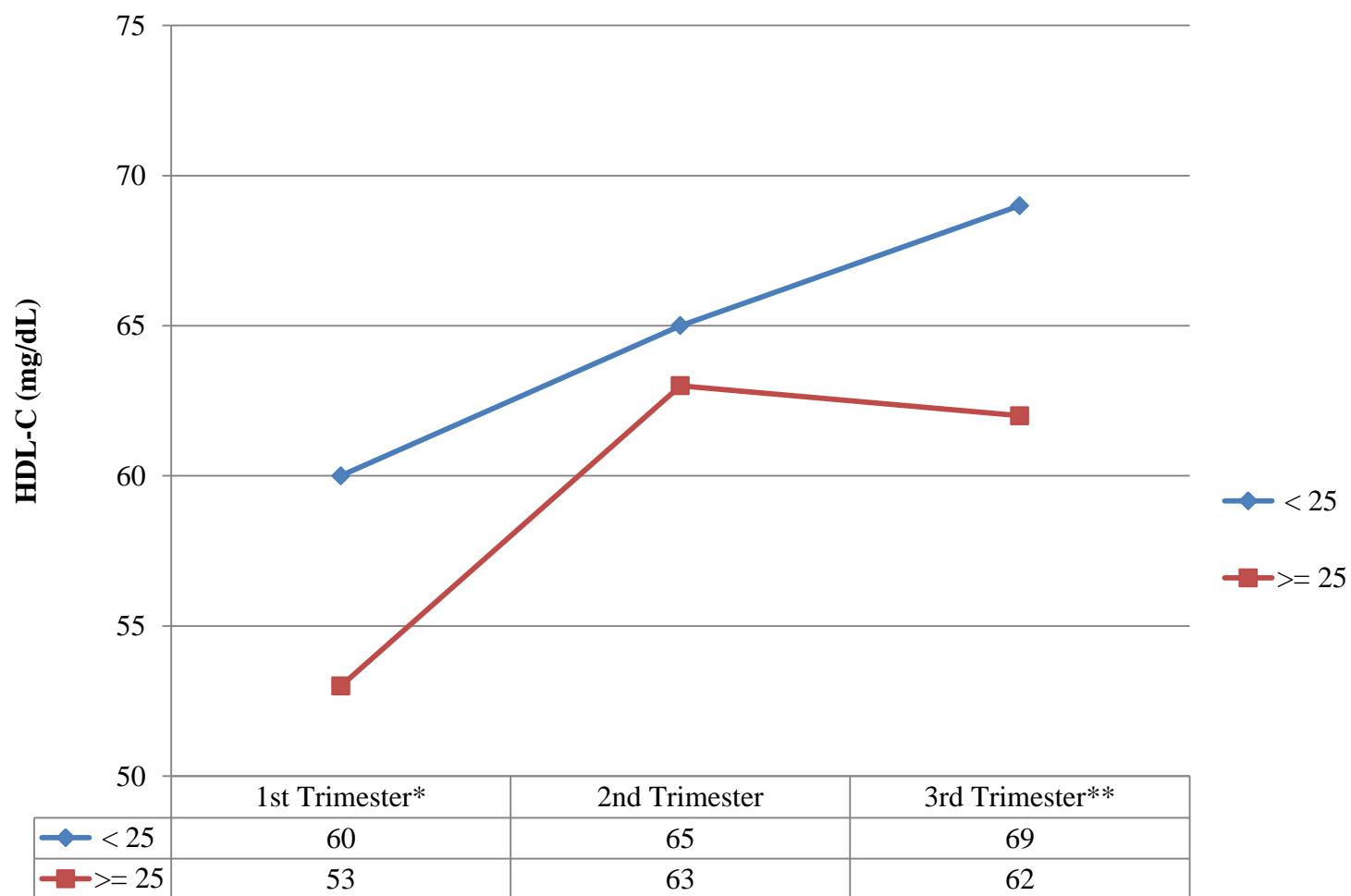
HDL-C: high density-lipoprotein cholesterol; BMI: body mass index
HDL-C levels statistically different across BMI categories
* p-value =0.001; ** p-value = 0.01

Figure 3.10b: Average LDL-C (mg/dL) levels in sample population by trimester stratified by maternal pre-pregnancy BMI

	1st Trimester	2nd Trimester*	3rd Trimester
< 25	83	109	132
>= 25	82	96	121
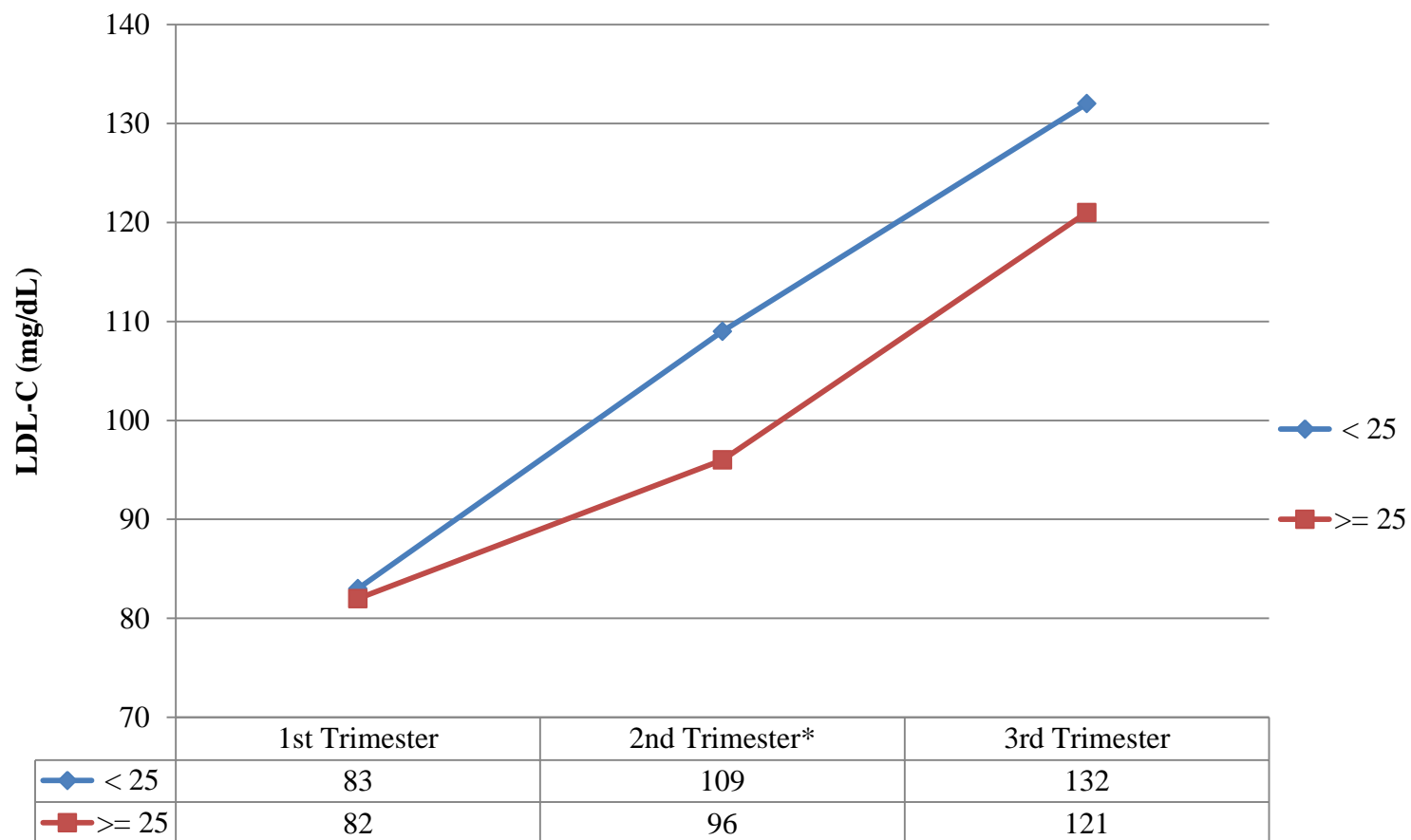
LDL-C: low density-lipoprotein cholesterol; BMI: body mass index
LDL-C levels statistically different across BMI categories
* p-value =0.03

Figure 3.10c: Average TC (mg/dL) levels in sample population by trimester stratified by maternal pre-pregnancy BMI

	1st Trimester	2nd Trimester*	3rd Trimester**
< 25	162	210	240
>= 25	158	192	223
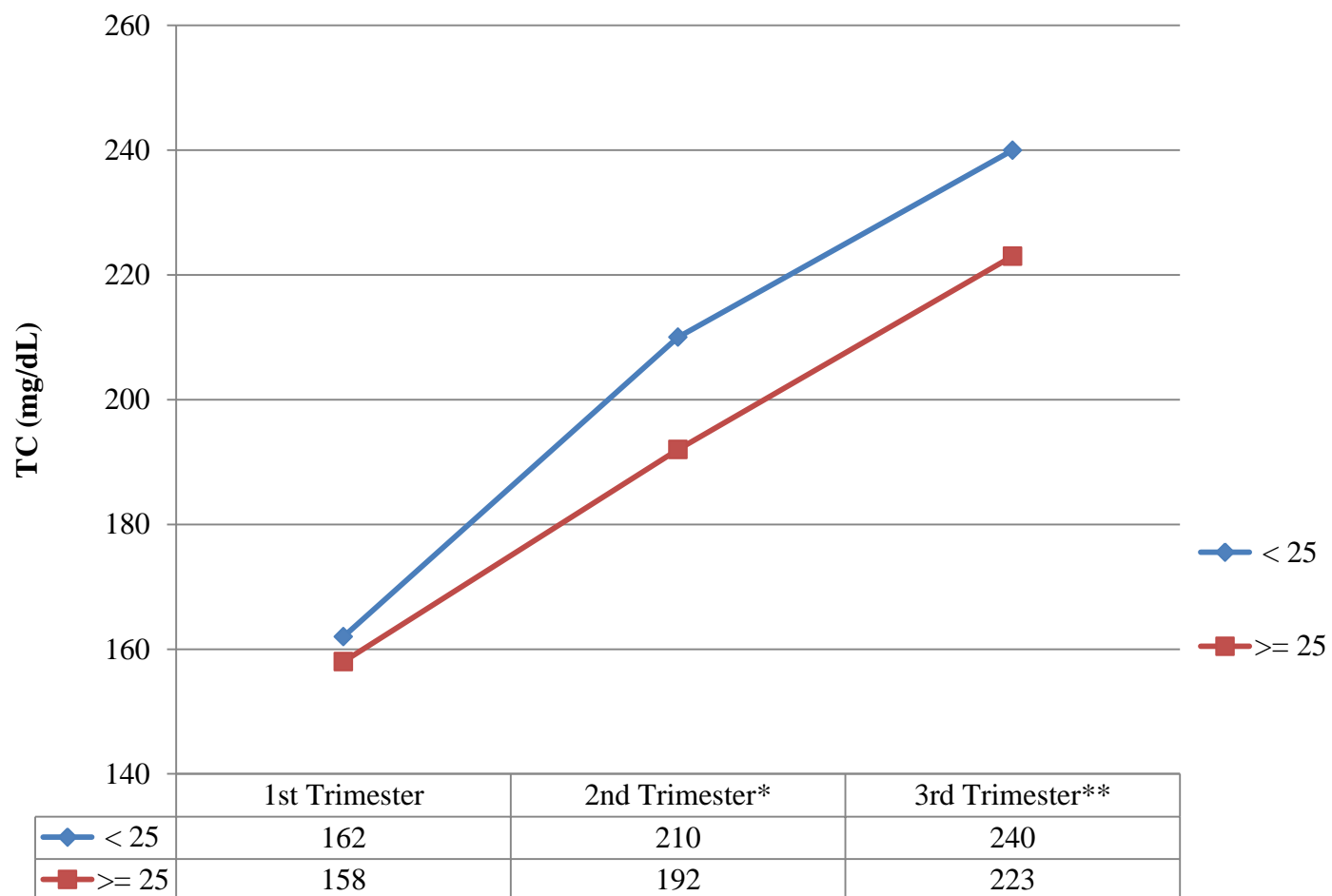
TC: total cholesterol; BMI: body mass index
TC levels statistically different across BMI categories
* p-value =0.009; ** p-value = 0.03

Figure 3.11a: Average HDL-C (mg/dL) levels in sample population by trimester stratified by maternal postpartum BMI

	1st Trimester	2nd Trimester	3rd Trimester
<18.5			
18.5- <25	57	63	65
25- <30	56	63	67
>= 30	56	64	64
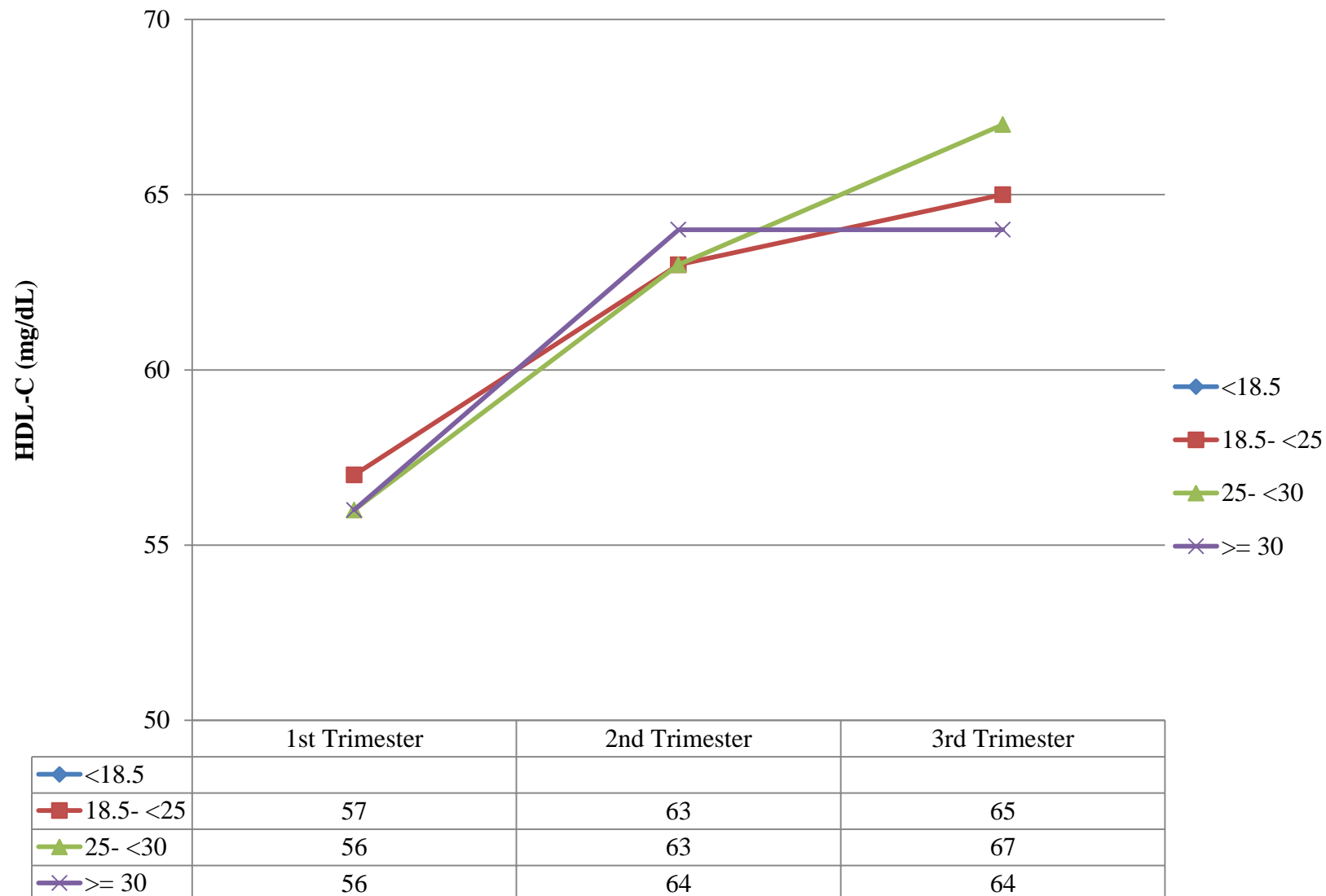
HDL-C: high density-lipoprotein cholesterol; BMI: body mass index

Figure 3.11b: Average LDL-C (mg/dL) levels in sample population by trimester stratified by maternal postpartum BMI

	1st Trimester	2nd Trimester*	3rd Trimester
<18.5			
18.5- <25	80	114	136
25- <30	84	111	133
>= 30	83	96	121
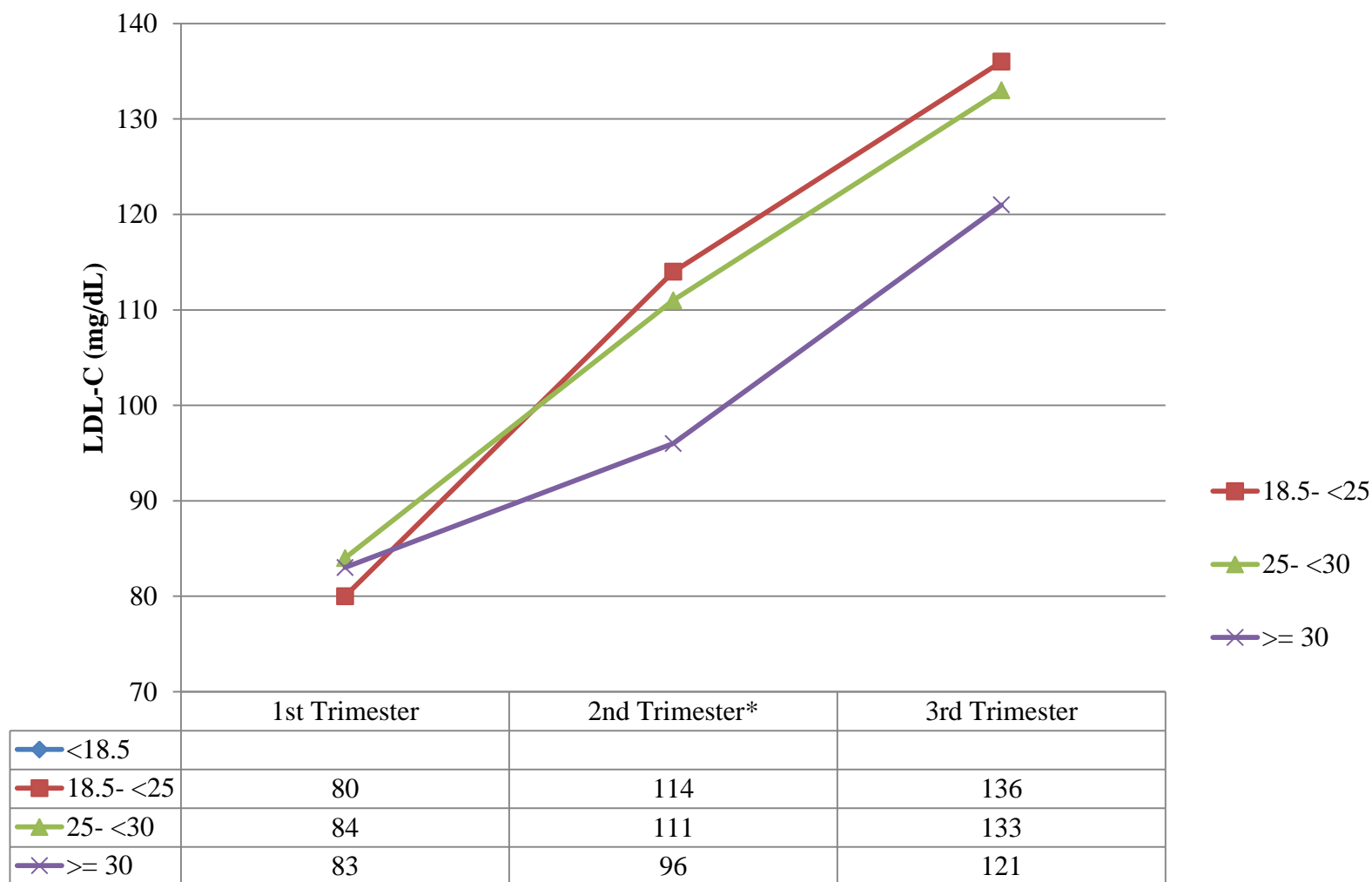
LDL-C: low density-lipoprotein cholesterol; BMI: body mass index
LDL-C levels statistically different across BMI categories
* p-value =0.001

Figure 3.11c: Average TC (mg/dL) levels in sample population by trimester stratified by maternal postpartum BMI

	1st Trimester	2nd Trimester	3rd Trimester
◆ <18.5			
■ 18.5- <25	160	209	234
▲ 25- <30	159	209	237
✳ >= 30	161	194	226
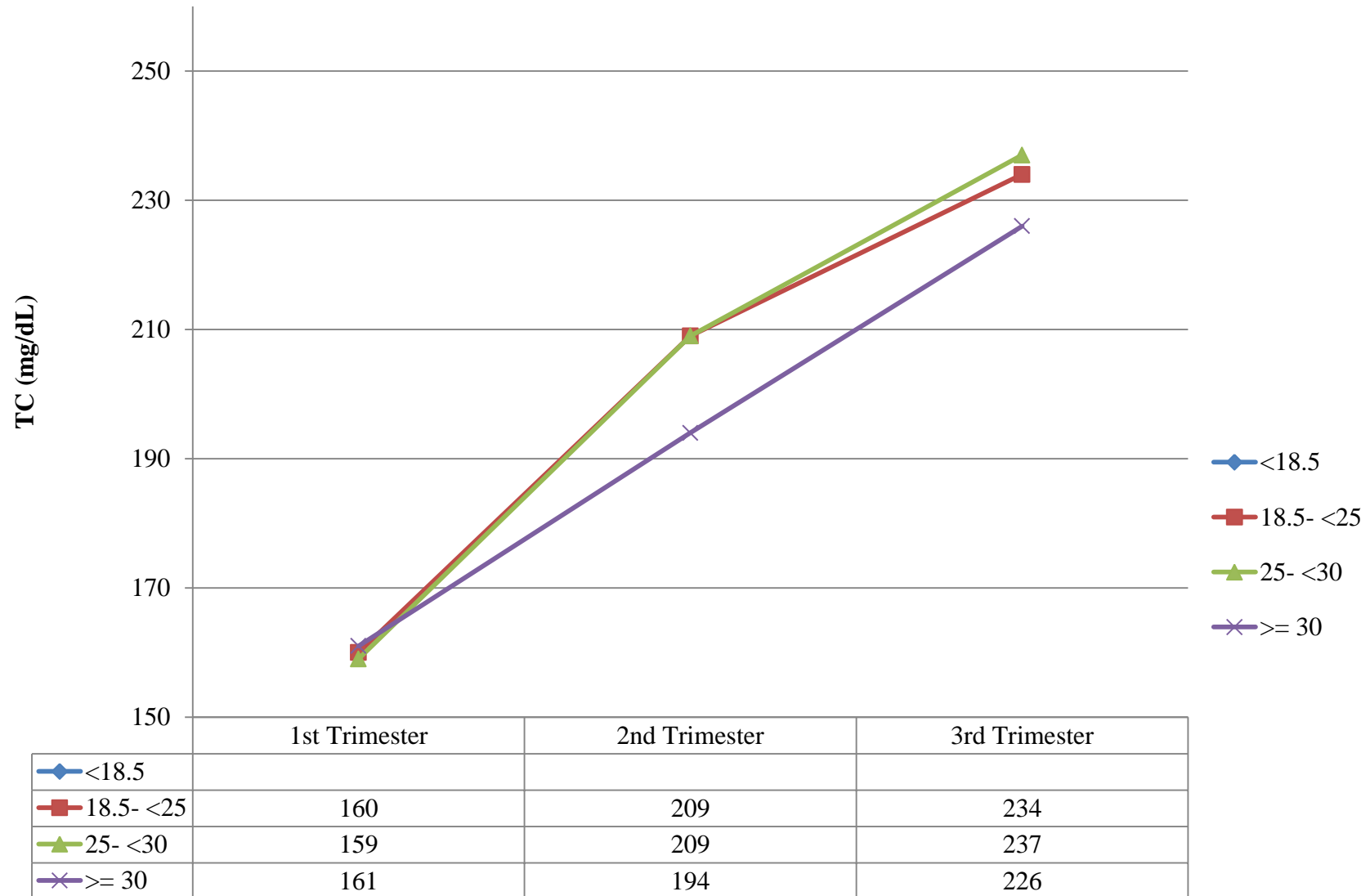
TC: total cholesterol; BMI: body mass index

Figure 3.12a: Average HDL-C (mg/dL) levels in sample population by trimester stratified by if this was mom's first pregnancy

	1st Trimester	2nd Trimester	3rd Trimester
Not First Pregnancy	56	65	67
First Pregnancy	56	62	63
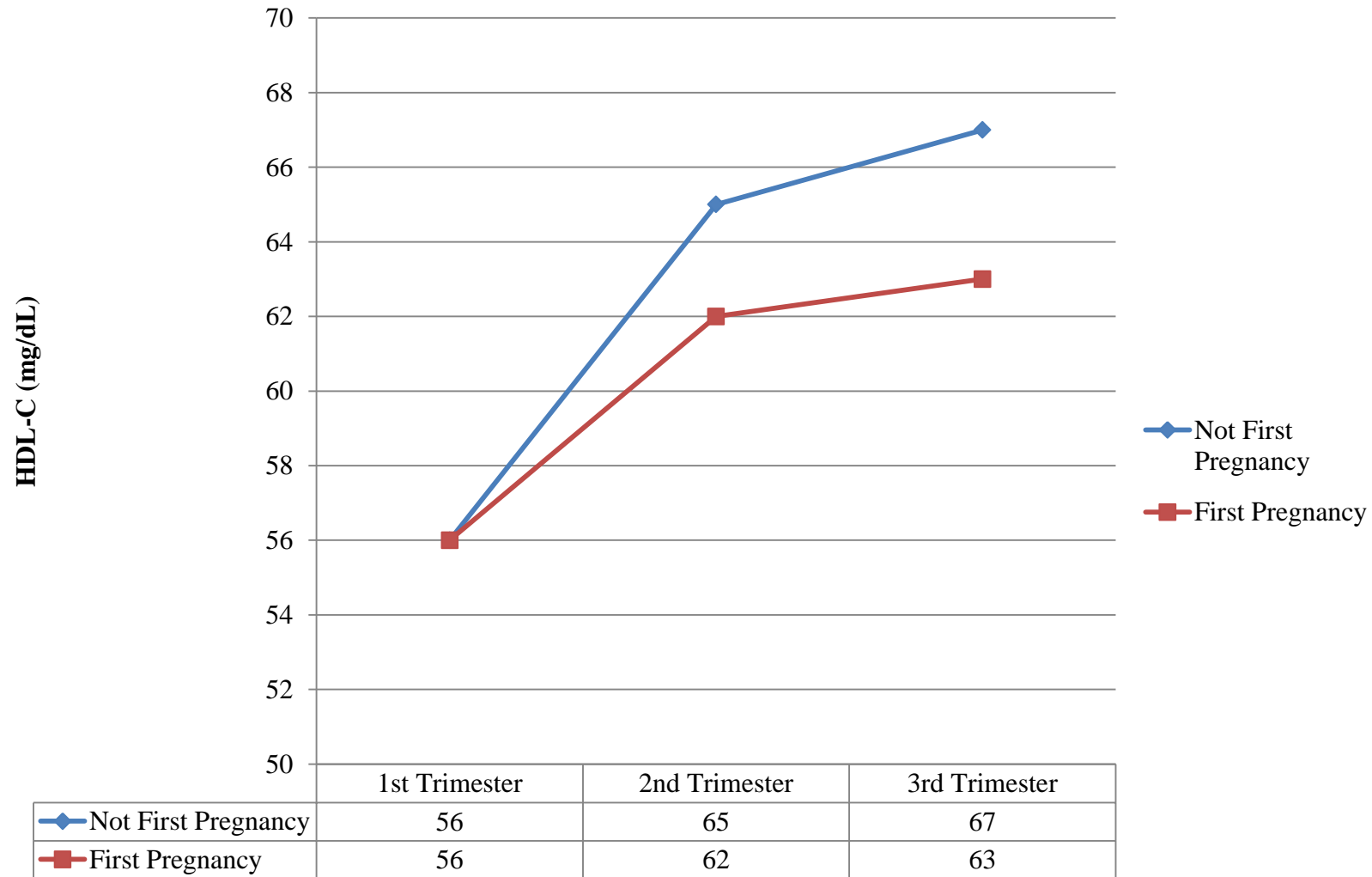
HDL-C: high density-lipoprotein cholesterol

Figure 3.12b: Average LDL-C (mg/dL) levels in sample population by trimester stratified by if this was mom's first pregnancy

	1st Trimester	2nd Trimester	3rd Trimester
Not First Pregnancy	80	99	124
First Pregnancy	84	105	127
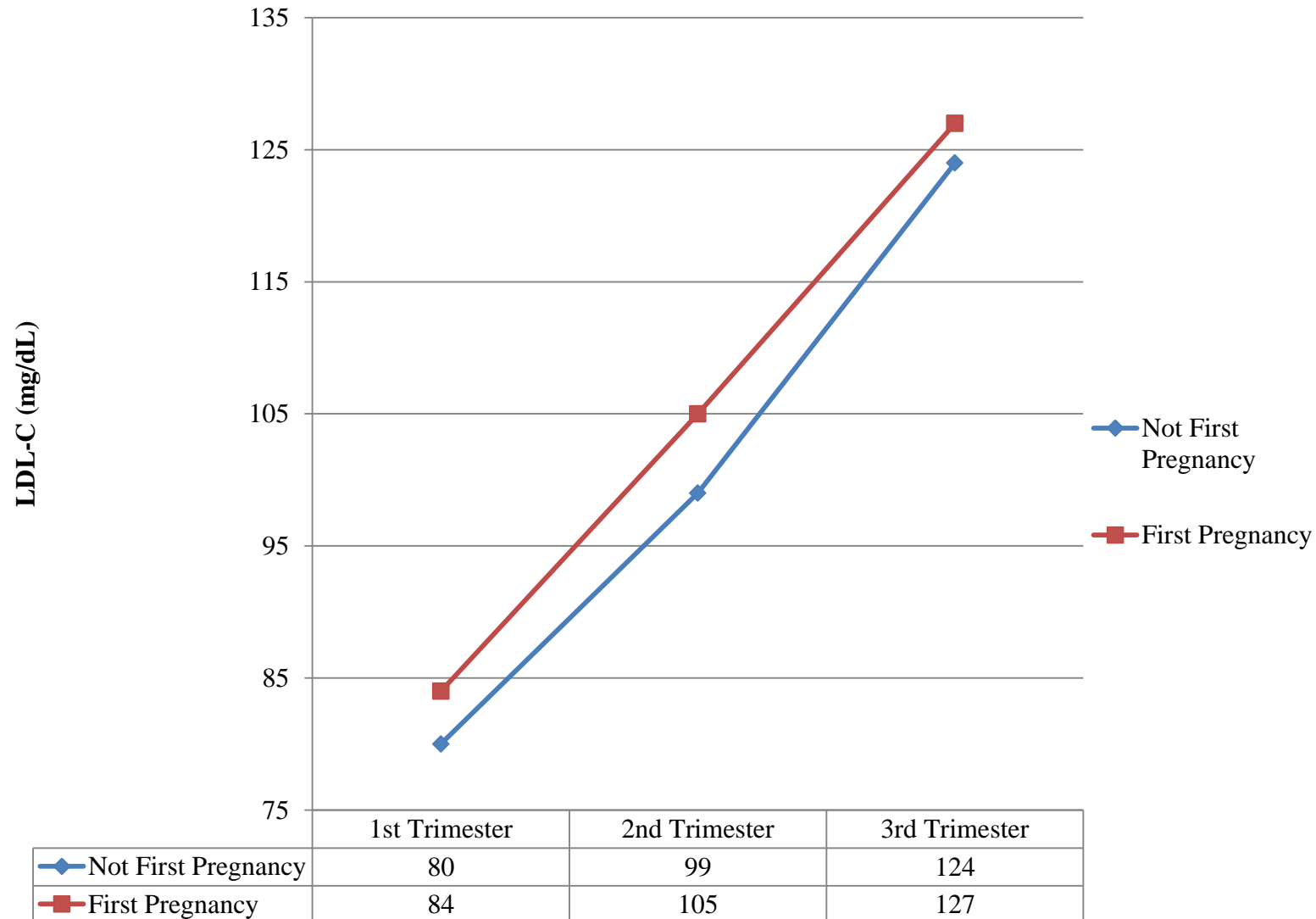
LDL-C: low density-lipoprotein cholesterol

Figure 3.12c: Average TC (mg/dL) levels in sample population by trimester stratified by if this was mom's first pregnancy

	1st Trimester	2nd Trimester	3rd Trimester
Not First Pregnancy	157	196	228
First Pregnancy	163	204	232
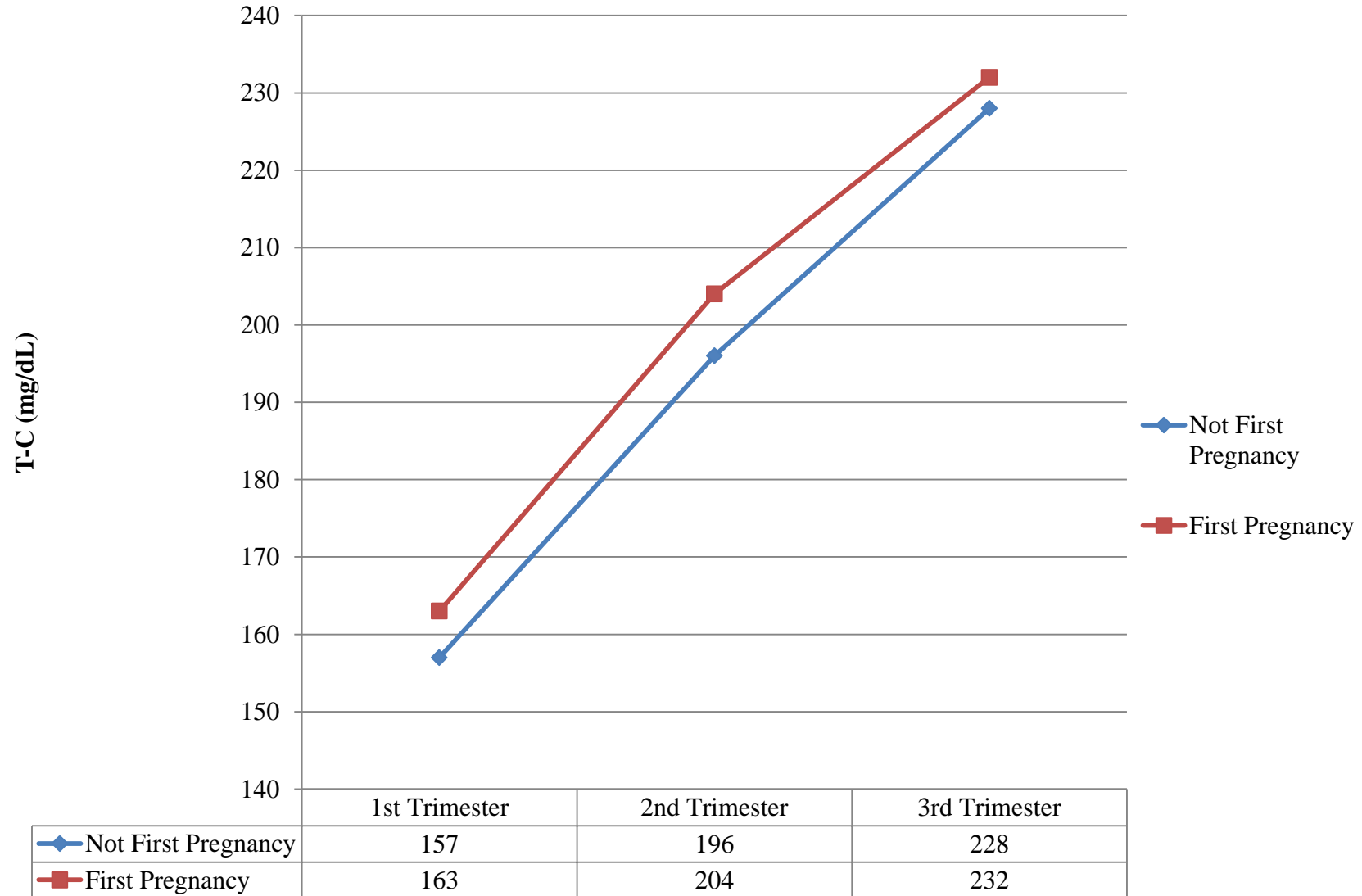
TC: total cholesterol

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CHAPTER FOUR
RELATIONSHIP BETWEEN CHANGES IN MATERNAL CHOLESTEROL DURING
GESTATION AND FETAL GROWTH

INTRODUCTION

Fetal growth complications are among the most common adverse outcomes of pregnancy.

Infants born small for gestational age (SGA) are at an increased risk of morbidity and mortality.

Infants born large for gestational age (LGA) are also at an increased risk of morbidity and

mortality. These complications, in both SGA and LGA infants, are often chronic and are thought

to be the cause of health complexities beginning at birth and extending into adulthood. SGA

infants are at a greater risk of fetal demise, neonatal mortality, and cognitive delay. SGA infants

are also at an increased risk of coronary artery disease, and stroke later in life [1 – 5]. LGA

infants have an increased risk of injury during birth, hypoglycemia after delivery, and

development of type two diabetes and obesity later in life [6 – 8]. Fetal growth complications,

SGA and LGA, are multifactorial and some research suggests their causes might be grouped into

three main categories, maternal insufficiencies, fetal insufficiencies, and placental insufficiencies

[5, 9].

It is well documented that during pregnancy maternal cholesterol levels undergo

significant changes. Research suggests that these are adaptive changes essential for proper fetal

development and growth [10 – 13]. In the developing fetus, cholesterol maintains cell integrity

and structure and also activates patterning proteins that have a role in the development of

essential organs [2, 14 – 15]. As discussed in the *Maternal Cholesterol and Fetal Growth* section

of chapter two of this dissertation, research proposes the relationship between maternal

cholesterol during gestation and SGA and LGA infants may reflect placental complications;

however, the exact relationship and biological mechanisms are still being studied. Previous studies, looking at maternal cholesterol levels at a single time point during pregnancy, have described a relationship between maternal cholesterol during gestation and fetal growth complications, although these findings are inconclusive.

Research assessing the relationship between maternal cholesterol at a single time point during pregnancy and adverse fetal outcomes may be missing valuable information related to the change in maternal cholesterol over time during pregnancy. Changes in maternal cholesterol may capture different biological responses to pregnancy compared to cholesterol levels from a single time point and may provide insight into an infant's risk of abnormal fetal growth. The aim of the proposed research is to study the relationship between change in maternal cholesterol levels (total cholesterol (TC), low density-lipoprotein cholesterol (LDL-C), and high density-lipoprotein cholesterol (HDL-C)) between two specimens collected during pregnancy and fetal growth. This will be one of the first studies to look at the relationship between changes in maternal cholesterol rather than maternal cholesterol levels at a single time point during pregnancy. The relationship between the exposure, change in maternal cholesterol levels, and both SGA infants and LGA infants will be studied.

METHODS

Study Population

This analysis was conducted using information from women participating in the Archive for Research on Child Health (ARCH) study. The ARCH study is described in greater detail in chapter 3 of this dissertation. Women were eligible for this analysis if they were active in the ARCH study, had an expected date of confinement, due date, on or before December 31, 2013,

and had a serum specimen collected at two different time points during pregnancy. 201 women were eligible for this analysis. ARCH study participants with singleton pregnancies and available birth certificate data were included in this analysis. 195 women were included in this analysis. Figure 3.2 in chapter 3 can be referenced for additional information on how the included population was selected. The demographics of the women included in this study are summarized in table 4.1.

Of note, the maternal questionnaire used to enroll participants into the ARCH study provided six different categories for maternal race, American Indian/Alaska Native, Native Hawaiian/Pacific Islander, Asian, Multiracial, White, and Black. For this analysis, given the small number of subjects in the Black, American Indian/Alaska Native, Native Hawaiian/Pacific Islander, Asian, and Multiracial categories, race was categorized into two groups, White (n=127) and non-White (n=54). Information on maternal race was missing from the enrollment questionnaire for 14 subjects.

Cholesterol

Per ARCH protocol, all serum specimens were sent to a laboratory at Michigan State University (MSU) and stored at -80° C. The 195 women included in this study had first and second serum samples archived in 500µl aliquots. Aliquots of the first and second specimen for each of the 195 participants were thawed, refrigerated, and transported to the chemical laboratory at Sparrow Hospital laboratories, in Lansing, Michigan, for cholesterol testing. All of the cholesterol testing procedures abided by the National Cholesterol Education Program performance criteria. Testing methods for TC were restricted to levels between 25 mg/dL and 700 mg/dL. HDL-C levels were valid from 2 mg/dL – 200 mg/dL. None of the women studied

for cholesterol levels, had TC or HDL-C levels outside of the testing range. LDL-C levels were calculated in the lab using the Friedwald equation, formula 4.1 [16].

Formula 4.1:

$$Total\ Cholesterol - [(High\ Density\ Lipoprotein\ Cholesterol) + \left(\frac{Triglycerides}{5}\right)]$$

Invalid LDL-C measurements occurred when triglyceride levels were greater than 400 mg/dL [16]. Five women had triglycerides greater than 400 mg/dL for either their first or second specimen. One participant had a second trimester LDL-C value of negative 7 mg/dL. These six specimens were excluded from LDL-C analyses. 189 of the 195 study participants were included in the LDL-C analysis.

The main exposure of interest for this analysis was the change in maternal cholesterol (TC, LDL-C, and HDL-C) levels between the first and second specimen. This percent change will be calculated individually for TC, LDL-C, and HDL-C using formula 4.2.

Formula 4.2:

$$\frac{2nd\ cholesterol\ level\ - 1st\ cholesterol\ level}{1st\ cholesterol\ level} \times 100$$

The first serum specimen was to be collected at the time of enrollment. Of the women included in this analysis, 71% had their first specimen collected during the first trimester. 28% of women included in this analysis had their first specimen collected during the second trimester. Two women, 1%, had their first specimen collected during the third trimester. The average gestational age at the time of collection for the first serum specimen was 12 weeks. Second trimester serum specimens were to be collected at the time of the glucose tolerance testing although this was not always possible due to missed visits or participants and staff forgetting to send the specimen to the MSU laboratory. 29% of women included in this analysis had their second specimen

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collected during the second trimester. The remaining 71% of women included in this analysis had their second specimen collected in the third trimester. The average gestational age at the time of collection for the second serum specimen was 28 weeks.

Fetal growth

The primary outcome of interest for this chapter was fetal growth, analyzed as a continuous variable and as a categorical variable. For this dissertation, fetal growth was calculated using birthweight, corrected gestational age at birth, and sex of the infant, all obtained from the birth certificate. Using methodologies described elsewhere in the literature, a corrected gestational age was calculated for each of the 195 ARCH participants [17 – 18]. In short, the clinical estimate of gestational age was compared to the calculated gestational age using last menstrual period (LMP) recorded on the birth certificate. If both LMP and clinical estimate were available and both estimates were within two weeks of each other, the LMP was compared to the birth weight z-score to see if plausible. A plausible z-score for term births was between negative five and five. A plausible z-score for preterm births was between negative four and three. If plausible, LMP estimate was used. If implausible, the clinical estimate for gestational age was compared to the birth weight z-score. If the clinical estimate was within an acceptable range, the clinical estimate for gestational age was used in place of LMP. The acceptable range for term births was a z-score between negative five and five. For preterm births, the acceptable z-score range was between negative three and two. If the discrepancy between LMP and clinical estimate for gestational age was greater than two weeks, the clinical estimate was first examined in relation to the birth weight z-score. LMP was used if in range for the birth weight z-score and if the clinical estimate was not in range for the birth weight z-score. In the study population, corrected gestational age ranged from 32 weeks gestation to 43 weeks gestation.

To assess fetal growth continuously, birth weight z-scores were used (formula 4.3). Expected birth weights were calculated using the population standard from the 2009 and 2010 US live birth files maintained by the National Center for Health Statistics [17]. Table 4.2 summarizes the expected birthweights for male and female infants ranging in gestational age from 32 weeks to 42 weeks.

Formula 4.3:

$$\frac{Observed\ birth\ weight - Expected\ birth\ weight}{Birth\ weight\ standard\ deviation\ from\ population\ standard}$$

To measure fetal growth as a categorical variable, infants were categorized as SGA, average for gestational age (AGA), or LGA. SGA infants were those whose birth weight was in the lowest 10th percentile for gestational age and sex. LGA infants were those whose birth weight was in the highest 10th percentile for gestational age and sex.

Analytics

Multiple regression models, using the purposeful selection process, were generated to investigate the proposed hypotheses. The purposeful selection process is described in detail elsewhere in the literature and outlined below [19]. SAS, version 9.2, was utilized for all analyses. Six different models were developed following the purposeful selection methodology to address three different hypotheses. These models include analyzing the relationship between changes in LDL-C and fetal growth as both a categorical and a continuous variable, changes in HDL-C and fetal growth as both a categorical and continuous variable, and changes in TC and fetal growth again as a categorical and continuous variable. Below, analytic methods have been broken out into two sections, one for fetal growth as a categorical variable and the second for fetal growth as a continuous variable.

Hypothesis 1a: Women with rates of change in LDL-C from first to second specimen in the lowest quartile will give birth to infants with decreased measures of fetal growth.

Hypothesis 1b: Women with rates of change in TC from first to second specimen in the lowest quartile will give birth to infants with decreased measures of fetal growth.

Hypothesis 2a: Women with rates of change in LDL-C from first to second specimen in the highest quartile will give birth to infants with increased measures of fetal growth.

Hypothesis 2b: Women with rates of change in TC from first to second specimen in the highest quartile will give birth to infants with increased measures of fetal growth.

Hypothesis 3: Changes in maternal HDL-C from first to second specimen will not be significantly associated with infants being in the highest or lowest quartile for fetal growth.

Fetal Growth- Continuous

Step one of the purposeful selection methodology was to run a univariate analysis between important covariates, as identified in the literature, and the outcome of interest, fetal growth. Covariates with a type three analysis of effects chi-square p-value less than or equal to 0.25 in the univariate models were included in the multivariate model building. If any covariates had a chi-square p-value greater than 0.25 but were thought to have a clinically significant relationship with fetal growth then they were included in the model building phase. For this step, maternal race, postpartum body mass index (BMI), net maternal weight gain during pregnancy, maternal education level, and household income all had significant relationships with fetal growth. Net maternal weight gain was calculated as the maternal weight at delivery minus self-reported maternal pre-pregnancy weight minus the birthweight of the baby. Net maternal weight gain was used instead of maternal weight gain, calculated as weight at delivery minus self-reported pre-pregnancy weight, as literature suggests when looking at two variables where one is

a sum containing the other (birthweight and maternal weight gain), bias can be introduced resulting in an inflated correlation between the two variables [20]. Since fetal growth is a measure that includes birthweight, net maternal weight gain was used instead of maternal weight gain to reduce bias. Maternal pre-pregnancy BMI did not have a significant relationship with fetal growth in this patient population (p-value = 0.41). This univariate analysis is summarized in table 4.3.

The next step, summarized in tables 4.4a, 4.4b, and 4.4c, was to run a multiple linear regression model including the main independent variable, change in maternal cholesterol, and the five significant covariates identified in step one. Because both postpartum BMI and net maternal weight gain during pregnancy are measures of maternal weight and therefore correlated, it was decided to only keep one of the two covariates in the model. A review of the literature highlighted a significant relationship between maternal weight gain during pregnancy and fetal growth. Given this finding, net maternal weight gain during pregnancy was included in step two and postpartum BMI was excluded.

From this full model, a reduced model was generated by removing covariates that had p-values greater than 0.10 in the full model. Sample sizes for the full and reduced models were the same. The reduced models for change in LDL-C, change in HDL-C, and change in TC did not include any additional covariates. The reduced linear regression models were compared to the full models using likelihood ratio testing. For this testing, the null hypothesis represented the reduced model with q degrees of freedom. The alternate hypothesis represented the full model with p degrees of freedom. P-values calculated from a chi-square model with p-q degrees of freedom were used. For each of the three analyses, LDL-C, HDL-C, and TC, the full linear

regression models were not significantly different from the reduced liner regression models. Reduced linear regression models were used for subsequent steps.

Step three was to check if any of the variables removed from the full model were important for providing a necessary adjustment of the effect of the variables in the reduced model. This was done by comparing the estimated coefficient of LDL-C, HDL-C, and TC (main independent variables) from the full model to the estimated coefficients of the main independent variables in the reduced model. If any of the estimated coefficients for the main independent variables in the reduced model changed by 20% or more, the removed variables were individually added back into the reduced model. Variables were added back one by one until the estimated coefficients of the main independent variables did not differ by more than 20% from what was calculated for the full model. For the LDL-C model, maternal race and household income were added back to the model. For HDL-C, no covariates were added back to the reduced model. For TC, maternal race, education, household income, and net maternal weight gain were added back to the model.

For step four, covariates that were not statistically significant from the univariate analysis were individually added to the full linear regression model. This was done to see if any covariates significantly impacted the linear regression model as a whole, but may not have individually had a significant relationship with fetal growth. No additional covariates were added to the linear regression models for LDL-C, HDL-C, or TC.

Step five; the linear relationship between fetal growth and each of the continuous variables in the full model were examined with Loess procedures. Change in maternal LDL-C, HDL-C, and TC as well as net maternal weight gain were the only continuous covariates included in the full linear regression models. Smoothed plots provided information regarding the

parametric relationship between each LDL-C, HDL-C, and TC and fetal growth. Linear

relationships were supported for each of the four parametric relationships; therefore, all four

covariates remained as continuous variables in the model.

The final step was to evaluate interaction terms between the main independent variable,

change in maternal cholesterol, and each covariate. Likelihood ratio testing compared the full

model from step four to models with each interaction term. No significant interaction terms were

identified. Final linear regression models for each of the three models are summarized in tables

4.5a, 4.5b, and 4.5c.

Fetal Growth- Categorical

Table 4.6 stratifies maternal characteristics on fetal growth category, SGA, AGA, and

LGA. A univariate analysis was completed looking at the relationship between relevant

covariates and fetal growth. Covariates were included in the univariate analysis based on

findings in the literature. A covariate was determined to be significant, and therefore included in

the model building, if the type three analysis of effects chi-square p-value was less than or equal

to 0.25. If the p-value was greater than 0.25 but literature supported a strong relationship

between the covariate of interest and fetal growth, these variables were considered for inclusion

into the model building. When looking at the relationship between fetal growth categories and

maternal covariates, maternal race, gestational diabetes, maternal education, household income,

and net maternal weight gain were statistically significant. In a sub-data set, excluding women

with missing data for the covariates of interest (n=172), the univariate relationship between fetal

growth categories and tobacco use during pregnancy became statistically significant (p-value=

0.20) for this step. Given this finding and support of the literature, tobacco use during pregnancy

was included in the logistic regression models. All other univariate relationships in this sub-data

set matched the univariate relationships in the full data set. Table 4.7 summarizes the univariate analysis for the full dataset.

In step two, full multinomial logistic regression models were developed to analyze the relationship between changes in maternal cholesterol and fetal growth as a categorical variable with three levels, SGA, AGA, and LGA. Three multinomial models including maternal cholesterol (LDL-C, HDL-C, or TC) and the six covariates from step one were developed. As a result of the lack of participants with a household income of $75,000 or greater in the SGA and LGA groups, all three full models failed to converge as a result of quasi-complete separation. Household income was then transformed into having three outcomes rather than the original four (> $25,000, $25,000 - $49,999, and greater than or equal to $50,000). Step two for LDL-C, HDL-C, and TC full models are summarized in tables 4.8a, 4.8b, and 4.8c.

Reduced models were generated by removing covariates from the full model that had type three analysis of effects chi-square p-values greater than 0.10. The reduced model for change in LDL-C contained net maternal weight gain in addition to change in LDL-C. The reduced model for change in HDL-C contained gestational diabetes, tobacco use during pregnancy, net maternal weight gain, and change in HDL-C. The reduced model for TC contained gestational diabetes, maternal education, net maternal weight gain, and change in TC. Sample sizes for the full and reduced models were the same. Likelihood ratio testing compared the full logistic regression model to the reduced model. For this testing, the null hypothesis represented the reduced model with q degrees of freedom. The alternate hypothesis represented the full model with p degrees of freedom. P-values calculated from a chi-square model with p-q degrees of freedom were used. The full logistic regression model for change in LDL-C was statistically different from the reduced model, therefore the full model was used. For change in

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HDL-C and change in TC the full logistic models were not significantly different from the reduced logistic models, therefore the reduced models were used in these analyses.

Step three was to compare the estimated coefficients for change in LDL-C, change in HDL-C, and change in TC from the full model to the estimated coefficients from the reduced model. This was to check if any of the variables removed from the full model for the reduced model were important for providing a necessary adjustment of the effect of the main independent variables in the reduced model. If any of the estimated coefficients for the main independent variables in the reduced model changed by 20% or more, the removed variables were individually added back into the reduced model. Since the change in LDL-C model was the full model, no covariates were added back to this model. For the change in HDL-C model, no covariates were added back to the reduced model. For the change in TC model, maternal race and household income were added back.

In step four of the purposeful selection model building, covariates that were not statistically significant from the univariate analysis were individually added to the reduced models to see if any covariates significantly impacted the logistic regression model as a whole. The covariates reevaluated for significance were ethnicity, if this was the mother's first pregnancy, gestational hypertension, previous preterm birth, maternal age, and marital status. Likelihood ratio testing compared the reduced model with each new model. None of the six aforementioned variables were added back to the models.

The final step in building the best fit logistic regression model was to check for interaction terms between change in maternal cholesterol and each of the covariates included in the model. Models with interaction terms were compared to the base logistic regression models defined in step four using likelihood ratio testing. None of the tested interaction terms were

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statistically significant; therefore none were included in the model. Final logistic regression

models for each of the three models are summarized in tables 4.9a, 4.9b, and 4.9c.

EXPLORATORY ANALYSIS

Upon completion of the development of the primary analysis regression models, it was

determined that change in maternal cholesterol should be adjusted for the number of weeks

between the two serum samples. The change in maternal cholesterol was subsequently calculated

as the percent change per gestational week (formula 4.4) and the mg/dL unit change per

gestational week (formula 4.5).

Formula 4.4:

$$\frac{\left(\frac{2nd\ cholesterol\ level\ -\ 1st\ cholesterol\ level}{1st\ cholesterol\ level} \times 100\right)}{Number\ of\ weeks\ between\ 1st\ and\ 2nd\ cholesterol\ level}$$

Formula 4.5:

$$\frac{2nd\ cholesterol\ level\ -\ 1st\ cholesterol\ level}{Number\ of\ weeks\ between\ 1st\ and\ 2nd\ cholesterol\ level}$$

It was also decided to look at the relationship between first and second maternal cholesterol

levels and fetal growth. Using the three new methods for maternal cholesterol, purposeful

selection methods were followed and additional models were developed. Tables 4.10a, 4.10b,

4.10c show the final multiple linear regression models for the percent change per week with fetal

growth as a continuous outcome variable. Tables 4.11a, 4.11b, and 4.11c show the final multiple

logistic regression models for the percent change per week with fetal growth as a categorical

outcome variable. Tables 4.12a, 4.12b, and 4.12c show the final multiple linear regression

models for the mg/dL unit change per week with fetal growth as a continuous outcome variable. Tables 4.13a, 4.13b, and 4.13c show the final multiple logistic regression models for the mg/dL unit change per week with fetal growth as a categorical outcome variable. Tables 4.14a, 4.14b, and 4.14c show the final multiple linear regression models for first and second cholesterol levels with fetal growth as a continuous outcome variable. Tables 4.15a, 4.15b, 4.15c show the final multiple logistic regression models for first and second cholesterol levels with fetal growth as a categorical outcome variable.

RESULTS

The distribution of birth weight z-scores is shown in figure 4.1. The incidence of SGA infants in the ARCH study population was 10.8 per 100 infants, n = 21 women. The incidence of infants meeting criteria for LGA was 11.3 per 100 infants, n = 22 women. 152 of the 195 included infants were classified as AGA.

LDL-C

Of the 195 women in this ARCH study population, 189 had valid first and second specimen LDL-C levels. The median change in maternal LDL-C from first to second specimen was 33%, the average change was 39%. Change in maternal LDL-C ranged from a 51% decrease from first to second specimen to a 150% increase. The average increase in LDL-C per gestational week was 2.4%. The average unit increase per gestational week was 2.03 mg/dL. The variations in change in maternal LDL-C are depicted in figures 4.2a – 4.2c. It was hypothesized that women with rates of change in LDL-C from first to second specimen that fall below the lowest quartile will give birth to infants with decreased measures of fetal growth. It was also

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hypothesized that women with rates of change in LDL-C that fall above the highest quartile will give birth to larger infants.

Fetal Growth Continuous

In the unadjusted analysis, change in maternal LDL-C was not significantly associated with birthweight z-scores with a p-value of 0.41, table 4.3. Table 4.5a summarizes the final adjusted model for the relationship between change in maternal LDL-C and birthweight z-scores. In this model maternal race, maternal education, and household income were controlled for. The sample size for this model was 168 women. This model shows a negative relationship between maternal change in LDL-C and fetal growth, although not statistically significant (p-value = 0.10).

The univariate analysis for the percent change in LDL-C per gestational week and fetal growth as a continuous variable was not statistically significant (p-value = 0.15). In addition, the univariate analysis for the unit change in LDL-C per gestational week and fetal growth as a continuous variable was not statistically significant (p-value = 0.29). Using purposeful selection methodology, no additional covariates were selected to be adjusted for in the final linear regression models for the percent change in LDL-C per gestational week and the unit change in LDL-C per gestational week. The included sample size for both models was 189 women. Table 4.10a shows the final model for the percent change in LDL-C per gestational week and table 4.12a shows the final model for the unit change in LDL-C per gestational week.

Lastly, the relationship between the first LDL-C specimen and fetal growth as not statistically significant (p-value = 0.11) and the relationship between the second LDL-C specimen and fetal growth was not statistically significant (p-value = 0.63). The multiple linear regression model that adjusted for the first and second LDL-C level also controlled for maternal

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race, net maternal weight gain, maternal education, household income, and a statistically significant interaction between the first LDL-C level and maternal education, table 4.14a. These significant findings indicate in this population, women who were high school graduates or had their GED had a -0.27 decrease in their BW Z-score for every one unit increase in the first LDL-C measurement compared to women who did not finish high school (95% Confidence Interval (CI): -0.05, -0.007, p-value= 0.008).

Fetal Growth Categorical

Figure 4.3a shows the average LDL-C values for the first and second specimen by the three fetal growth categories. This data shows that the average maternal LDL-C is lower in SGA pregnancies for both the first and second specimen, although not statistically significant in this population. This data also suggests that maternal LDL-C has a higher rate of change in AGA pregnancies compared to SGA and LGA pregnancies. Table 4.6 shows the average change in LDL-C by the three fetal growth categories. In the univariate analysis with fetal growth measured categorically as SGA, AGA, or LGA, table 4.7 shows LDL-C was not significantly associated with fetal growth (p-value = 0.29). The final adjusted multinomial logistic regression model included 167 women and is summarized in table 4.9a. In the adjusted multinomial model, change in LDL-C trended towards significance with infants born LGA (Odds Ratio (OR) = 0.98, 95% CI = 0.97, 1.0, p-value = 0.07). This relationship suggests that for every one unit increase in the percent change in LDL-C the multinomial log-odds for LGA to AGA infants would be expected to decrease by 0.02.

The percent change in LDL-C per gestational week was not significantly associated with fetal growth as a categorical variable (p-value = 0.50) in the unadjusted model. After adjusting for maternal race, tobacco use during pregnancy, maternal education, and net maternal weight

gain, the percent increase in LDL-C per gestational week is significantly associated with LGA infants (OR= 0.78, 95% CI = 0.63, 0.98, p-value = 0.03). This relationship suggests that for every one unit increase in the percent change in LDL-C the multinomial log-odds for LGA to AGA infants would be expected to decrease by 0.22. Table 4.11a summarizes this final model which included 170 women.

The unadjusted relationship between the unit change in LDL-C per gestational week and fetal growth as a categorical variable was not statistically significant (p-value =0.62). The final logistic regression model, table 4.13a, included 167 women and controlled for maternal race, gestational diabetes, tobacco use during pregnancy, maternal education, household income, and net maternal weight gain. Unit change in LDL-C per gestational week trended towards significance with infants born LGA (OR= 0.83, 95% CI = 0.66, 1.03, p-value = 0.10).

The relationship between the categorical fetal growth measurement and the first LDL-C level as well as the second LDL-C trended towards significance, first LDL-C p-value= 0.09 and second LDL-C p-value = 0.07. The full model, table 4.15a, controlled for maternal race, gestational diabetes, tobacco use during pregnancy, maternal education, household income, and net maternal weight gain. In the final full model, which included 167 women, the first and second LDL-C levels were not significantly associated with infants born either SGA or LGA.

HDL-C

All 195 study participants had valid first and second HDL-C levels and birth certificate data for the pregnancy of interest. The median change in maternal HDL-C from first to second specimen was 10% and the average change was 12%. Change in maternal HDL-C ranged from a 34% decrease from first to second specimen to an 82% increase. The average increase in HDL-C per gestational week was 0.65%. The average unit increase per gestational week was 0.32

mg/dL. Figures 4.4a – 4.4c highlight the variation in maternal change in HDL-C. It was hypothesized that changes in maternal HDL-C from first to second specimen will not be significantly associated with infants being in the highest or lowest quartile for fetal growth.

Fetal Growth Continuous

The unadjusted relationship between change in maternal HDL-C and fetal growth as a continuous variable was not statistically significant, table 4.3 (p-value = 0.20). The unadjusted relationship between the percent change in HDL-C per gestational week and fetal growth as a continuous variable was not statistically significant, (p-value= 0.39). The unadjusted relationship between the unit change in HDL-C per gestational week and fetal growth as a continuous variable was not statistically significant. In each of the three aforementioned analyses, 195 women were included. Tables 4.5b, 4.10b, and 4.12b show the final models for the relationships between maternal HDL-C and fetal growth as a continuous variable. In each of the three final models, no additional covariates were adjusted for. The change in HDL-C, the percent change in HDL-C per gestational week, and the unit change in HDL-C per gestational week are not associated with fetal growth as a continuous variable in this population.

Looking at both first and second HDL-C levels, neither the first nor the second measurement was significantly associated with fetal growth as a continuous variable, p-values = 0.11 and 0.46 respectively. The final multiple linear regression model controlled for first and second HDL-C levels as well as net maternal weight gain, maternal education, and household income. In this final adjusted model of 185 women, the first HDL-C level was not significantly associated with fetal growth, p-value= 0.11. The same for the second HDL-C level, there is no significant association with fetal growth, p-value= 0.98. Table 4.14b summarizes this final model.

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Fetal Growth Categorical

Figure 4.3b shows the average HDL-C values for the first and second specimen by the three fetal growth categories. SGA pregnancies have lower average HDL-C values for both the first and second specimen, but the rate of change is similar to that seen in AGA and LGA pregnancies. Table 4.6 summarizes the average change in HDL-C by the three fetal growth categories. In table 4.7, the univariate analysis with fetal growth measured categorically as SGA, AGA, or LGA, HDL-C was not significantly associated with fetal growth (p-value= 0.96). The final model for the relationship between change in maternal HDL-C and fetal growth as a categorical variable included 193 women and is summarized in table 4.9b. Net maternal weight gain, gestational diabetes, and tobacco use during pregnancy were controlled for in this model. This model showed the relationship between maternal change in HDL-C and fetal growth as a categorical variable was not significant (SGA OR = 0.99, 95% CI = 0.97, 1.02, LGA OR= 1.0, 95% CI = 0.98, 1.03).

The univariate analysis for the percent change in HDL-C per gestational week and fetal growth as a categorical variable was not statistically significant (p-value= 0.85). The final model for the relationship between the percent change in HDL-C per gestational week and fetal growth as a categorical variable controlled for maternal race, gestational diabetes, net maternal weight gain, and tobacco use during pregnancy. This final model included 179 women. As summarized in table 4.11b, there was no significant relationship between change in HDL-C per gestational week and fetal growth as a categorical variable.

The unadjusted analysis looking at the unit increase in HDL-C per gestational week and fetal growth as a categorical variable was not statistically significant, p-value= 0.85. The final

logistic regression model, n=193 women, controlled for gestational diabetes, net maternal weight gain, and tobacco use. In the final model, table 4.13b, there were no significant relationships between the unit change in HDL-C per gestational week and the SGA and LGA fetal growth categories.

The association between the first HDL-C levels and the categorical fetal growth outcome was not statically significant, p-value= 0.89. The association between the second HDL-C levels and fetal growth as a categorical variable was also not statistically significant, p=value 0.81. The final adjusted model, table 4.15b, controlled for maternal race, gestational diabetes, net maternal weight gain, maternal education, household income, and tobacco use. The final model of 172 women found no statistically significant relationship between neither the first nor the second HDL-C levels and the fetal growth categories.

TC

All 195 women had first and second TC values as well as corresponding birth certificate data. The median change in maternal TC from first to second specimen was 30%, the average change was 34%. Change in maternal TC ranged from a 13% decrease from first to second specimen to a 104% increase. The average percent change in TC per gestational week was 2.05%. The average unit increase in TC per gestational week was 3.35 mg/dL. The variations in change in maternal TC are depicted in figures 4.5a – 4.5c. It was hypothesized that women with first to second specimen rates of change in TC that fall below the lowest quartile will have infants that have decreased measures of fetal growth. Women with first to second specimen rates of change in TC that are above the highest quartile, it was hypothesized that they would give birth to infants that are large for gestational age.

Fetal Growth Continuous

In the unadjusted analysis, the relationship between change in TC and fetal growth was not statistically significant, p-value= 0.73, table 4.3. Table 4.5c shows the final model for the relationship between change in maternal TC and fetal growth as a continuous variable, n= 172 women. In this model, maternal race, net maternal weight gain, maternal education, and household income were controlled for. With this model, there was no significant relationship between maternal change in TC and fetal growth, p-value = 0.58.

The univariate analysis between the percent change in TC per gestational week and fetal growth as a continuous variable was not statistically significant, p-value= 0.63. The final linear regression model included 177 women and controlled for maternal race and maternal education. In the final model, table 4.10c, there was no significant relationship between percent change in TC per gestational week and fetal growth as a continuous variable.

For the unit change in TC per gestational week, in the unadjusted model there was no significant association with fetal growth as a continuous variable, p-value= 0.64. In 177 women, the unit change in TC per gestational week was not significantly associated with fetal growth after controlling for maternal race and maternal education, p-value= 0.31, table 4.12c.

The unadjusted relationship between the first TC level and fetal growth was not statistically significant, p-value= 0.39. Also, the unadjusted relationship between the second TC level and fetal growth was not statistically significant, p-value= 0.39. The final model for the relationship between the individual TC levels (first and second) and fetal growth, table 4.14c, controlled for maternal race, net maternal weight gain, maternal education, household income, and a statistically significant interaction between the first TC level and education. The final

model included 172 women. These findings indicate in this population, women who were high school graduates or had their GED had a -0.02 decrease in their BW Z-score for every unit increase in the first TC measurement compared to women who did not finish high school (p-value= 0.02). The final model did not show a significant relationship between the second TC level and fetal growth, p-value= 0.49.

Fetal Growth Categorical

Figure 4.3c shows the average TC values for the first and second specimen by the three fetal growth categories. SGA pregnancies have significantly lower average TC values for both the first and second specimen (p-value= 0.05). The rate of change from first to second TC levels in SGA pregnancies is similar to that seen in AGA and LGA pregnancies. Table 4.6 summarizes the average change in TC by the three fetal growth categories. In the univariate analysis, table 4.7, with fetal growth measured categorically as SGA, AGA, or LGA, TC was not significantly associated with fetal growth. Table 4.9c shows the final model for the relationship between change in maternal TC and fetal growth as a categorical variable. This model, n=172, controlled for maternal race, net maternal weight gain, gestational diabetes, maternal education, and household income. Change in maternal TC is not significantly associated with fetal growth as a categorical variable.

The univariate analysis for the percent change in TC per gestational week and fetal growth as a categorical variable was not statistically significant (p-value= 0.85). The final logistic regression model, table 4.11c, controlled for maternal race, gestational diabetes, net maternal weight gain, maternal education, and tobacco use during pregnancy. The sample size for the final adjusted model was 175 women. In this population there was no significant relationship between the percent change in TC per gestational week and fetal growth.

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In the unadjusted analysis, the relationship between the unit change in TC per gestational week and fetal growth as a categorical variable was not statistically significant (p-value= 0.88). After adjusting for maternal race, gestational diabetes, net maternal weight gain, maternal education, and tobacco use during pregnancy the relationship between the unit change in TC per gestational week and fetal growth as a categorical variable was not statistically significant, n= 175 women, table 4.13c.

The unadjusted relationship between the first TC level and fetal growth was not statistically significant in this population, but trended toward statistical significance with a p-value of 0.16. The unadjusted relationship between the second TC level and fetal growth as a categorical variable was statistically significant (SGA OR = 0.99, 95% CI = 0.98, 1.0, LGA OR = 1.0, 95% CI = 0.99, 1.0, type three analysis of effects p-value= 0.05). The multiple logistic regression model, table 4.15c, controlled for maternal race, gestational diabetes, net maternal weight gain, maternal education, household income, and tobacco use during pregnancy. The final model included 172 women and found no significant relationship between the first TC levels and fetal growth categories as well as no significant relationship between the second TC levels and fetal growth categories.

DISCUSSION

Unadjusted first and second LDL-C, HDL-C, and TC levels were lower in pregnancies resulting in SGA babies compared to pregnancies resulting in AGA and LGA babies. However, only the second TC levels were statistically different between SGA, AGA, and LGA babies. Because second TC levels were collected at different time points in pregnancy, this significant relationship was further explored. When looking at TC levels based on the trimester they were collected, TC levels were lower in SGA pregnancies, although no longer statistically significant.

The finding that SGA pregnancies have lower maternal cholesterol is in line with the findings presented in table 2.5 of chapter two in this dissertation. Reproducing these results in a larger study population is an important next step to further investigate this finding.

The unadjusted relationships between change in maternal cholesterol (LDL-C, HDL-C, and TC) and fetal growth were not statistically significant within this study population. This was true for both fetal growth as a continuous variable as well as a categorical variable. After adjusting for covariates deemed significant through purposeful selection modeling, the relationship between the change in maternal cholesterol levels and fetal growth, continuous and categorical, remained not statistically significant. The adjusted relationship between change in maternal LDL-C and fetal growth as a continuous variable trended towards significance, p-value = 0.10, and showed increases in the change in LDL-C resulted in a decreased BW Z-score.

Exploratory analyses led to the development of 18 additional models to investigate the relationship between maternal cholesterol levels and fetal growth. These additional models evaluated maternal cholesterol in three different ways. The first set of models used percent change in maternal cholesterol (LDL-C, HDL-C, and TC) per gestational week as the main independent variable. The univariate analyses for this main variable were not statistically significant. The adjusted logistic regression model for the percent change in LDL-C per gestational week found a statistically significant negative relationship between the percent change in LDL-C and LGA infants, p-value= 0.03. This multivariable model adjusted for maternal race, tobacco use during pregnancy, maternal education, and net maternal weight gain.

The next set of models used unit change in maternal cholesterol (LDL-C, HDL-C, and TC) per gestational week as the main independent variable. Both the univariate and adjusted

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analyses for these models found no statistically significant relationships between maternal cholesterol and fetal growth.

The final set of exploratory models looked at the first and second cholesterol levels and did not transform them into a new variable. In the univariate analyses, LDL-C, HDL-C, and TC were not significantly associated with fetal growth as a continuous variable. When looking at fetal growth as a categorical variable, in the univariate analysis the first and second LDL-C levels were significantly associated with SGA infants (p-values= 0.03). First and second HDL-C levels were not significantly associated with fetal growth as a categorical outcome. Second TC levels were significantly associated with SGA infants and the relationship between the first TC levels and SGA infants trended towards significance (p-value= 0.06). In the final adjusted models, both the first LDL-C and the first TC had statistically significant interactions with maternal education, specifically women whose highest level of education was high school graduation or a GED. This interaction between maternal education and LDL-C and TC being significantly associated with fetal growth was not an expected result and has not been reported elsewhere in the literature. The biological plausibility of this interaction is unclear. Two additional models were run excluding this interaction. In the LDL-C model, the first LDL-C levels remained significantly associated with the BW Z-score (first LDL-C estimate: 0.0089, 95% CI: 0.001, 0.17, p-value= 0.03). Although the relationship between the first LDL-C levels and BW-Z score is significant in the adjusted model and not the univariate model, the estimates for the first LDL-C levels in the two models are not significantly different. The 95% confidence intervals for the two estimates overlap and the difference between the two estimates is only 0.004. Both the univariate and adjusted analyses should be run in a population with greater variability in BW Z-scores. In the TC model, when the interaction with maternal education is

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removed, first TC levels are no longer significantly associated with the BW Z-score (first TC estimate: 0.0025, 95% CI: -0.0028, 0.0078, p-value= 0.35). This result seems more plausible and warrant additional research.

In pregnancies resulting in SGA babies, lower maternal cholesterol levels were found at single time points in pregnancy. However, high levels of cholesterol, specifically LDL-C, during pregnancy may increase the plaque buildup in the placental blood vessels, thereby reducing blood flow to the placenta causing placental insufficiencies and increasing levels of oxidative stress [10, 21 – 22]. It is proposed this reduction in blood flow yields a reduction in the oxygen and nutrients required for normal fetal development and growth. The findings of this dissertation that increased changes in LDL-C are associated with reduced fetal growth support this biological process. Increased rates of change in LDL-C may indicate the body is creating additional LDL-C at a faster pace compared to those with lower rates of increase. Perhaps this increased rate of LDL-C production causes greater plaque buildup at a more rapid pace giving the body less time to adapt, adjust, and possibly even counteract the plaque.

At the time of the dissertation, there were no results in the literature looking at changes in maternal LDL-C with relationship to fetal growth. The findings from this research highlight the need for additional studies focusing specifically on changes in maternal cholesterol during pregnancy. Chapter two of this dissertation highlighted current research that suggests that low LDL-C levels at a single time point during pregnancy are associated with smaller infants. Maternal cholesterol at a single time point does not capture how cholesterol levels change during pregnancy. This dissertation added to the literature by focusing on the change in maternal cholesterol levels measured three different ways. The change in maternal cholesterol during

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pregnancy captures how maternal cholesterol levels are responding to pregnancy and may provide a different, more accurate, picture of fetal growth.

Attempts to replicate the significant results of this analysis should be completed. These results suggest there is importance in monitoring the change of maternal cholesterol levels during pregnancy to help identify women who may be at risk of delivering an infant with reduced fetal growth. Women with elevated changes in maternal LDL-C should be monitored closely during pregnancy, fetal growth should be measured frequently during pregnancy, and interventions should be discussed if applicable.

LIMITATIONS

A few limitations have been identified for this study. The incidence of SGA and LGA infants within the ARCH study population is 11% and 11%, respectively. Because the overall sample size for this analysis is relatively small, only 21 infants were SGA and 22 infants were LGA. This research should be repeated in a sample with larger numbers of SGA and LGA infants. In addition to a larger sample size of SGA and LGA infants, additional research should also include a more racially diverse patient population. Reported alcohol use during pregnancy, pre-pregnancy hypertension, pre-pregnancy diabetes, and pre-pregnancy high cholesterol, although significant maternal characteristics, did not provide any information for this research. The lack of variability in participant responses made controlling for these variables unbeneficial, table 4.16. These four variables were therefore excluded from the purposeful selection model building.

Although the maternal questionnaire at enrollment was robust, no information was available on maternal diet and nutritional intake during gestation. Maternal weight gain could be

used as a proxy for maternal diet, however, future studies should control for this variable as maternal diet may strongly influence maternal cholesterol levels during pregnancy. Lastly, the ARCH database is a rich data set with self-reported information, information from the birth certificate, and biological information. Due to the nature of the ARCH project, the study population does not include women with clinically categorized high risk pregnancies. If a woman's pregnancy was deemed high risk by her doctor, her care was transferred to a specialized clinic in the Lansing area. Once transferred, ARCH was no longer able to collect additional biological specimens during gestation from the participant; however, specimens at the time of delivery were still collected in these women if they delivered at the appropriate hospital.

Despite this wealth of information, these findings lack external generalizability. It is important to acknowledge the lack of clinical, geographical, racial, and socioeconomic diversity in those women with first and second trimester cholesterol levels. This Lansing, Michigan study focuses on maternal cholesterol levels in a predominantly low socioeconomic population with 59% of enrolled women being white. The findings presented here may not be applicable to women of different races and other socioeconomic standings.

This study had maternal cholesterol levels at two time points during pregnancy, however, there was variability in the timing of specimen collection and whether or not women were fasting at the time of specimen collection or not. A more controlled study that collected specimens at exact time points during pregnancy, perhaps even more than two serum specimens, and encouraged all specimens to be either fasting or non-fasting may provide more specific information on the exact timing of expected changes in maternal cholesterol and may even be able to link adverse outcomes to maternal cholesterol levels at specific points of time during pregnancy.

Additional research on this topic is needed to help further explore the relationship between maternal cholesterol levels during pregnancy and fetal growth. Future study populations should include greater participant diversity, have larger sample sizes, and increased incidence in fetal growth, allowing for more robust analysis.

APPENDICES

APPENDIX A

Tables

Table 4.1: Demographics of ARCH study population with a singleton pregnancy, cholesterol at two time points during pregnancy, and corresponding birth certificates

	N (%)
NUMBER OF PARTICIPANTS	195
MATERNAL AGE AT BIRTH OF BABY	
18-24 years	91 (46.7)
25-30 years	69 (35.4)
31-40 years	34 (17.4)
>40 years	1 (0.5)
Missing data	0 (0)
MATERNAL RACE	
Black or African American	37 (19)
White	127 (65.1)
Other (American Indian, Alaska Native, Native Hawaiian, Pacific Islander, Asian, or Multiracial)	17 (8.7)
Missing data	14 (7.2)
MATERNAL ETHNICITY	
Hispanic or Latino	26 (13.3)
Not Hispanic or Latino	169 (86.7)
Missing data	0 (0)
MATERNAL EDUCATION	
Did not finish high school	25 (12.8)
High school graduate or GED	61 (31.3)
Some college	58 (29.7)
College graduate or more	47 (24.1)
Missing data	4 (2.1)
HOUSEHOLD INCOME	
Under $25,000	130 (66.7)
$25,000 to $49,999	34 (17.4)
$50,000 to $74,999	15 (7.7)
$75,000 or above	11 (5.6)
Missing data	5 (2.6)
MARITAL STATUS	
Married, living with the baby's father	60 (30.8)
Married	10 (5.1)
Unmarried, living with the baby's father	62 (31.8)
Unmarried	63 (32.3)
Missing data	0 (0)

ARCH: Archive for Research on Child Health

Table 4.2: Expected birthweight (grams) used for calculating birth weight z-scores based on gestational age and sex of the fetus

Gestational Age (weeks)	Male (grams)	Female (grams)
32	1882	1800
33	2126	2033
34	2382	2296
35	2653	2560
36	2905	2799
37	3149	3028
38	3337	3209
39	3465	3333
40	3547	3417
41	3624	3486
42	3648	3512

Table 4.3: Unadjusted univariate analysis between fetal growth as continuous variable and each covariate being evaluated for inclusion in the multiple regression model building

	Mean BW grams	Mean BW Z-score	R-Square	Type 3 P-value
TC change	-	-	0.0006	0.73
LDL-C change	-	-	0.0036	0.41
HDL-C change	-	-	0.008	**0.20***
Race			0.019	**0.06***
White	3426.1	0.03		
Non-white	3262.3	-0.27		
Missing data (n=14)	3521.1	0.17		
Ethnicity			0.0003	0.95
Not Hispanic or Latino	3387.4	-0.04		
Hispanic or Latino	3388.8	-0.05		
Maternal age	-	-	0.003	0.47
Maternal education			0.03	**0.13***
Did not finish high school	3313.6	-0.30		
High school graduate/GED	3321.4	-0.21		
Some college	3445.1	0.09		
College graduate or more	3414.7	0.11		
Household income			0.04	**0.08***
< $25, 000	3346.8	-0.17		
$25,000 - $49,999	3351.2	0.09		
$50,000 - $74,999	3657.9	0.47		
≥ $75,000	3475.6	0.10		
Marital status			0.01	0.55
Married	3505.3	0.29		
Married living w/ baby's father	3394.9	0.03		
Unmarried living w/ baby's father	3384.6	-0.15		
Unmarried	3364.8	-0.06		

Table 4.3 (cont'd)

First pregnancy				0.002	0.52
	No	3347.8	-0.09		
	Yes	3431.1	0.01		
Tobacco use				0.005	0.31
	No	3393.5	-0.004		
	Yes	3365.4	-0.18		
Gestational diabetes				0.0006	0.73
	No	3393.8	-0.05		
	Yes	3149.4	0.11		
Gestational hypertension				0.0009	0.68
	No	3398.4	-0.05		
	Yes	3206.3	0.08		
Previous preterm birth				0.003	0.48
	No	3395.8	-0.05		
	Yes	2863.3	0.37		
Pre-pregnancy BMI (con)		-	-	0.004	0.40
Pre-pregnancy BMI (cat)				0.01	0.56
	<18.5	3219.2	-0.44		
	18.5 -<25	3386.7	-0.03		
	25- <30	3420.4	0.008		
	≥ 30	3405.8	0.02		
Postpartum BMI (con)		-	-	0.011	**0.14***
Postpartum BMI (cat)				0.054	**0.01***
	<18.5	2452.5	-1.79		
	18.5 -<25	3253.0	-0.32		
	25- <30	3371.3	-0.08		
	≥ 30	3455.2	0.10		
Net maternal weight gain		-	-	0.022	**0.04***

* p-value < 0.25, used in multivariate model. P-value is from type 3 analysis of effects.

BW: birthweight; TC: total cholesterol; LDL-C: low density-lipoprotein cholesterol; HDL-C: high density-lipoprotein cholesterol;

BMI: body mass index; con: continuous variable; cat: categorical variable

Table 4.4a: Multiple linear regression model, full model, for fetal growth as a continuous outcome variable including change in maternal LDL-C and the significant covariates from step one of purposeful selection

	Estimate	95% CI		P-value
LDL-C change	-0.003	-0.007	0.001	**0.10***
Race				
Non-white	-0.25	-0.60	0.10	0.16
Education level				
High school graduate or GED	-0.16	0.67	0.35	0.53
Some college	0.21	-0.30	0.72	0.41
College graduate or more	0.06	-0.51	0.63	0.83
Household income				
$25,000 - $49,999	0.10	-0.30	0.51	0.62
$50,000 - $74,999	0.41	-0.20	1.02	0.19
$75,000 or above	0.15	-0.54	0.84	0.67
Net maternal weight gain	0.007	-0.002	0.02	0.15

* Type 3 Analysis of Effects p-value ≤ 0.10, used in reduced multivariate model

LDL-C: low density-lipoprotein cholesterol; CI: confidence interval

Table 4.4b: Multiple linear regression model, full model, for fetal growth as a continuous outcome variable including change in maternal HDL-C and the significant covariates from step one of purposeful selection

	Estimate	95% CI		P-value
HDL-C change	0.004	-0.003	0.01	0.27
Race				
Non-white	-0.23	-0.57	0.11	0.18
Education level				
High school graduate or GED	-0.18	-0.69	0.32	0.48
Some college	0.16	-0.35	0.67	0.53
College graduate or more	0.007	-0.56	0.58	0.98
Household income				
$25,000 - $49,999	0.11	-0.30	0.51	0.61
$50,000 - $74,999	0.35	-0.26	0.95	0.27
$75,000 or above	0.91	0.87	1.08	0.73
Net maternal weight gain	0.007	-0.002	0.02	0.13

* Type 3 Analysis of Effects p-value ≤ 0.10, used in reduced multivariate model

HDL-C: High density-lipoprotein cholesterol; CI: confidence interval

Table 4.4c: Multiple linear regression model, full model, for fetal growth as a continuous outcome variable including change in maternal TC and the significant covariates from step one of purposeful selection

	Estimate	95%CI		P-value
TC change	-0.002	-0.007	0.004	0.58
Race				
Non-white	-0.24	-0.58	0.10	0.17
Education level				
High school graduate/GED	-0.16	-0.67	0.34	0.53
Some college	0.20	-0.32	0.71	0.45
College graduate or more	0.04	-0.53	0.61	0.88
Household income				
$25,000 - $49,999	0.10	-0.30	0.51	0.63
$50,000 - $74,999	0.36	-0.25	0.97	0.25
$75,000 or above	0.12	-0.57	0.82	0.73
Net maternal weight gain	0.007	-0.002	0.02	0.12

* Type 3 Analysis of Effects p-value ≤ 0.10, used in reduced multivariate model

TC: Total cholesterol; CI: confidence interval

Table 4.5a: Final multiple linear regression model for fetal growth as a continuous outcome variable including change in maternal LDL-C and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
LDL-C change	-0.003	-0.007	0.001	0.10
Race				
Non-white	-0.25	-0.60	0.10	0.16
Education level				
High school graduate or GED	-0.18	-0.69	0.33	0.49
Some college	0.15	-0.36	0.66	0.57
College graduate or more	0.02	-0.55	0.60	0.96
Household income				
$25,000 - $49,999	0.12	-0.28	0.53	0.55
$50,000 - $74,999	0.51	-0.09	1.12	0.10
$75,000 or above	0.20	-0.49	0.90	0.57

* p-value ≤ 0.05

LDL-C: low density-lipoprotein cholesterol; CI: confidence interval

Table 4.5b Final multiple linear regression model for fetal growth as a continuous outcome variable including change in maternal HDL-C and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
HDL-C change	0.005	-0.003	0.01	0.20

* p-value ≤ 0.05

HDL-C: High density-lipoprotein cholesterol; CI: confidence interval

Table 4.5c: Final multiple linear regression model for fetal growth as a continuous outcome variable including change in maternal TC and the covariates determined to be significant through purposeful selection

	Estimate	95%CI		P-value
TC change	-0.002	-0.007	0.004	0.58
Race				
Non-white	-0.24	-0.58	0.10	0.17
Education level				
High school graduate/GED	-0.16	-0.67	0.34	0.53
Some college	0.20	-0.32	0.71	0.45
College graduate or more	0.04	-0.53	0.61	0.88
Household income				
$25,000 - $49,999	0.10	-0.30	0.51	0.63
$50,000 - $74,999	0.36	-0.25	0.97	0.25
$75,000 or above	0.12	-0.57	0.82	0.73
Net maternal weight gain	0.007	-0.002	0.02	0.12

* p-value ≤ 0.05

TC: Total cholesterol; CI: confidence interval

Table 4.6: Average change in maternal cholesterol and demographics of the ARCH study population included in this chapters analysis stratified by fetal growth categories

	SGA N (%)	AGA N (%)	LGA N (%)	Total N (%)
NUMBER OF PARTICIPANTS	21	152	22	195
AVERAGE CHANGE IN LDL-C	35.1%	41.9%	29%	
AVERAGE CHANGE IN HDL-C	11.2%	12.1%	12.9%	
AVERAGE CHANGE IN TC	29.9%	34.9%	30.8%	
AVERAGE CHANGE IN LDL-C PER WEEK GESTATION	2.6%	2.5%	1.8%	
AVERAGE CHANGE IN HDL-C PER WEEK GESTATION	0.75%	0.62%	0.75%	
AVERAGE CHANGE IN TC PER WEEK GESTATION	2.1%	2.1%	1.9%	
MATERNAL RACE				
Black or African American	5 (23.8)	31 (20.4)	1 (4.5)	37 (19)
White	13 (61.9)	97 (63.8)	17 (77.3)	127 (65.1)
Other (American Indian, Alaska Native, Native Hawaiian, Pacific Islander, Asian, or Multiracial)	2 (9.5)	14 (9.2)	1 (4.5)	17 (8.7)
Missing data	1 (4.8)	10 (6.6)	3 (13.6)	14 (7.2)
MATERNAL ETHNICITY				
Hispanic or Latino	2 (9.5)	22 (14.5)	2 (9.1)	26 (13.3)
Not Hispanic or Latino	19 (90.5)	130 (85.5)	20 (90.9)	169 (86.7)
MATERNAL AGE AT BIRTH OF BABY				
18-24 years	9 (42.9)	72 (47.4)	10 (45.5)	91 (46.7)
25-30 years	8 (38.1)	55 (36.2)	6 (27.3)	69 (35.4)
31-40 years	4 (19.1)	24 (15.8)	6 (27.3)	34 (17.4)
>40 years	0 (0)	1 (0.7)	0 (0)	1 (0.5)
MATERNAL EDUCATION				
Did not finish high school	5 (23.8)	19 (12.5)	1 (4.6)	25 (12.8)
High school graduate or GED	4 (19.1)	54 (35.5)	3 (13.6)	61 (31.3)
Some college	9 (42.9)	38 (25)	11 (50)	58 (29.7)
College graduate or more	3 (14.3)	37 (24.3)	7 (31.8)	47 (24.1)
Missing data	0 (0)	4 (2.6)	0 (0)	4 (2.1)

Table 4.6 (cont'd)

HOUSEHOLD INCOME				
Under $25,000	18 (85.7)	102 (67.1)	10 (45.5)	130 (66.7)
$25,000 to $49,999	2 (9.5)	28 (18.4)	4 (18.1)	34 (17.4)
$50,000 to $74,999	1 (4.8)	8 (5.3)	6 (27.2)	15 (7.7)
$75,000 or above	0 (0)	10 (6.6)	1 (4.5)	11 (5.6)
Missing data	0 (0)	4 (2.6)	1 (4.5)	5 (2.6)
MARITAL STATUS				
Married, living with the baby's father	5 (23.8)	47 (30.9)	8 (36.4)	60 (30.8)
Married	1 (4.8)	7 (4.6)	2 (9.1)	10 (5.1)
Unmarried, living with the baby's father	7 (33.3)	49 (32.2)	6 (27.3)	62 (31.8)
Unmarried	8 (38.1)	49 (32.2)	6 (27.3)	63 (32.3)
FIRST PREGNANCY				
No	13 (61.9)	78 (52)	10 (45.5)	102 (52.3)
Yes	8 (38.1)	73 (48)	12 (54.6)	93 (47.7)
TOBACCO USE				
No	14 (66.7)	121 (79.6)	19 (86.4)	154 (79)
Yes	7 (33.3)	31 (30.4)	3 (13.6)	41 (21)
GESTATIONAL DIABETES				
No	20 (95.2)	150 (98.7)	20 (90.9)	190 (197.4)
Yes	1 (4.8)	2 (1.3)	3 (9.1)	5 (2.6)
GESTATIONAL HYPERTENSION				
No	20 (95.2)	144 (94.7)	20 (90.9)	184 (94.4)
Yes	1 (4.8)	8 (5.3)	2 (9.1)	11 (5.6)
PREVIOUS PRETERM BIRTH				
No	21 (100)	149 (98)	22 (100)	192 (98.5)
Yes	0 (0)	3 (2)	0 (0)	3 (1.5)
PRE-PREGNANCY BMI				
< 18.5	2 (9.5)	8 (5.3)	1 (4.5)	11 (5.6)
18.5 - < 25	6 (28.6)	51 (33.6)	9 (40.9)	66 (33.8)
25 - < 30	6 (28.6)	50 (32.9)	6 (27.3)	62 (31.8)
≥ 30	6 (28.6)	42 (27.6)	6 (27.3)	54 (27.7)

Table 4.6 (cont'd)

	Missing data	1 (4.8)	1 (0.7)	0 (0)	2 (1.0)
POSTPARTUM BMI					
	< 18.5	2 (9.5)	0 (0)	0 (0)	2 (1)
	18.5 - < 25	3 (14.3)	22 (14.5)	2 (9.1)	27 (13.8)
	25 - < 30	6 (28.6)	49 (32.2)	6 (27.3)	61 (31.3)
	≥ 30	9 (42.9)	81 (53.3)	14 (63.6)	104 (53.3)
	Missing data	1 (4.8)	0 (0)	0 (0)	1 (0.5)

SGA: small for gestational age; AGA: average for gestational age; LGA: large for gestational age; LDL-C: low density-lipoprotein cholesterol; HDL-C: high density-lipoprotein cholesterol; TC: total cholesterol; BMI: body mass index

Table 4.7: Unadjusted univariate analysis between fetal growth as a categorical variable and each covariate being evaluated for inclusion in the multiple regression model building. (AGA is referent group)

	Type 3 P-value	SGA Estimate	SGA OR	SGA 95% CI		LGA Estimate	LGA OR	LGA 95% CI	
TC Change	0.58	-0.008	0.99	0.97	1.01	-0.007	0.99	0.98	1.01
LDL-C Change	0.29	-0.005	1.0	0.98	1.01	**-0.01**	0.99	0.98	1.0
HDL-C change	0.96	-0.003	1.0	0.97	1.02	0.002	1.0	0.98	1.03
Race	**0.18***								
White		Ref				Ref			
Non-white		0.07	1.61	0.43	3.11	-0.67	0.25	0.06	1.15
Ethnicity	0.68								
Not Hispanic or Latino		Ref				Ref			
Hispanic or Latino		-0.24	0.62	0.14	2.86	-0.26	0.59	0.13	2.71
Maternal age	0.71	0.008	1.01	0.93	1.10	0.03	1.04	0.95	1.12
Maternal education	**0.05***								
Did not finish high school		Ref				Ref			
High school graduate/GED		-0.63	0.28	0.07	1.16	-0.71	1.06	0.10	10.77
Some college		0.53	0.90	0.27	3.06	0.95	5.50	0.66	45.8
College graduate or more		-0.54	0.31	0.07	1.43	0.52	3.60	0.41	31.39
Household income	**0.05***								
< $25, 000		Ref				Ref			
$25,000 - $49,999		2.27	0.41	0.09	1.85	-0.23	1.46	0.43	5.0
$50,000 - $74,999		2.83	0.71	0.08	6.01	1.43	7.65	2.21	26.49
≥ $75,000		-8.27	<0.001	<0.001	>999.9	-0.59	1.02	0.12	8.81
Marital status	0.93								
Married		0.04	1.0	0.11	9.39	0.55	2.33	0.39	13.91
Married living with baby's father		-0.25	0.74	0.22	2.52	0.04	1.39	0.45	4.31
Unmarried living with baby's father		Ref				Ref			
Unmarried		0.17	1.14	0.39	3.40	-0.29	1.0	0.30	3.31
First pregnancy	0.55								
No		Ref				Ref			

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Table 4.7 (cont'd)

	p-value								
Yes		-0.20	0.67	0.26	1.70	0.13	1.30	0.53	3.19
Tobacco use	0.27								
No		Ref				Ref			
Yes		0.33	1.95	0.73	5.25	-0.24	0.62	0.17	2.22
Gestational diabetes	**0.14***								
No		Ref				Ref			
Yes		0.66	3.75	0.33	43.26	1.01	7.50	1.0	56.24
Gestational hypertension	0.76								
No		Ref				Ref			
Yes		-0.05	0.90	0.11	7.58	0.29	1.80	0.36	9.08
Previous preterm birth	0.99								
No		Ref							
Yes		-6.2	<0.001	<0.001	>999.9	-6.2	<0.001	<0.001	>999.9
Pre-pregnancy BMI (con)	0.95	-0.005	1.0	0.93	1.07	-0.01	0.99	0.93	1.06
Pre-pregnancy BMI (cat)	0.97								
<18.5		0.51	1.75	0.30	10.27	-0.11	0.88	0.09	8.29
18.5 -<25		-0.24	0.82	0.25	2.74	0.24	1.24	0.41	3.75
25- <30		-0.22	0.84	0.25	2.80	-0.15	0.84	0.25	2.80
≥ 30		Ref				Ref			
Postpartum BMI (con)	0.67	-0.02	0.98	0.91	1.05	0.02	1.02	0.96	1.09
Postpartum BMI (cat)	0.98								
<18.5		12.58	>999.9	<0.001	>999.9	0.11	0.84	<0.001	>999.9
18.5 -<25		-4.09	1.23	0.31	4.92	-0.35	0.53	0.11	2.49
25- <30		-4.20	1.10	0.37	3.29	-0.05	0.71	0.26	1.97
≥ 30		Ref				Ref			
Net maternal weight gain	**0.02***	-0.03	0.97	0.94	1.01	0.03	1.03	1.0	1.05

*p-value < 0.25, used in multivariate model. P-value is from type 3 analysis of effects.

SGA: small for gestational age; AGA: average for gestational age; LGA: large for gestational age; CI: confidence interval; TC: total cholesterol; LDL-C: low density-lipoprotein cholesterol; HDL-C: high density-lipoprotein cholesterol; BMI: body mass index; con: continuous variable; cat: categorical variable

Table 4.8a: Multinomial logistic regression model for fetal growth as a categorical outcome variable including change in maternal LDL-C and the significant covariates from step one of purposeful selection, with household income collapsed into three categories

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
LDL-C Change	-0.002	0.80	-0.02	0.08
Maternal Race				
Non-white	0.01	0.98	-0.56	0.19
Education Level				
High school graduate/GED	-0.67	0.17	-0.65	0.36
Some college	0.40	0.34	1.27	0.01
College graduate or more	-0.20	0.74	-0.54	0.45
Household Income				
$25,000 - $49,999	-0.33	0.60	-0.66	0.22
$50,000 or above	-0.23	0.78	0.98	0.11
Tobacco Use	0.42	0.15	-0.58	0.19
Gestational Diabetes	0.92	0.24	1.52	0.06
Net maternal weight gain	-0.02	0.24	0.04	0.02*

* Type 3 Analysis of Effects p-value ≤ 0.10, used in reduced multivariate model

LDL-C: low density-lipoprotein cholesterol; HDL-C: high density-lipoprotein cholesterol; TC: total cholesterol; SGA: small for gestational age; LGA: large for gestational age;

Table 4.8b: Multinomial logistic regression model for fetal growth as a categorical outcome variable including change in maternal HDL-C and the significant covariates from step one of purposeful selection, with household income collapsed into three categories

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
HDL-C Change	-0.006	0.67	0.009	0.56
Maternal Race				
Non-white	0.02	0.95	-0.47	0.26
Education Level				
High school graduate/GED	-0.73	0.14	-0.76	0.27
Some college	0.39	0.34	1.18	0.01
College graduate or more	-0.16	0.79	-0.45	0.51
Household Income				
$25,000 - $49,999	-0.30	0.63	-0.49	0.35
$50,000 or above	-0.27	0.74	0.70	0.22
Tobacco Use	0.45	0.13	-0.58	0.18*
Gestational Diabetes	0.95	0.23	1.96	0.01*
Net maternal weight gain	-0.02	0.25	0.04	0.01*

* Type 3 Analysis of Effects p-value ≤ 0.10, used in reduced multivariate model

SGA: small for gestational age; LGA: large for gestational age; HDL-C: high density-lipoprotein cholesterol

Table 4.8c Multinomial logistic regression model for fetal growth as a categorical outcome variable including change in maternal TC and the significant covariates from step one of purposeful selection, with household income collapsed into three categories

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
TC Change	-0.004	0.73	-0.01	0.36
Maternal race				
Non-white	0.006	0.98	-0.50	0.23
Education Level				
High school graduate/GED	-0.73	0.13	-0.70	0.31
Some college	0.39	0.34	1.21	0.01*
College graduate or more	-0.16	0.79	-0.45	0.51
Household Income				
$25,000 - $49,999	-0.32	0.61	-0.58	0.27
$50,000 or above	-0.24	0.77	0.80	0.17
Tobacco Use	0.43	0.14	-0.56	0.20
Gestational Diabetes	0.95	0.22	1.70	0.04*
Net maternal weight gain	-0.02	0.27	0.04	0.01*

* Type 3 Analysis of Effects p-value ≤ 0.10, used in reduced multivariate model

SGA: small for gestational age; LGA: large for gestational age; TC: total cholesterol;

Table 4.9a: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including change in maternal LDL-C and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
LDL-C Change	-0.002	0.80	-0.02	0.08
Maternal race				
Non-white	0.01	0.98	-0.56	0.19
Education Level				
High school graduate/GED	-0.67	0.17	-0.65	0.36
Some college	0.40	0.34	1.27	0.01*
College graduate or more	-0.20	0.74	-0.54	0.45
Household Income				
$25,000 - $49,999	-0.33	0.60	-0.66	0.22
$50,000 or above	-0.23	0.78	0.98	0.11
Tobacco Use	0.42	0.15	-0.58	0.19
Gestational Diabetes	0.92	0.24	1.52	0.06
Net maternal weight gain	-0.02	0.24	0.04	0.02*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; LDL-C: low density-lipoprotein cholesterol

Table 4.9b: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including change in maternal HDL-C and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
HDL-C Change	-0.006	0.67	0.004	0.75
Tobacco Use	0.45	0.09	-0.60	0.12
Gestational Diabetes	0.28	0.67	1.46	0.01*
Net maternal weight gain	-0.03	0.10	0.04	0.004*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; HDL-C: high density-lipoprotein cholesterol

Table 4.9c: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including change in maternal TC and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
TC change	-0.004	0.73	-0.01	0.34
Maternal race				
Non-white	-0.02	0.94	-0.45	0.28
Education Level				
High school graduate/GED	-0.65	0.17	-0.76	0.26
Some college	0.40	0.32	1.15	0.01*
College graduate or more	-0.39	0.50	-0.21	0.74
Household Income				
$25,000 - $49,999	-0.35	0.58	-0.57	0.27
$50,000 or above	-0.20	0.80	0.72	0.19
Gestational Diabetes	1.09	0.16	1.60	0.03*
Net maternal weight gain	-0.02	0.34	0.03	0.04*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; TC: total cholesterol

Table 4.10a: Final multiple linear regression model for fetal growth as a continuous outcome variable including percent change in maternal LDL-C per week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
LDL-C percent change per week	-0.04	-0.10	0.01	0.15

* p-value ≤ 0.05

LDL-C: low density-lipoprotein cholesterol; CI: confidence interval

Table 4.10b: Final multiple linear regression model for fetal growth as a continuous outcome variable including percent change in maternal HDL-C per week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
HDL-C percent change per week	0.05	-0.06	0.15	0.39

* p-value ≤ 0.05

HDL-C: High density-lipoprotein cholesterol; CI: confidence interval

Table 4.10c: Final multiple linear regression model for fetal growth as a continuous outcome variable including percent change in maternal TC per week and the covariates determined to be significant through purposeful selection

	Estimate	95%CI		P-value
TC percent change per week	-0.05	-0.14	0.04	0.25
Maternal race				
Non-white	-0.30	-0.63	0.02	0.07
Education level				
College graduate or more	0.15	-0.36	0.67	0.56
Some college	0.23	-0.27	0.73	0.36
High school graduate/GED	-0.14	-0.64	0.36	0.58

* p-value ≤ 0.05

TC: Total cholesterol; CI: confidence interval

Table 4.11a: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including percent change in maternal LDL-C per week and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
LDL-C percent change per week	0.04	0.67	-0.24	0.03*
Maternal race				
Non-white	0.08	0.78	-0.76	0.08
Education Level				
High school graduate/GED	-0.54	0.24	-1.02	0.14
Some college	0.34	0.39	1.37	0.004*
College graduate or more	-0.35	0.51	-0.03	0.95
Tobacco Use	0.48	0.09	-0.65	0.13
Net maternal weight gain	-0.03	0.12	0.04	0.01*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; LDL-C: low density-lipoprotein cholesterol

Table 4.11b: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including percent change in maternal HDL-C per week and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
HDL-C percent change per week	-0.02	0.90	0.16	0.47
Maternal race				
Non-white	0.13	0.61	-0.61	0.12
Tobacco Use	0.55	0.05*	-0.42	0.28
Gestational Diabetes	0.86	0.26	1.65	0.01*
Net maternal weight gain	-0.03	0.15	0.04	0.01*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; HDL-C: high density-lipoprotein cholesterol

Table 4.11c: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including percent change in maternal TC per week and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
TC percent change per week	0.07	0.68	-0.19	0.26
Maternal race				
Non-white	0.12	0.68	-0.60	0.16
Education Level				
High school graduate/GED	-0.63	0.18	-0.95	0.15
Some college	0.38	0.34	1.33	0.005*
College graduate or more	-0.37	0.49	-0.15	0.78
Tobacco Use	0.44	0.12	-0.61	0.15
Gestational Diabetes	1.04	0.17	161	0.03*
Net maternal weight gain	-0.02	0.19	0.05	0.004*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; TC: total cholesterol

Table 4.12a: Final multiple linear regression model for fetal growth as a continuous outcome variable including unit (mg/dL) change in maternal LDL-C per week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
LDL-C unit change per week	-0.03	-0.09	0.03	0.29

* p-value ≤ 0.05

LDL-C: low density-lipoprotein cholesterol; CI: confidence interval

Table 4.12b: Final multiple linear regression model for fetal growth as a continuous outcome variable including unit (mg/dL) change in maternal HDL-C per week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
HDL-C unit change per week	0.04	-0.13	0.21	0.65

* p-value ≤ 0.05

HDL-C: High density-lipoprotein cholesterol; CI: confidence interval

Table 4.12c: Final multiple linear regression model for fetal growth as a continuous outcome variable including unit (mg/dL) change in maternal TC per week and the covariates determined to be significant through purposeful selection

	Estimate	95%CI		P-value
TC unit change per week	-0.02	-0.07	0.02	0.31
Maternal race				
Non-white	-0.30	-0.63	0.02	0.07
Education level				
College graduate or more	0.15	-0.37	0.66	0.58
Some college	0.23	-0.27	0.73	0.38
High school graduate/GED	-0.14	-0.64	0.36	0.58

* p-value ≤ 0.05

TC: Total cholesterol; CI: confidence interval

Table 4.13a: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including unit (mg/dL) change in maternal LDL-C per week and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
LDL-C unit change per week	0.001	0.99	-0.19	0.10
Maternal race				
Non-white	0.02	0.96	-0.60	0.18
Education Level				
High school graduate/GED	-0.67	0.17	-0.68	0.33
Some college	0.38	0.36	1.29	0.01*
College graduate or more	-0.19	0.76	-0.59	0.39
Household income				
$25,000 - $49,999	-0.31	0.62	-0.58	0.26
$50,000 or above	-0.27	0.74	0.78	0.17
Tobacco Use	0.43	0.15	-0.62	0.16
Gestational Diabetes	0.95	0.22	1.59	0.04*
Net maternal weight gain	-0.02	0.24	0.04	0.01*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; LDL-C: low density-lipoprotein cholesterol

Table 4.13b: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including unit (mg/dL) change in maternal HDL-C per week and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
HDL-C unit change per week	0.02	0.94	0.22	0.50
Tobacco Use	-0.44	0.10	0.60	0.12
Gestational Diabetes	0.32	0.63	1.50	0.01*
Net maternal weight gain	-0.03	0.11	0.04	0.004*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; HDL-C: high density-lipoprotein cholesterol

Table 4.13c: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including unit (mg/dL) change in maternal TC per week and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
TC unit change per week	0.001	0.99	-0.10	0.25
Maternal race				
Non-white	0.11	0.70	-0.61	0.16
Education Level				
High school graduate/GED	-0.63	0.18	-0.96	0.15
Some college	0.40	0.32	-0.96	0.005*
College graduate or more	-0.38	0.49	-0.16	0.77
Tobacco Use	-0.44	0.12	0.60	0.15
Gestational Diabetes	1.01	0.18	1.63	0.03*
Net maternal weight gain	-0.02	0.20	0.05	0.004*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; TC: total cholesterol

Table 4.14a: Final multiple linear regression model for fetal growth as a continuous outcome variable including first and second maternal LDL-C levels and the covariate determined to be significant through purposeful selection

	Estimate	95% CI		P-value
First LDL-C	0.02	0.003	0.04	0.02*
Second LDL-C	-0.004	-0.01	0.001	0.10
First LDL-C and education level interaction				
High school graduate or GED	-0.03	-0.15	-0.01	0.01*
Some college	-0.002	-0.02	0.02	0.86
College graduate or more	-0.01	-0.03	0.01	0.13
Maternal race				
Non-white	-0.22	-0.56	0.11	0.19
Education level				
High school graduate or GED	2.17	0.38	3.96	0.02*
Some college	0.32	-1.31	1.95	0.70
College graduate or more	1.33	-0.48	3.14	0.15
Household income				
$25,000 - $49,999	0.04	-0.36	0.43	0.86
$50,000 - $74,999	0.49	-0.11	1.10	0.11
$75,000 or above	0.17	-0.50	0.84	0.62
Net maternal weight gain	0.01	-0.004	0.01	0.23

* p-value ≤ 0.05

LDL-C: low density-lipoprotein cholesterol; CI: confidence interval

Table 4.14b: Final multiple linear regression model for fetal growth as a continuous outcome variable including first and second maternal HDL-C levels and the covariate determined to be significant through purposeful selection

	Estimate	95% CI		P-value
First HDL-C	-0.01	-0.03	0.003	0.11
Second HDL-C	-0.0002	-0.01	0.01	0.98
Education level				
High school graduate or GED	0.07	-0.39	0.52	0.78
Some college	0.38	-0.09	0.85	0.11
College graduate or more	0.25	-0.28	0.78	0.36
Household income				
$25,000 - $49,999	0.22	-0.16	0.60	0.25
$50,000 - $74,999	0.48	-0.10	1.06	0.11
$75,000 or above	0.22	-0.45	0.89	0.52
Net maternal weight gain	0.01	-0.0001	0.02	0.05*

* p-value ≤ 0.05

HDL-C: High density-lipoprotein cholesterol; CI: confidence interval

Table 4.14c: Final multiple linear regression model for fetal growth as a continuous outcome variable including first and second maternal TC levels and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
First TC	0.01	-0.001	0.02	0.07
Second TC	-0.001	-0.01	0.003	0.49
First TC and education level interaction				
High school graduate or GED	-0.02	-0.03	-0.002	0.02*
Some college	-0.004	-0.02	0.01	0.61
College graduate or more	-0.01	-0.03	0.002	0.10
Maternal race				
Non-white	-0.20	-0.54	0.13	0.24
Education level				
College graduate or more	2.18	-0.42	4.79	0.10
Some college	0.79	-1.63	3.22	0.52
High school graduate or GED	2.73	0.17	5.29	0.04*
Household income				
$25,000 - $49,999	0.06	-0.34	0.45	0.78
$50,000 - $74,999	0.37	-0.24	0.98	0.23
$75,000 or above	0.10	-0.59	0.78	0.78
Net maternal weight gain	0.01	-0.002	0.02	0.14

* p-value ≤ 0.05

TC: Total cholesterol; CI: confidence interval

Table 4.15a: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including first and second LDL-C levels and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
First LDL-C	-0.02	0.18	0.02	0.30
Second LDL-C	-0.01	0.54	-0.02	0.10
Maternal race				
Non-white	-0.09	0.77	-0.57	0.18
Education Level				
High school graduate/GED	-0.72	0.15	-0.66	0.35
Some college	0.39	0.36	1.25	0.01*
College graduate or more	-0.02	0.7	-0.48	0.49
Household income				
$25,000 - $49,999	-0.22	0.73	-0.62	0.25
$50,000 or above	-0.53	0.52	0.86	0.16
Tobacco Use	0.35	0.25	-0.56	0.20
Gestational Diabetes	1.05	0.21	1.37	0.10
Net maternal weight gain	-0.02	0.19	0.04	0.03*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; LDL-C: low density-lipoprotein cholesterol

Table 4.15b: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including first and second HDL-C levels and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
First HDL-C	0.01	0.75	-0.03	0.31
Second HDL-C	-0.01	0.75	0.01	0.77
Maternal race				
Non-white	0.02	0.95	-0.43	0.32
Education Level				
High school graduate/GED	-0.71	0.14	-0.81	0.26
Some college	0.39	0.34	1.22	0.01*
College graduate or more	-0.19	0.76	-0.44	0.52
Household Income				
$25,000 - $49,999	-0.32	0.61	-0.43	0.40
$50,000 or above	-0.24	0.76	0.75	0.19
Tobacco Use	0.45	0.13	-0.68	0.14
Gestational Diabetes	0.98	0.22	2.22	0.01*
Net maternal weight gain	-0.02	0.26	0.04	0.01*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; HDL-C: high density-lipoprotein cholesterol

Table 4.15c: Final multinomial logistic regression model for fetal growth as a categorical outcome variable including first and second TC levels and the covariates determined to be significant through purposeful selection

	SGA		LGA	
	Estimate	**P-value**	**Estimate**	**P-value**
First TC	-0.01	0.39	0.002	0.88
Second TC	-0.01	0.28	-0.01	0.31
Maternal race				
Non-white	-0.09	0.77	-0.55	0.20
Education Level				
High school graduate/GED	-0.76	0.13	-0.76	0.28
Some college	0.41	0.33	1.21	0.01*
College graduate or more	0.02	0.97	-0.36	0.60
Household income				
$25,000 - $49,999	-0.22	0.72	-0.52	0.33
$50,000 or above	-0.45	0.59	0.68	0.24
Tobacco use	-0.38	0.20	0.54	0.21
Gestational diabetes	1.08	0.21	1.76	0.04*
Net maternal weight gain	-0.02	0.31	0.04	0.02*

*p-value ≤ 0.05

SGA: small for gestational age; LGA: large for gestational age; TC: total cholesterol

Table 4.16: Significant covariates, based on a review of the literature, that were excluded from this analysis due to a lack of variability in participant responses

	Yes	No	Blank
Maternal alcohol use	0 (0%)	195 (100%)	0 (0%)
Pre-pregnancy diabetes	0 (0%)	195 (100%)	0 (0%)
Pre-pregnancy hypertension	0 (0%)	195 (100%)	0 (0%)
Pre-pregnancy high cholesterol	2 (1%)	126 (64.6%)	67 (34.4%)

APPENDIX B

Figures

Figure 4.1: Distribution of birth weight z-scores for the 195 ARCH participants included in the study population
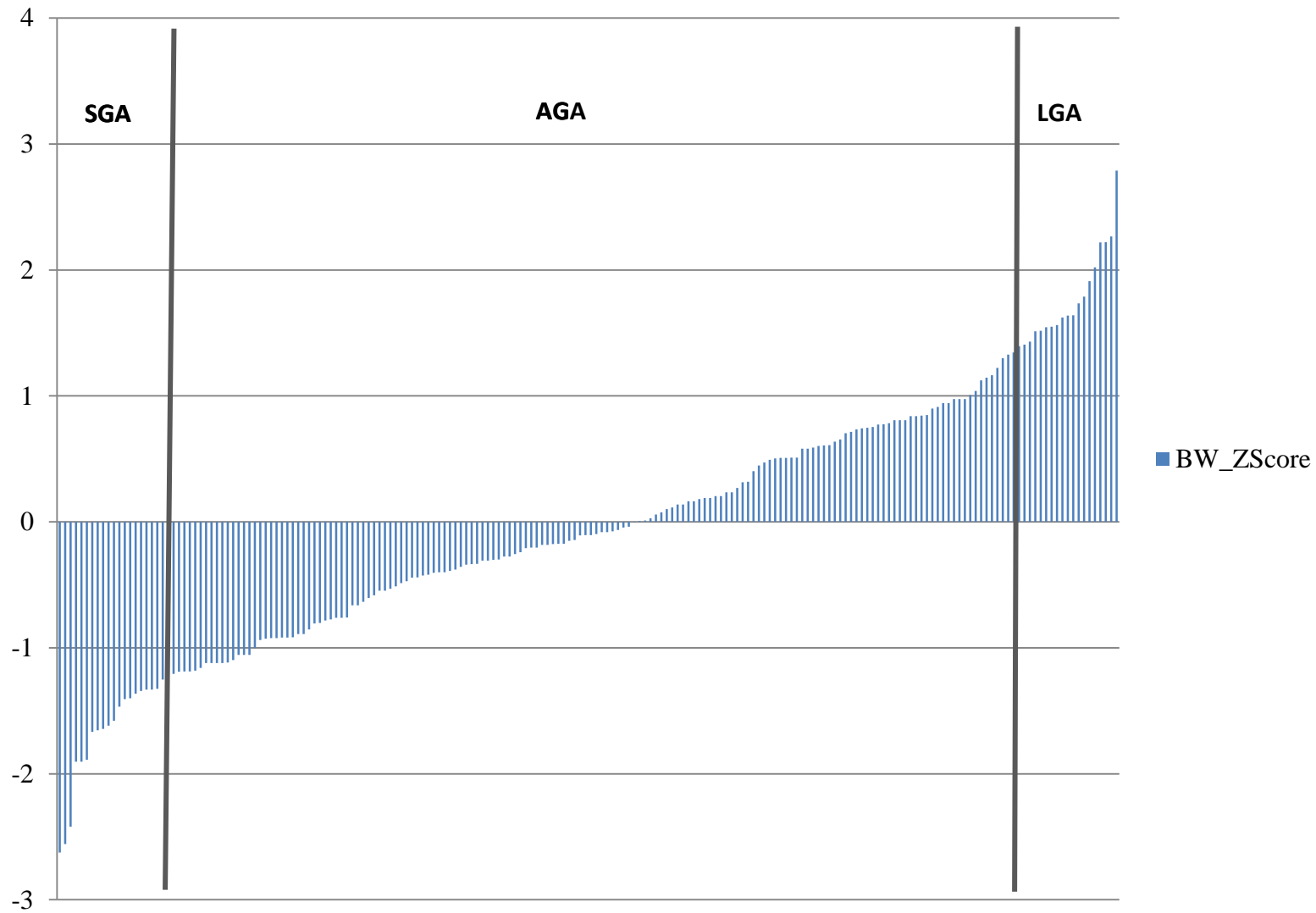
SGA: small for gestational age; AGA: average for gestational age; LGA: large for gestational age

Figure 4.2a: Percent change in maternal LDL-C from first to second specimen for each study subject

Each of the 189 study participants represents a bar in the graph below
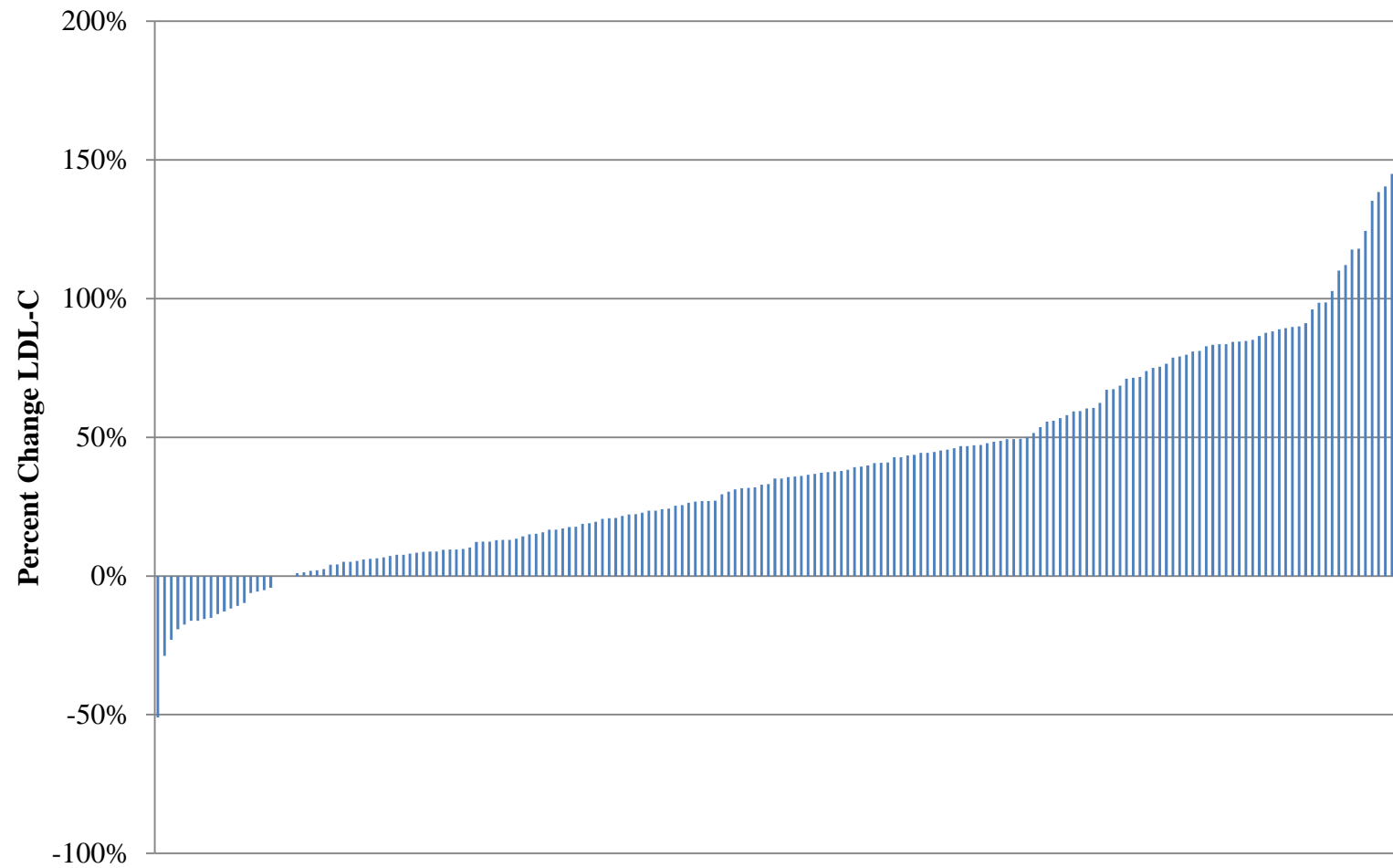
LDL-C: low density-lipoprotein cholesterol

Figure 4.2b: Percent change in maternal LDL-C per gestational week from first to second specimen for each study subject
Each of the 189 study participants represents a bar in the graph below
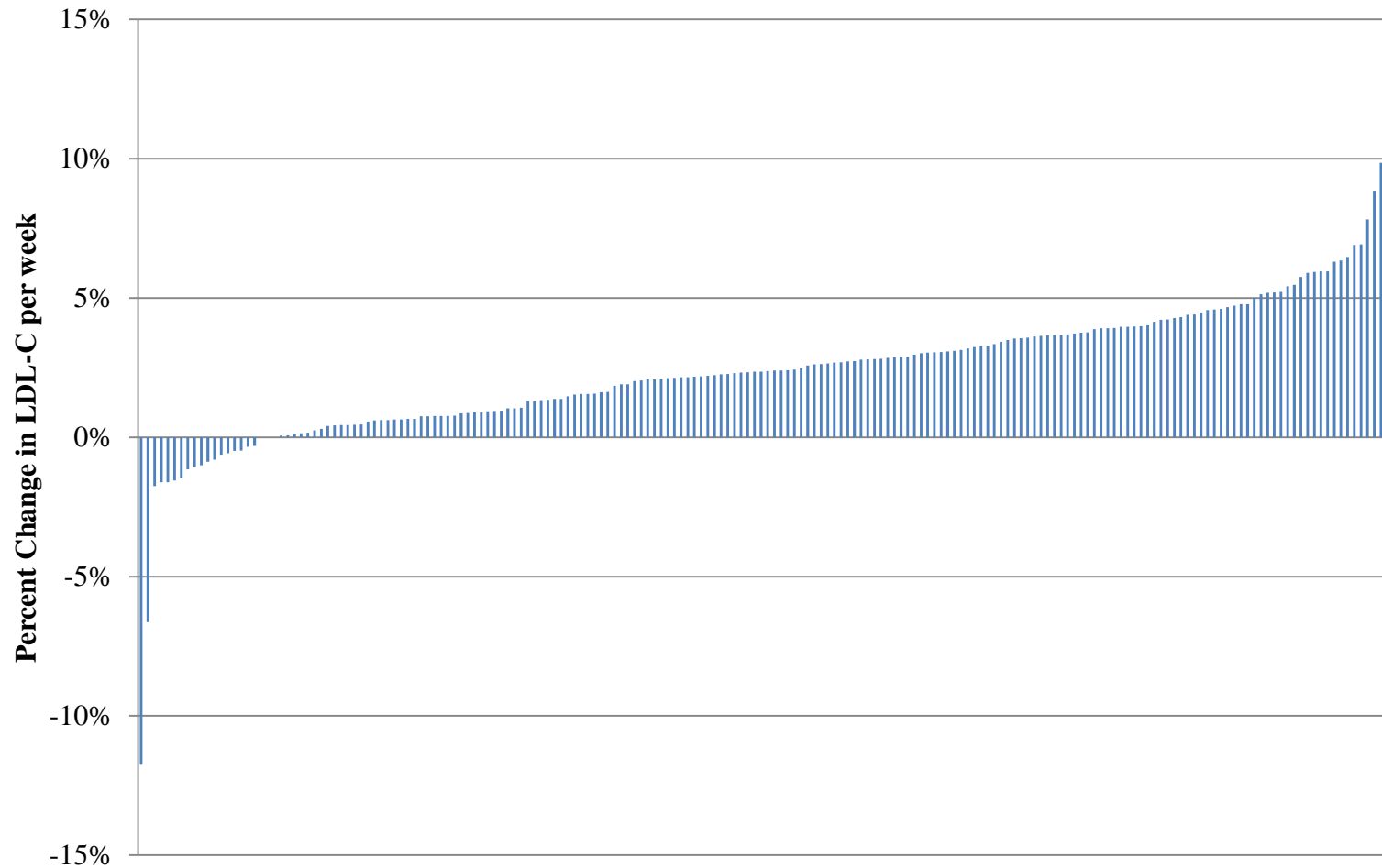
LDL-C: low density-lipoprotein cholesterol

Figure 4.2c: Unit change in maternal LDL-C per gestational week from first to second specimen for each study subject
Each of the 189 study participants represents a bar in the graph below
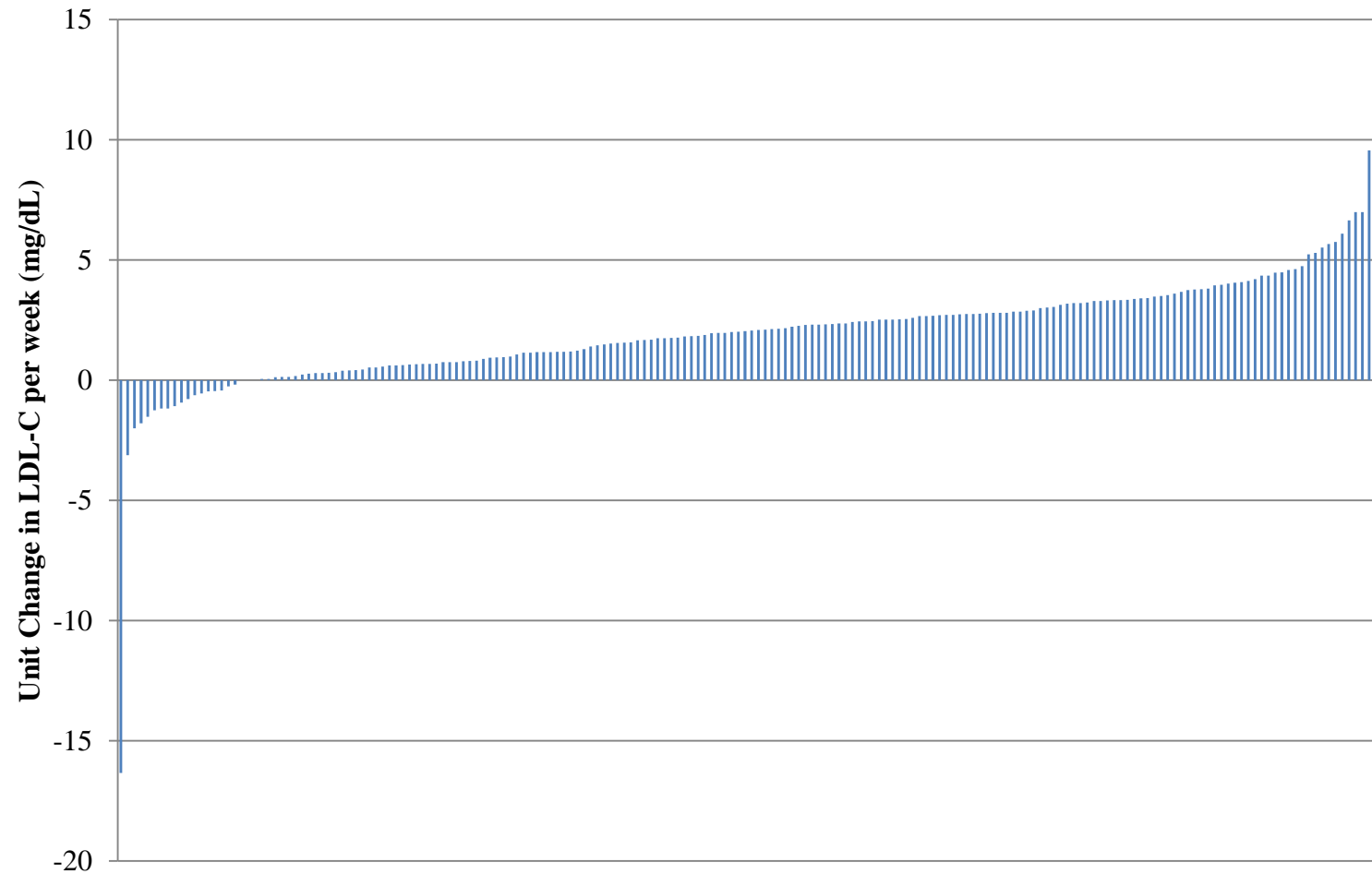
LDL-C: low density-lipoprotein cholesterol

Figure 4.3a: First and second specimen averages for maternal change in LDL-C for 189 study subjects stratified by fetal growth category

	1st Average LDL-C	2nd Average LDL-C
SGA	76.2	102.2
AGA	88	122.8
LGA	89.8	114.1
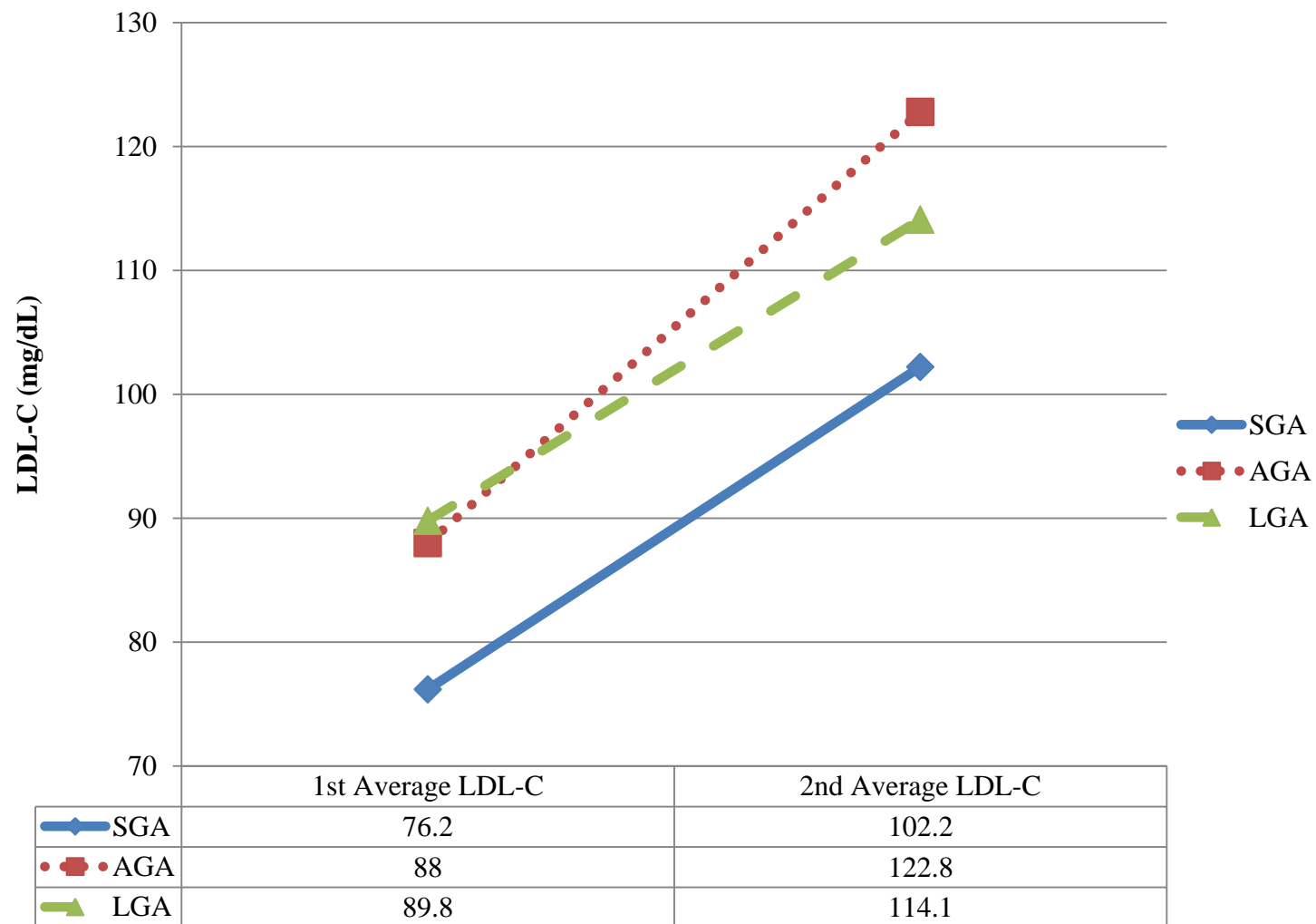
LDL-C: low density-lipoprotein cholesterol

Figure 4.3b: First and second specimen averages for maternal change in HDL-C for 195 study subjects stratified by fetal growth category

	1st Average HDL-C	2nd Average HDL-C
SGA	57	62.6
AGA	58.4	64.7
LGA	58.5	65
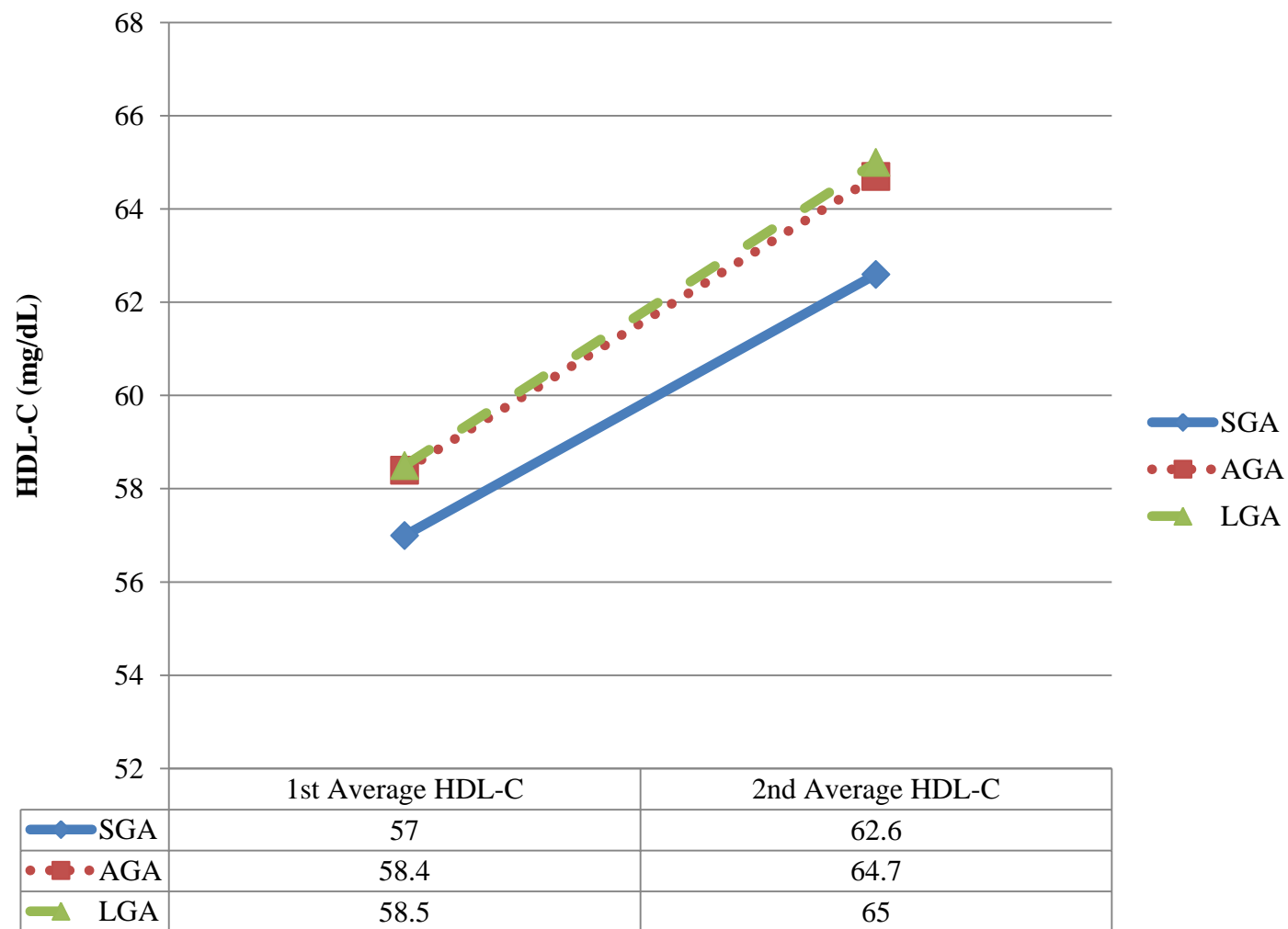
HDL-C: high density-lipoprotein cholesterol

Figure 4.3c: First and second specimen averages for maternal change in TC for 195 study subjects stratified by fetal growth category

	1st Average TC	2nd Average TC*
SGA	156.2	200.8
AGA	170.5	226.8
LGA	172.7	221.5
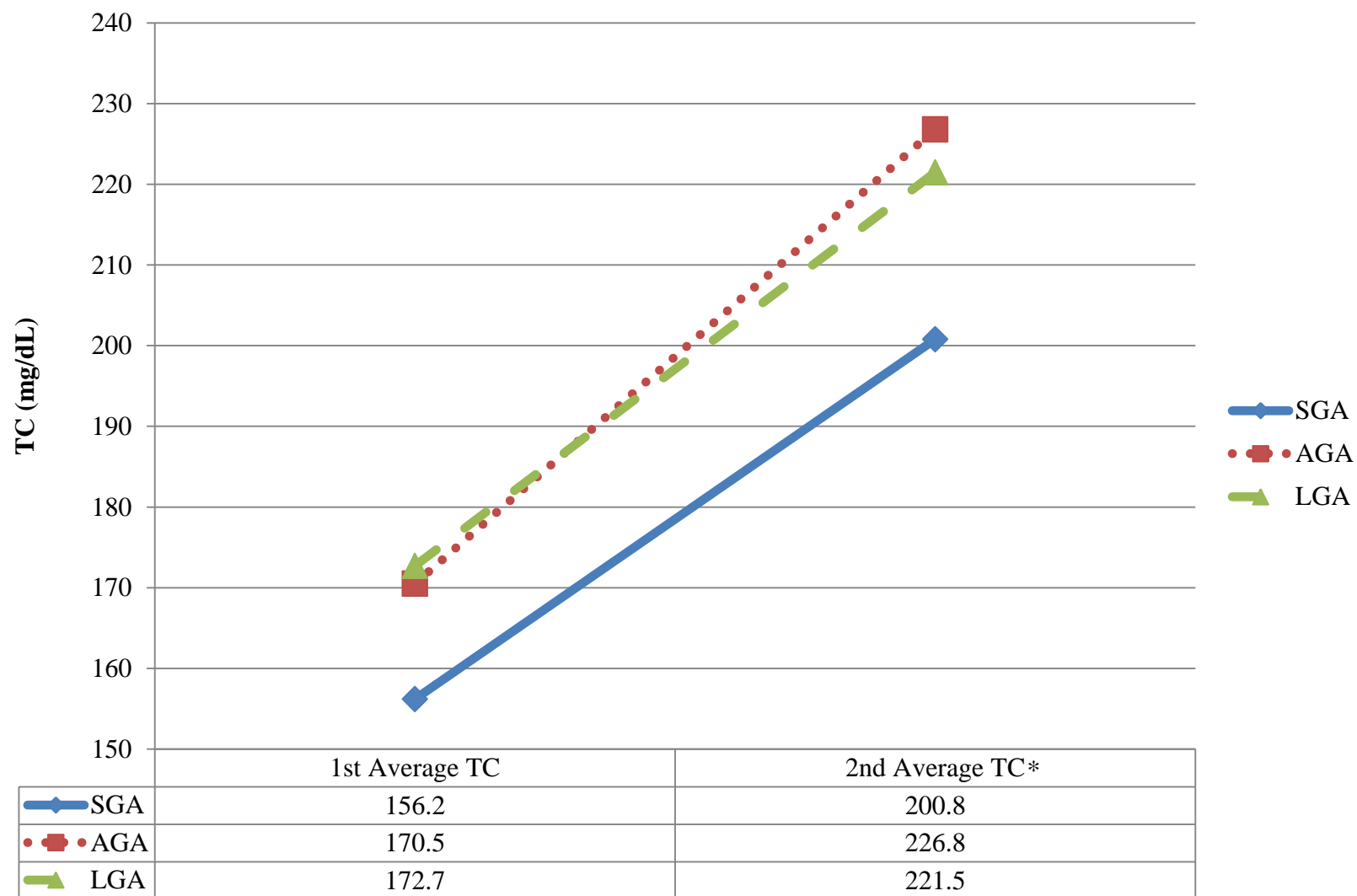
TC: total cholesterol
TC levels statistically different across fetal growth categories
* p-value = 0.05

Figure 4.4a: Percent change in maternal HDL-C from first to second specimen for each study subject
Each of the 195 study participants represents a bar in the graph below
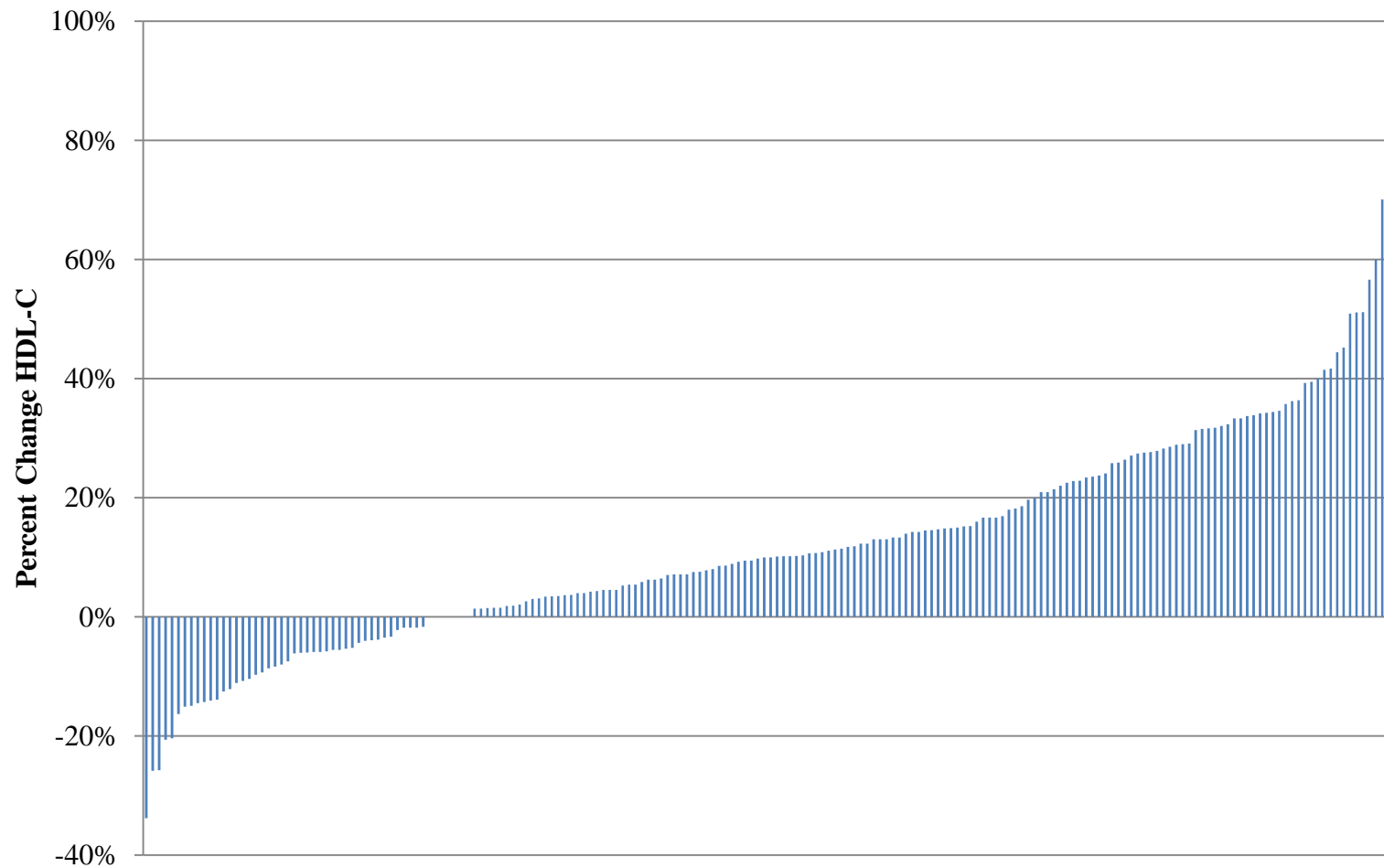
HDL-C: high density-lipoprotein cholesterol

Figure 4.4b: Percent change in maternal HDL-C per gestational week from first to second specimen for each study subject
Each of the 195 study participants represents a bar in the graph below
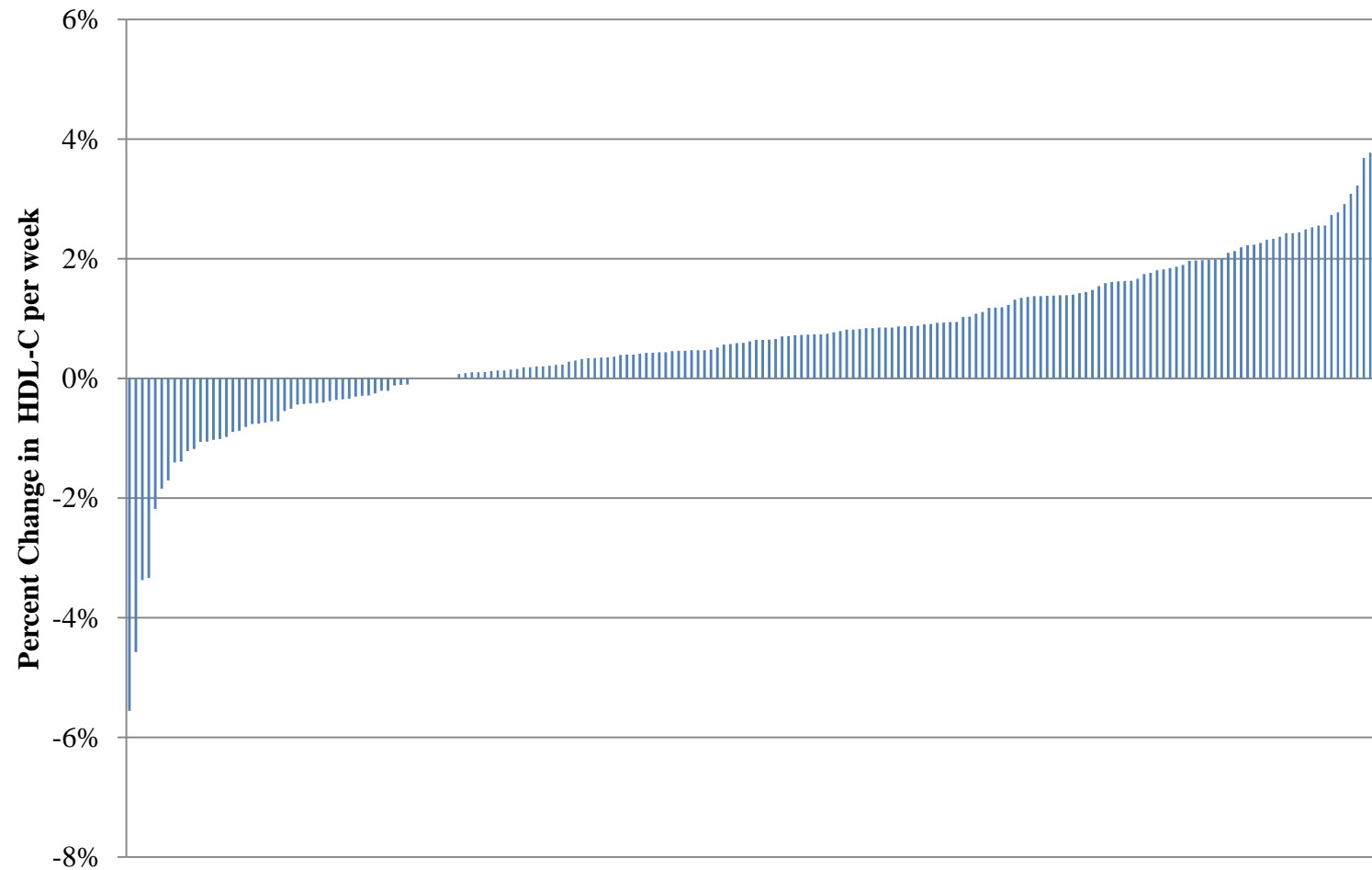
HDL-C: high density-lipoprotein cholesterol

Figure 4.4c: Unit change in maternal HDL-C per gestational week from first to second specimen for each study subject
Each of the 195 study participants represents a bar in the graph below
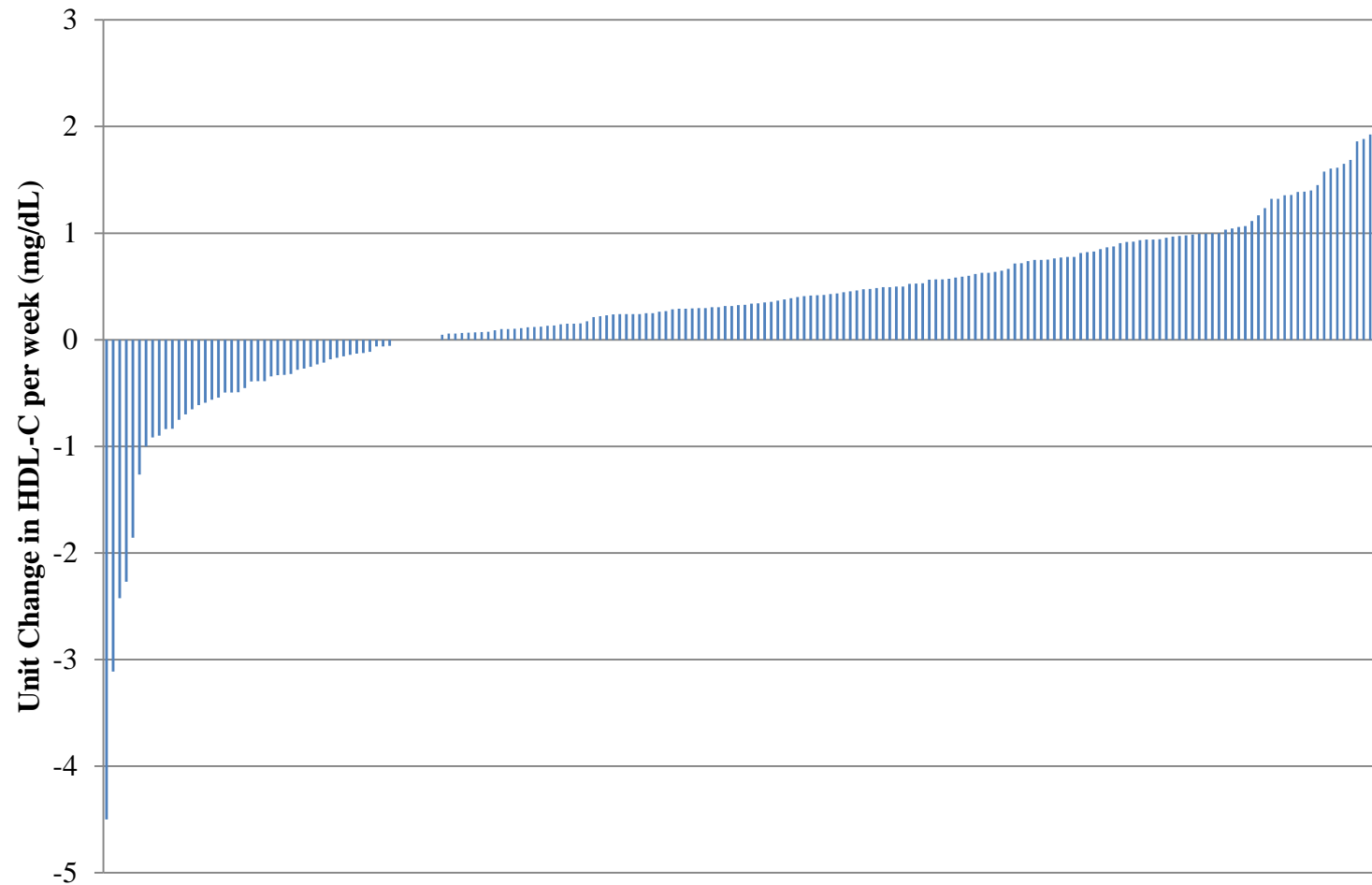
HDL-C: high density-lipoprotein cholesterol

Figure 4.5a: Percent change in maternal TC from first to second specimen for each study subject
Each of the 195 study participants represents a bar in the graph below
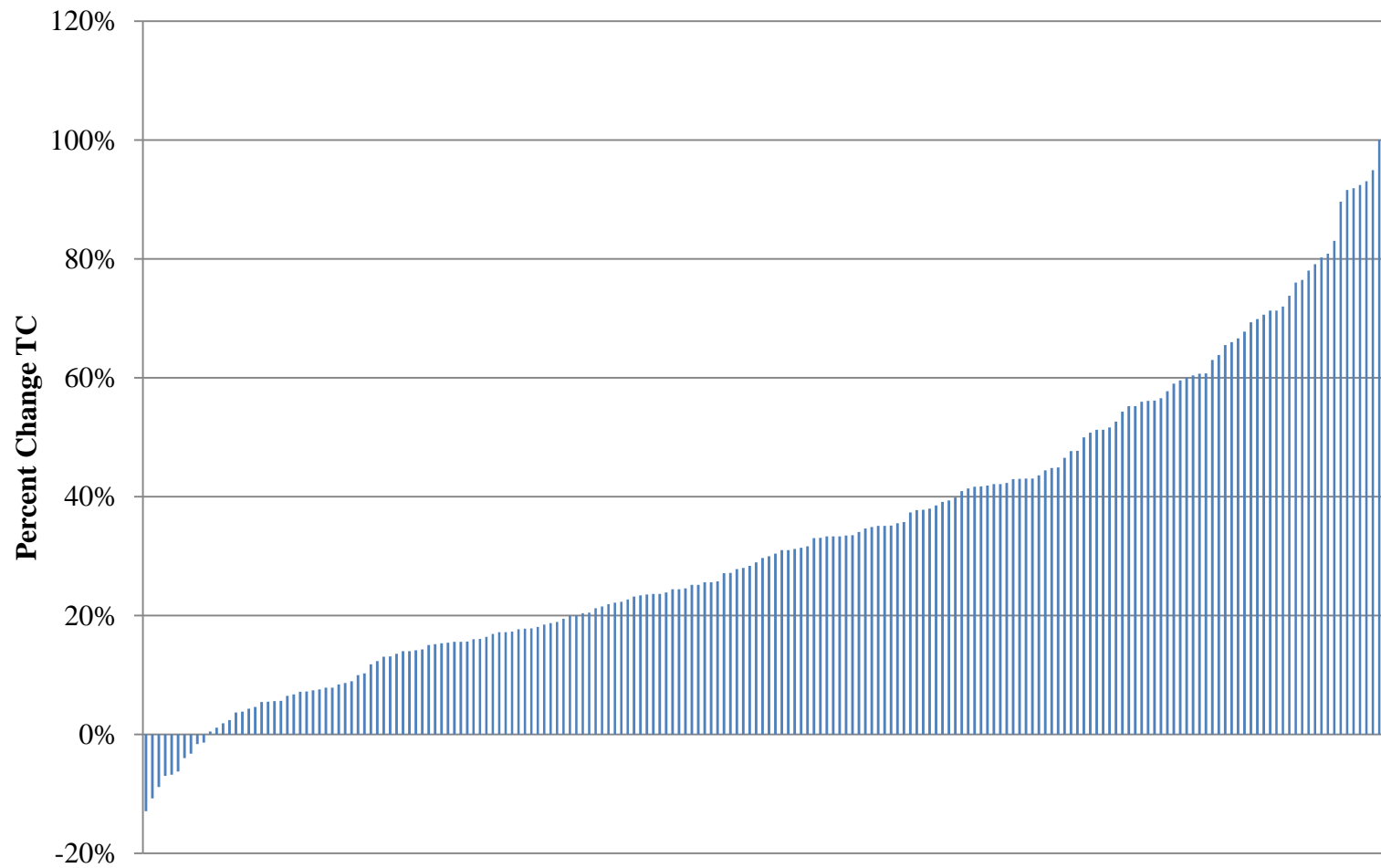
TC: total cholesterol

Figure 4.5b: Percent change in maternal TC per gestational week from first to second specimen for each study subject
Each of the 195 study participants represents a bar in the graph below
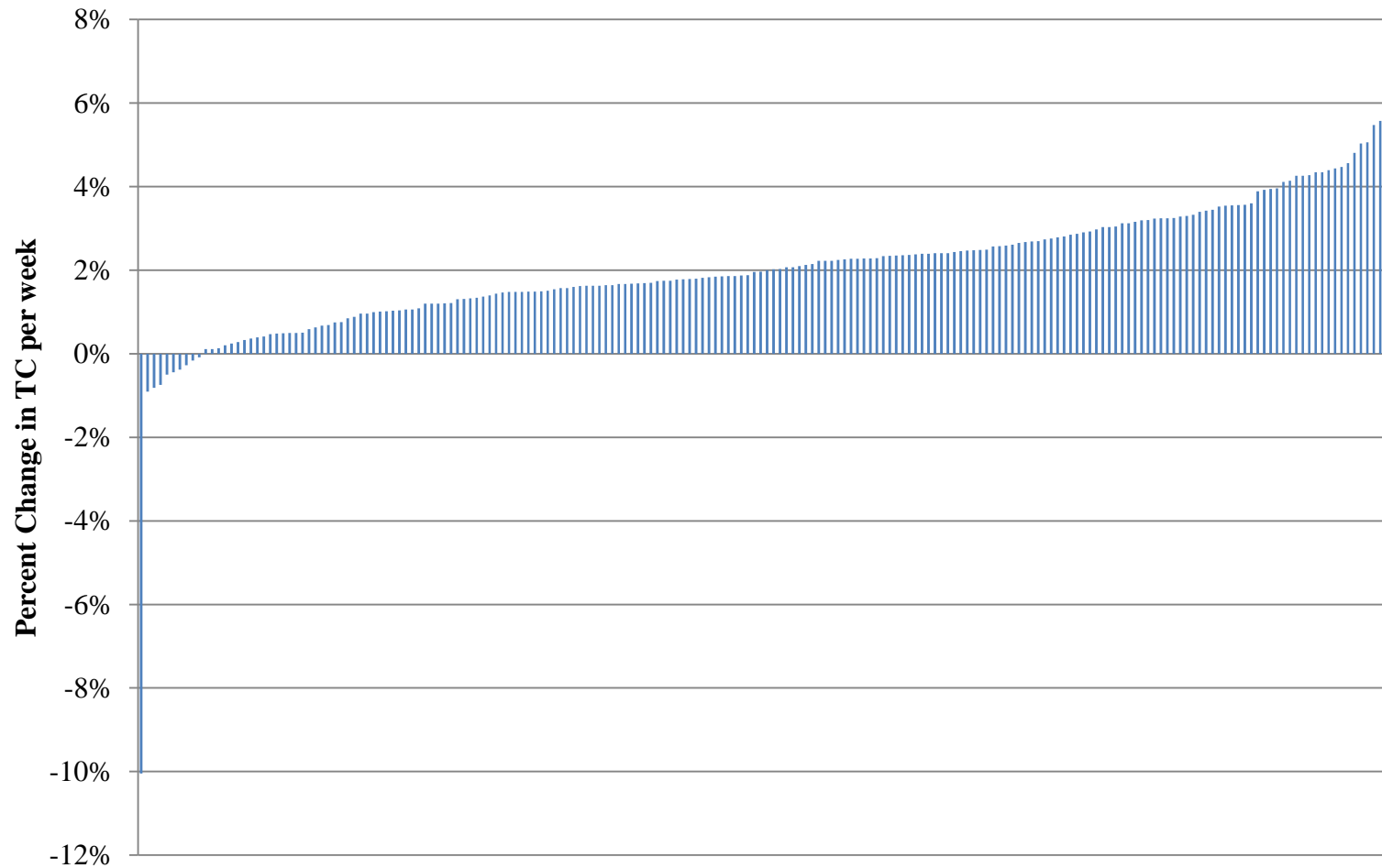
TC: total cholesterol

Figure 4.5c: Unit change in maternal TC per gestational week from first to second specimen for each study subject
Each of the 195 study participants represents a bar in the graph below
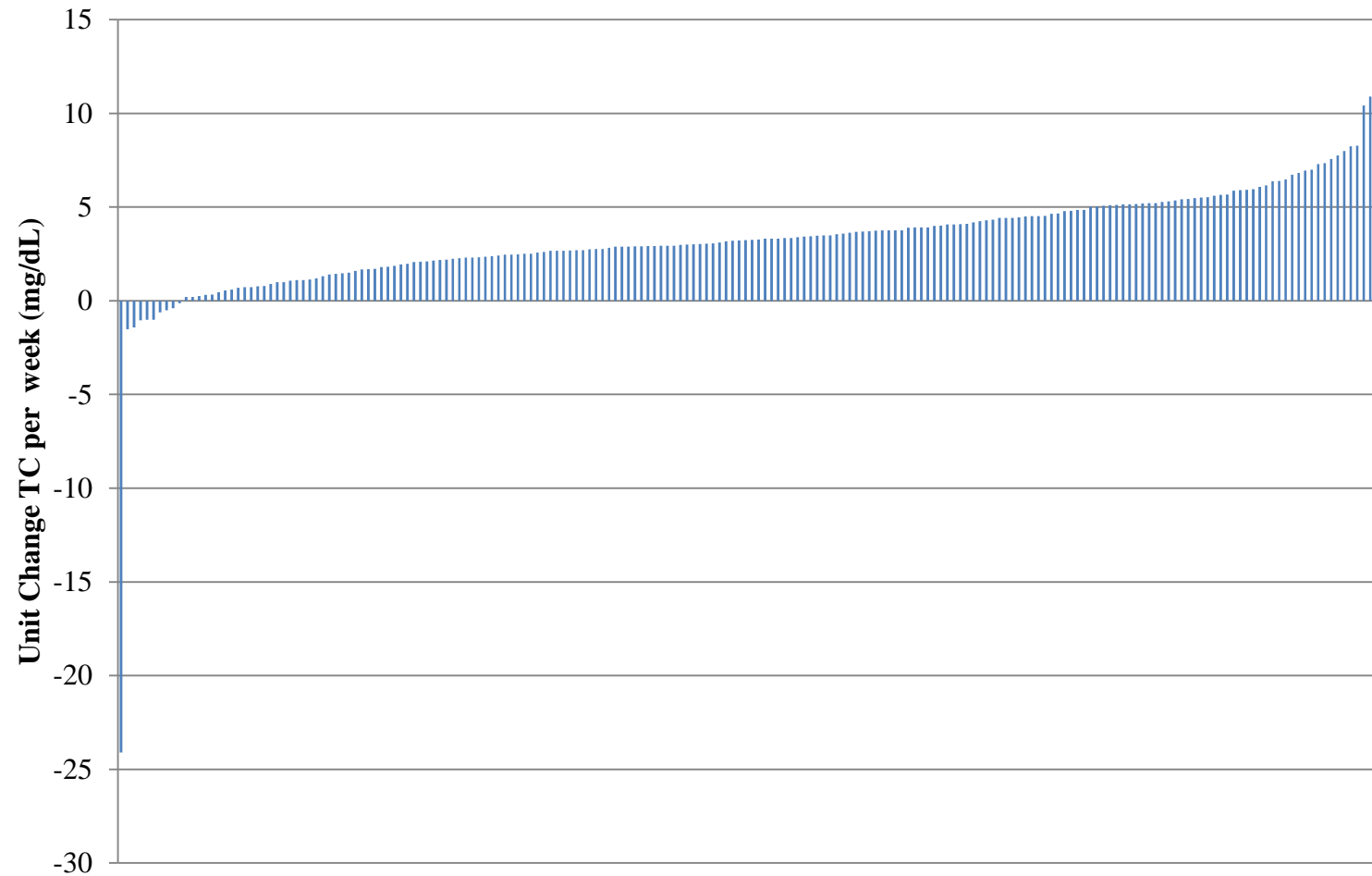
TC: total cholesterol

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CHAPTER FIVE
RELATIONSHIP BETWEEN CHANGES IN MATERNAL CHOLESTEROL DURING GESTATION AND GESTATIONAL AGE AT DELIVERY

INTRODUCTION

Preterm delivery, defined as giving birth to an infant less than 37 weeks of gestation, is the leading cause of infant mortality in the world, contributing to roughly 75% of all neonatal deaths and 60% of all infant deaths [1, 2]. In the United States the 2015 rate of preterm births was 9.63%, a slight increase from the 2014 preterm birth rate of 9.57% [3]. Preterm births disproportionately affect non-Hispanic Black women. The 2015 rate in Black women was 13.41%, the Hispanic rate was 9.14%, compared to the rate in non-Hispanic White women of 8.88% [3]. Infants born prematurely are at an increased risk of developing chronic lung disease, nosocomial infections, and neurocognitive complications, including cerebral palsy and mental retardation [4, 5].

Clinically, preterm births are divided into two categories, spontaneous preterm deliveries (sPTB), which includes premature rupture of membranes (PROM) and spontaneous labor, and medically indicated premature deliveries (mPTB) [6]. Research suggests multiple causes of sPTB, including infection, inflammation, vascular disease, and uterine over distension, although the pathogenesis is not yet well understood [6]. mPTB cases are those where a medical professional deems it safer for the mother and/or fetus to induce labor and deliver the infant than to leave the fetus in utero. It is estimated that 70% of preterm births are sPTB and the remaining 30% are mPTB [6, 7].

The etiology of preterm birth is unknown, although some research has suggested common risk factors between sPTB and cardiovascular disease [6]. As discussed in chapter two

of this dissertation, elevated cholesterol is a risk factor for cardiovascular disease and maternal cholesterol levels increase during gestation. However, as highlighted in the *Maternal Cholesterol Levels and Gestational Age at Delivery* section of this dissertation, contradicting evidence exists about the relationship between maternal cholesterol levels during gestation and the risk of preterm delivery.

Some literature suggests an association between maternal cholesterol during pregnancy and preterm birth; however, highly selective and non-generalizable study populations make comparing results across studies challenging. Maternal cholesterol levels are continually changing during pregnancy, yet most studies look at cholesterol as a single data point during pregnancy. Changes in maternal cholesterol may capture different biological responses to pregnancy than cholesterol levels from a single time point, and may provide insight into the relationship between maternal cholesterol and preterm delivery. The purpose of the proposed research is to analyze the association between changes in maternal low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) levels, and total cholesterol (TC) levels between two specimens collected during pregnancy and gestational age at delivery. This will be one of the few studies to have looked at a rate of change in maternal cholesterol in relation to preterm birth rather than examining maternal cholesterol levels at single time points during pregnancy. It is hypothesized that women with rates of change in LDL-C that fall below the 25th percentile or above the 75th percentile will be at an increased risk of delivering an infant with a lower gestational age. Secondly, it is hypothesized that women with rates of change in TC that fall below the 25th percentile or above the 75th percentile will be at an increased risk of delivering an infant with a lower gestational age. Lastly, it is hypothesized that no association between changes in maternal HDL-C and gestational age will be identified.

METHODS

Study Population

The Archive for Research on Child Health (ARCH) study, as described in detail in chapter 3, was utilized for this analysis. Active ARCH participants with serum specimens collected at two different time points during pregnancy and expected dates of confinement on or before December 31, 2013 were considered eligible for inclusion (n=201). Of the 201 eligible pregnancies, only singleton pregnancies with corresponding birth certificates were included (n=195). Table 5.1 summarizes the demographics of the included study population. Figure 3.2 in chapter 3 can be referenced for additional information on how the included population was selected.

For maternal race, the enrollment questionnaire for ARCH provided participants six different racial categories to select from, American Indian/Alaska Native, Native Hawaiian/Pacific Islander, Asian, Multiracial, White, and Black. Of the 195 study subjects for this analysis 37 women indicated their race as Black, six women indicated their race as Asian, one woman indicated American Indian/Alaska Native, zero women selected Native Hawaiian/Pacific Islander, and ten selected multiple races. For this analysis, given the small number of subjects in the Black, American Indian, Alaska Native, Native Hawaiian, Pacific Islander, Asian, and Multiracial categories, race was re-categorized into two groups, White (n= 127), and non-White (n=54). Information on maternal race was missing from the enrollment questionnaire for 14 subjects.

Cholesterol

All specimens from the ARCH study were sent to a laboratory at Michigan State University and stored at -80° C. Aliquots of the first and second specimen for each of the 195

participants were thawed, refrigerated, and transported to the chemical laboratory at Sparrow

Hospital laboratories, in Lansing, Michigan, for cholesterol testing. Testing methods for TC were

restricted to levels between 25 mg/dL and 700 mg/dL. HDL-C levels were valid from $2 - 200$

mg/dL. None of the women studied for cholesterol levels, had levels outside of the testing range.

The Friedwald equation, formula 5.1, was used to calculate LDL-C levels [8].

Formula 5.1:

$$Total\ Cholesterol - [(High\ Density\ Lipoprotein\ Cholesterol) + \left(\frac{Triglycerides}{5}\right)]$$

Invalid LDL-C measurements occurred when triglyceride levels were greater than 400 mg/dL

[8]. In five cases women had triglyceride levels greater than 400 mg/dL for either their first or

second specimen, therefore valid LDL-C levels were unable to be calculated. One participant had

a second trimester LDL-C value of negative 7 mg/dL. These six women were excluded from

LDL-C analyses. Procedures used to measure cholesterol levels abided by the National

Cholesterol Education Program performance criteria.

The change in maternal cholesterol (TC, LDL-C, and HDL-C) levels from the first to the

second specimen was the main exposure. Percent change was calculated individually for TC,

LDL-C, and HDL-C using formula 5.2.

Formula 5.2:

$$\frac{2nd\ cholesterol\ level\ -\ 1st\ cholesterol\ level}{1st\ cholesterol\ level} X\ 100$$

For the women in this study, the average gestational age when the first specimen was collected

was 12 weeks. 71% of included women had their first serum specimen collected during the first

trimester, 28% had their first serum specimen collected in the second trimester, and 1% had their

first serum specimen collected in the third trimester. The average gestational age when the

second specimen was collected was 28 weeks. 29% of included women had their second specimen collected during the second trimester and the remaining 71% of women had their second specimen collected during the third trimester.

Gestational Age at Birth

A corrected gestational age was calculated for each of the included 195 ARCH participants. Methodologies for correcting gestational age at birth are described elsewhere in the literature and summarized in chapter four of this dissertation [9-10]. Corrected gestational ages were divided into three groups, preterm birth (corrected gestational age less than 37 weeks), term birth (corrected gestational age between 37 and 40 weeks), and post-term birth (corrected gestational age greater than 40 weeks). Given the low incidence of preterm birth in this study population, it was decided to use gestational age as a continuous outcome variable, rather than a categorical outcome of preterm birth, term birth, and post-term birth, to identify any relationships between maternal cholesterol and changes in length of gestation.

Analytics

Multiple linear regression models were developed to analyze the given data. Purposeful selection, as described in detail by Hosmer and Lemeshow, was used to develop multiple linear regression models and to test each of the three hypotheses associated with maternal cholesterol and gestational age at birth [11]. Details of this method are described below. Analyses were completed using SAS, version 9.2

Hypothesis 1a: Women with rates of change in LDL-C from first to second specimen in the lowest quartile will be at an increased risk of delivering an infant with a smaller gestational age.

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Hypothesis 1b: Women with rates of change in TC from first to second specimen in the lowest quartile will be at an increased risk of delivering an infant with a smaller gestational age.

Hypothesis 2a: Women with rates of change in LDL-C from first to second specimen in the highest quartile will be at an increased risk of delivering an infant with a smaller gestational age.

Hypothesis 2b: Women with rates of change in TC from first to second specimen in the highest quartile will be at an increased risk of delivering an infant with a smaller gestational age.

Hypothesis 3: Changes in maternal HDL-C will not be significantly associated with a smaller gestational age.

The first step of purposeful selection model building was to conduct a univariate analysis with all relevant covariates, as determined by the literature, analyzing their relationship with the dependent variable, corrected gestational age. Table 5.2 summarizes the univariate analysis. Covariates in the univariate analysis that had a type three analysis of effects chi-square p-value less than or equal to 0.25 were determined to be potentially important and included in building a multiple regression model. Sex of the infant, if this was the mother's first pregnancy, the presence of gestational diabetes, the presence of gestational hypertension, history of preterm birth, and maternal age at time of birth had p-values less than or equal to 0.25. In a sub-data set, excluding women with missing data for the covariates of interest (n=172), the univariate relationship between corrected gestational age and sex of the infant became insignificant (p-value= 0.37) for this step. In addition, when further excluding women with missing LDL-C data the univariate relationship between maternal race and corrected gestational age became

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statistically significant (p-value= 0.22). Given these findings, sex of the infant was excluded

from the linear regression model building and maternal race was included in the linear regression

model building for the LDL-C model only. The main independent variables, change in maternal

LDL-C, HDL-C, and TC, were each included in their own model as the main independent

variable.

The second step of purposeful selection was to run a multiple linear regression model

with the main independent variable and the six significant covariates for the change in LDL-C

model and the five significant covariates for the change in HDL-C and change in TC models

identified in step one. These models were the full models. Tables 5.3a – 5.3e summarize the full

models for change in LDL-C, HDL-C, and TC. Of note, given results found in step five of the

purposeful selection model building, described in detail later in this section, changes in HDL-C

and TC are modeled as continuous variables as well as categorical variables. Changes in LDL-C

are only modeled as a continuous variable. Of the included covariates in the full model, those

with a p-value less than or equal to 0.10 remained in the regression model. This new model will

be referred to as the reduced model. For all five reduced models, the presence of gestational

diabetes, the presence of gestational hypertension, and history of preterm birth, remained

significant with a p-value less than or equal to 0.10. Likelihood ratio testing compared the full

multiple linear regression model to the reduced model. Sample sizes for the full and reduced

models were the same. For this testing, the null hypothesis represented the reduced model with q

degrees of freedom. The alternate hypothesis represented the full model with p degrees of

freedom. P-values calculated from a chi-square model with p-q degrees of freedom were used.

For all five analyses, the reduced models were not significantly different from the full models

with p-values less than or equal to 0.05. Therefore the reduced models were used in each analysis.

The next step, step three, was to check if any of the variables removed from the full model were important for providing a necessary adjustment of the effect of the variables in the reduced model. This was done by comparing the estimated coefficient of the main independent variable from the full model to the estimated coefficients of the main variable in the reduced model. If any of the estimated coefficients for the main variable in the reduced model changed by 20% or more, the removed variables were individually added back into the reduced model. Variables were added back one by one until the estimated coefficients of the main independent variables did not differ by more than 20% from what was calculated for the full model. In the change in LDL-C model, maternal race and maternal age were added back to the model. No variables were added back to the change in HDL-C models. In the change in TC model where change in TC was a continuous variable, no variables were added back to the model. In the change in TC model where change in TC was a categorical variable, whether or not this was the woman's first pregnancy was added back to the model.

Step four; covariates that were not statistically significant from the univariate analysis were individually added to each of the linear regression models from step three to see if any covariates significantly impacted the model as a whole, but may not have individually had a significant relationship with corrected gestational age. No additional covariates were added to the linear regression models for LDL-C, HDL-C, or TC.

In step five, Loess procedures were used to look at the linear relationship between corrected gestational age and each of the continuous variables deemed significant in in the reduced model. Changes in LDL-C, HDL-C, and TC as well as maternal age at birth of baby

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were the continuous variables identified for this analysis. Smoothed plots provided additional information regarding the parametric relationship between each continuous covariate and the corrected gestational age. LDL-C was found to have a linear relationship with the corrected gestational age and was analyzed as a continuous variable. HDL-C was found to have a semi-inverse U-shaped relationship with the corrected gestational age. The non-linear relationship supports the hypothesis to categorize HDL-C in to quartiles to look for a relationship between both the lowest quartile and highest quartile of HDL-C and corrected gestational age. Two models for HDL-C will be developed, a model with HDL-C as a continuous measure and a model with HDL-C broken into three groups, lowest 25th percentile, middle 50th percentile as a reference group, and highest 25th percentile. With TC, there is also evidence of a non-linear relationship with corrected gestational age. Although not quite as prominent as with HDL-C, there still appeared to be some inverse U-shaped relationship. Two models for TC will be developed, one with TC as a continuous variable and the second where TC will be categorized into two groups, lowest 25th percentile and the remaining 75th percentile as a reference group. Maternal age at birth of baby had a linear relationship with corrected gestational age and was included in each model as a single continuous variable.

In step six, each model was evaluated for interaction terms between the main independent variable, change in maternal cholesterol, and the included covariates. Likelihood ratio testing was used to compare the models with no interaction terms to the models with interaction terms added. No significant interaction terms were identified for the change in LDL-C model. For the change in HDL-C as a continuous variable model, two significant interaction terms were identified. These were the interaction between change in HDL-C and gestational diabetes and the interaction between change in HDL-C and previous preterm birth. For the change in HDL-C as a

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categorical variable, the change in HDL-C had a significant interaction with previous preterm birth. For the continuous change in TC model, the change in TC significantly interacted with previous preterm birth. For the categorical change in TC model, there were no significant interaction terms. Final models, including these significant interaction terms, for each of the five models are in tables 5.4a – 5.4e.

EXPLORATORY ANALYSIS

In addition to the aforementioned analyses, change in maternal cholesterol was recalculated two additional ways, both adjusting for the number of weeks between a participants two serum samples. The first, calculated change in maternal cholesterol as the percent change per week (formula 5.3) and the second, calculated the change by mg/dL unit change per week (formula 5.4).

Formula 5.3:

$$\frac{\left(\frac{2nd\ cholesterol\ level\ -\ 1st\ cholesterol\ level}{1st\ cholesterol\ level} \times\ 100\right)}{Number\ of\ weeks\ between\ 1st\ and\ 2nd\ cholesterol\ level}$$

Formula 5.4:

$$\frac{2nd\ cholesterol\ level\ -\ 1st\ cholesterol\ level}{Number\ of\ weeks\ between\ 1st\ and\ 2nd\ cholesterol\ level}$$

It was also decided to look at the relationship between first and second maternal cholesterol levels and corrected gestational age. Using the new variables for maternal cholesterol, purposeful selection methods were followed and additional models were developed. Tables 5.5a – 5.5c show the final multiple linear regression models for the percent change per week with corrected

gestational age as a continuous outcome variable. Tables 5.6a – 5.6c show the final multiple

linear regression models for the mg/dL unit change per week with corrected gestational age as a

continuous outcome variable. Tables 5.7a – 5.7c show the final multiple linear regression models

for first and second cholesterol levels with corrected gestational age as a continuous outcome

variable.

RESULTS

The distribution of corrected gestational age is shown in figure 5.1. Figures 5.2a – 5.2c

show the average cholesterol for the first and second specimens broken out by preterm (less than

37 weeks gestation), term (37 – 40 weeks gestation), and post-term (greater than 40 weeks

gestation) births. First LDL-C levels and first TC levels were each significantly higher in women

who had a preterm birth compared to women who had a term or post-term birth. Second LDL-C,

first and second HDL-C, and second TC levels were higher for preterm births compared to term

and post-term births, although not statistically significant. The incidence of preterm birth in this

study population was 4.1%, n=8 women. 156 women delivered term (80.0%) and 54 women

delivered post-term (9.7%). Given the small number of women who delivered preterm and post-

term in this population, it was decided not to use a categorical outcome but rather to use the

continuous variable, corrected gestational age as the outcome of interest.

LDL-C

189 women had LDL-C values for both first and second specimens as well as

corresponding birth certificate data. The median change in maternal LDL-C from first to second

specimen was 33%, average change was 39%. Change in maternal LDL-C ranged from a 51%

decrease from first to second specimen to a 150% increase. Figure 5.3 shows the univariate

relationship between corrected gestational age and the percent change in LDL-C. The average increase in LDL-C per gestational week was 2.4%. The average unit increase per gestational week was 2.03 mg/dL.

It was hypothesized that women with rates of change in LDL-C that fall below the 25th percentile or above the 75th percentile will be at an increased risk of giving birth to a baby with a smaller gestational age. As shown in table 5.2, the univariate analysis between change in maternal LDL-C and corrected gestational age was not statistically significant (95% Confidence Interval (CI): -0.004, 0.008, p-value = 0.46). As determined in step five of purposeful selection, this data set did not support a U-shaped relationship between LDL-C and corrected gestational age. Therefore, LDL-C was only analyzed as a continuous variable. When controlling for race, gestational diabetes, gestational hypertension, previous preterm birth, and maternal age no significant relationship was identified between changes in maternal LDL-C as a continuous variable and corrected gestational age at birth (95% CI: -0.01, 0.005, p-value = 0.74). Table 5.4a shows the final model and summarizes this finding. The final adjusted model included 175 women.

The univariate analysis between the percent change in LDL-C per gestational week and corrected gestational age was not statistically significant (p-value= 0.87). The final multiple regression model, table 5.5a, included data for 189 women and adjusted for gestational diabetes, gestational hypertension, previous preterm birth, and an interaction between percent change in LDL-C per gestational week and previous preterm birth. The interaction between percent change in LDL-C per gestational week and previous preterm birth was statistically significant (95% CI 0.56, 7.15, p-value= 0.02). Among women with a previous preterm birth, compared to those with

no previous preterm birth, for every 1% increase in LDL-C per gestational week there is a 3.85 week increase in the corrected gestational age birth.

The univariate analysis between the unit change in LDL-C per gestational week and corrected gestational age was not statistically significant (p-value= 0.89). The final multiple regression model, table 5.6a, adjusted for maternal race, gestational diabetes, gestational hypertension, previous preterm birth, and an interaction between unit change in LDL-C per gestational week and previous preterm birth. The interaction between unit change in LDL-C per gestational week and previous preterm birth was statistically significant in the 175 women included in this analysis (95% CI 0.56, 4.28, p-value= 0.01). Among women with a previous preterm birth, compared to those with no previous preterm birth, for every one unit increase in LDL-C per gestational week there is a 2.42 week increase in the corrected gestational age at birth.

When looking at the relationship between the first LDL-C specimen and corrected gestational age, the unadjusted analysis was statistically significant (p-value= 0.05). The unadjusted relationship between the second LDL-C and corrected gestational age was not statistically significant (p-value= 0.58). The initial final adjusted model adjusted for maternal race, if this was the women's first pregnancy, gestational diabetes, gestational hypertension, previous preterm birth, maternal age at birth of baby, an interaction between the first LDL-C and previous preterm birth, an interaction between the first LDL-C and gestational diabetes, an interaction between the second LDL-C and previous preterm birth, and an interaction between the second LDL-C and gestational diabetes. At a significance level of 0.05, the interaction terms between the first LDL-C and previous preterm birth, the first LDL-C and gestational diabetes, and the second LDL-C and previous preterm birth were not statistically significant in the initial

final model. The interaction terms between the first LDL-C and gestational diabetes and the second LDL-C and previous preterm birth were removed from the final model. The final model had a sample size of 175 women and is summarized in table 5.7a. The interaction between the first LDL-C and a previous preterm birth was statistically significant in the final model (95% CI: 0.02, 0.19, p-value= 0.01). Among women with a history of preterm birth, compared to those with no history of preterm birth, for every 10 unit increase in the first LDL-C there is a 1.1 week increase in the corrected gestational age at birth. The interaction between the second LDL-C and gestational diabetes was statistically significant in the final model (95% CI: -0.28, -0.06, p-value=0.003). Among women with gestational diabetes, compared to those with no gestational diabetes, for every 10 unit increase in the second LDL-C there is a 1.7 week decrease in the corrected gestational age at birth.

HDL-C

195 women had first and second HDL-C levels as well as corresponding birth certificate data. The median change in maternal HDL-C from first to second specimen was 10%, average change was 12%. Change in maternal HDL-C ranged from a 34% decrease from first to second specimen to an 82% increase. The univariate relationship between corrected gestational age and the percent change in HDL-C is depicted in figure 5.4. The average increase in HDL-C per gestational week was 0.65%. The average unit increase per gestational week was 0.32 mg/dL. It was hypothesized that changes in maternal HDL-C will not be significantly associated with a smaller gestational age.

HDL-C Continuous

As shown in table 5.2, the univariate relationship between change in HDL-C as a continuous variable and corrected gestational age appeared to be trending towards significance

although not statistically significant (95% CI: -0.004, 0.02, p-value = 0.19). Table 5.4b shows the final multivariate model for the relationship between the change in maternal HDL-C as a continuous variable and corrected gestational age. This model included 195 women and controlled for gestational diabetes, gestational hypertension, previous preterm birth, and two statistically significant interaction terms. The first interaction term looked at the interaction between change in HDL-C and previous preterm birth (95% CI: -0.16, -0.03, p-value = 0.004). The second interaction term included in this model was between the change in HDL-C and gestational diabetes (95% CI: 0.09, 0.30, p-value = 0.0002). The results for this model were stratified into three separate groups. These groups include those women with no gestational diabetes and no previous preterm birth, women with no gestational diabetes and a positive history of previous preterm birth, and women with gestational diabetes and no previous preterm birth. No women within this study sample were positive for both gestational diabetes and previous preterm birth, so estimates for this group are not interpreted from the SAS output. Compared to women with no gestational diabetes and no previous preterm birth, in this study sample, women with no gestational diabetes and a positive history of previous preterm birth had a one week decrease in the corrected gestational age for every 10% increase in HDL-C. Using the same comparison group, in women with gestational diabetes and no previous preterm birth every 10% increase in HDL-C resulted in a two week increase in corrected gestational age. Figure 5.5a shows the relationship between these three patient scenarios.

The unadjusted relationship between the percent change in HDL-C per gestational week and corrected gestational age was not statistically significant, although was trending towards significance (p-value= 0.06). In the final adjusted model (n=195), gestational diabetes, gestational hypertension, previous preterm birth, an interaction between the percent change in

HDL-C per gestational week and previous preterm birth, and an interaction between the percent change in HDL-C per gestational week and gestational diabetes were controlled for. Table 5.5b summarizes this final model. The results for this model were stratified into three separate groups. These groups include those women with no gestational diabetes and no previous preterm birth, women with no gestational diabetes and a positive history of previous preterm birth, and women with gestational diabetes and no previous preterm birth. Compared to women with no gestational diabetes and no previous preterm birth, in women with no gestational diabetes and a positive history of previous preterm birth, every 1% increase in HDL-C per gestational week resulted in a 1.7 week decrease in the corrected gestational age. Using the same comparison group, in women with gestational diabetes and no previous preterm birth, every 1% increase in HDL-C per gestational week resulted in a 2.7 week increase in corrected gestational age. Figure 5.5b shows the relationship between these four patient scenarios.

The unadjusted relationship between the unit change in HDL-C per gestational week and corrected gestational age was statistically significant (95% CI: 0.01, 0.58, p-value= 0.04). In the final adjusted model 195 women were included. The final adjusted model controlled for gestational diabetes, gestational hypertension, previous preterm birth, and two significant interaction terms, table 5.6b. The first interaction was between the unit change in HDL-C per gestational week and previous preterm birth (95% CI: -4.86, -0.96, p-value= 0.004). The second interaction was between the percent change in HDL-C per gestational week and gestational diabetes (95% CI: 1.25, 4.42, p-value= 0.0004). Similar to prior results for HDL-C, the results for this model were also stratified into three separate groups, women with no gestational diabetes and no previous preterm birth, women with no gestational diabetes and a positive history of previous preterm birth, and women with gestational diabetes and no previous preterm birth.

Compared to women with no gestational diabetes and no previous preterm birth, in women with no gestational diabetes and a positive history of previous preterm birth, every one unit increase in HDL-C per gestational week resulted in a 2.9 week decrease in the corrected gestational age. Using the same comparison group, in women with gestational diabetes and no previous preterm birth, every one unit increase in HDL-C per gestational week resulted in a 2.8 week increase in corrected gestational age. Figure 5.5c shows the relationship between these three patient scenarios.

The unadjusted relationship between the first HDL-C and corrected gestational age was statistically significant (p-value= 0.02). The unadjusted relationship between the second HDL-C and corrected gestational age was not statistically significant (p-value= 0.34). In the final adjusted model, table 5.7b, gestational diabetes, gestational hypertension, previous preterm birth, and an interaction between the second HDL-C and previous preterm birth were adjusted for (n=195). In the final adjusted model, the relationship between the first LDL-C and corrected gestational age was significantly significant (95% CI: -0.06, -0.01, p-value= 0.003). The interaction between the second HDL-C and previous preterm birth was also significantly associated with corrected gestational age (95% CI: -0.28, -0.007, p-value=0.04). Among all women in this study sample, for every 10% increase in the first HDL-C, there is a 0.4 week decrease in the corrected gestational age at birth. Among women with a history of previous preterm birth, compared to those women with no history of previous preterm birth, for every 10% increase in the second HDL-C, there is a 1.4 week decrease in the corrected gestational age at birth.

HDL-C Categorical

Change in HDL-C was then categorized into quartiles. The second and third quartiles were combined, labeled as middle 50%, and used as the reference group. In the first quartile, lowest 25th percentile, change in HDL-C ranged from -34% to 0%. In the second and third quartiles combined, change in HDL-C ranged from 0% to 23%. In the fourth quartile, highest 25th percentile, change in HDL-C ranged from 23% to 81%. The univariate analysis looking at change in maternal HDL-C as a categorical variable and corrected gestational age found that women with a rate of change in HDL-C in the highest quartile did not have significantly different corrected gestational ages at birth compared to the referent group (95% CI: -0.61, 0.53, p-value = 0.89). Women with changes in HDL-C in the lowest quartile had babies with smaller corrected gestational ages compared to the reference group, 39.3 weeks gestation compared to 39.7 weeks gestation, respectively (p-value= 0.29). Table 5.2 summarizes these findings.

The multiple linear regression model for change in HDL-C as a categorical variable (n=195) controlled for gestational diabetes, gestational hypertension, previous preterm birth, if this was the woman's first pregnancy, and an interaction between change in HDL-C as a categorical variable and previous preterm birth. Compared to women with a change in HDL-C in the reference group and no history of preterm birth, there was no statistically significant interaction between a previous preterm birth and HDL-C in either the top 25th percentile nor the bottom 25th percentile, table 5.4c.

TC

195 women had TC values for both first and second TC levels as well as corresponding birth certificate data. The median change in maternal TC from first to second specimen was 30%, average change was 34%. Change in maternal TC ranged from a 13% decrease from first to

second specimen to a 104% increase. Figure 5.6 shows the univariate relationship between

corrected gestational age and the percent change in TC. The average percent change in TC per

gestational week was 2.05%. The average unit increase in TC per gestational week was 3.35

mg/dL. It was hypothesized that women with rates of change in TC that fall below the 25th

percentile or above the 75th percentile will be at an increased risk of giving birth to a baby with a

smaller gestational age.

TC Continuous

The univariate relationship between change in TC as a continuous variable and corrected

gestational age is shown in table 5.2. This relationship was not statistically significant (95% CI: -

0.004, 0.01, p-value = 0.26).   The initial final model for the relationship between the change in

TC as a continuous variable and corrected gestational age controlled for gestational diabetes,

gestational hypertension, and previous preterm birth. The interaction between the change in TC

and previous preterm birth was trending towards significance during the model building phase,

p-value= 0.056, and was statistically significant when added to the complete final model (p-

value= 0.05). The final model controlled for gestational diabetes, gestational hypertension,

previous preterm birth, and the interaction between the change in TC and previous preterm birth.

In women with a history of preterm birth, compared to women with no history of preterm birth,

for each 10 unit increase in TC, the corrected gestational age decreased by one week (95% CI: -

0.23, 0.001, p-value= 0.5). Table 5.4d summarizes the final model.

The univariate analysis between the percent change in TC per gestational week and

corrected gestational age was not statistically significant, p-value= 0.32. The final linear

regression model controlled for gestational diabetes, gestational hypertension, previous preterm

birth, and an interaction between the percent change in TC per gestational week and previous

preterm birth. The final model included 195women and found women with a history of preterm birth had a 6.2 week decrease in corrected gestational age for every 1% increase in TC per gestational week, compared to women with no history of preterm birth (95% CI: -12.15, -0.17, p-value= 0.04), table 5.5c.

For the unit change in TC per gestational week, in the unadjusted model there was no significant association with corrected gestational age, p-value= 0.51. The final model for the unit change in TC per gestational week controlled for gestational diabetes, gestational hypertension, previous preterm birth, if this was the woman's first pregnancy, and an interaction term between the unit change in TC per gestational week and gestational diabetes, table 5.6c. The final model included 195 women. Compared to women with no gestational diabetes, women with gestational diabetes had a 0.7 week increase in the corrected gestational age for every one unit increase in TC per gestational week (95% CI: 0.12, 1.18, p-value= 0.02).

The unadjusted relationship between the first TC level and corrected gestational age was statistically significant, p-value= 0.008. The unadjusted relationship between the second TC level and corrected gestational age was not statistically significant, p-value= 0.24. The final model for the relationship between the individual TC levels (first and second) and corrected gestational age, table 5.7c, controlled for gestational diabetes, gestational hypertension, previous preterm birth, if this was the woman's first pregnancy, and a significant interaction between the first TC and previous preterm birth. The final model included 195 women and showed, compared to women with no previous preterm birth, for every 10% increase in the first TC, women with previous preterm birth saw a 0.5 week increase in corrected gestational age (95% CI: 0.003, 0.09, p-value= 0.04).

TC Categorical

Table 5.2 summarizes the univariate relationship between corrected gestational age and change in TC as a categorical variable. Change in TC was broken into to two categories, change in TC in the lowest 25th percentile and the remaining 75% was used as the reference group. Compared to the referent group, women with change in TC in the lowest 25th percentile had a smaller corrected gestational age, 39.3 weeks compared to 39.7 weeks, although this finding was not statistically significant (95% CI: -0.91, 0.17, p-value = 0.18).

Table 5.4e summarizes the findings for the final model for the relationship between changes in TC as a categorical variable and corrected gestational age. Gestational diabetes, gestational hypertension, previous preterm birth, and if this was the woman's first pregnancy were controlled for in this model. This final model (n=195) showed no statistically significant relationship between the lowest 25th percentile of TC levels and corrected gestational age compared to women with TC levels in the referent group (p-value= 0.50).

DISCUSSION

As summarized in chapter two of this dissertation, most reviewed studies looked at the relationship between maternal cholesterol at a single time point in pregnancy and preterm birth. Two studies looked at a percent change in maternal cholesterol and preterm birth and neither study found a significant relationship [12, 13]. The reviewed studies assessing the relationship been maternal cholesterol and a single time point and gestational age had findings that varied. To address the gap in current literature of only looking at maternal cholesterol at a single time point, this study looked at the change in maternal cholesterol in relation to corrected gestational age. It is thought that the change in cholesterol levels better captures the biological response to

pregnancy and the developing fetus in comparison to cholesterol levels assessed at a single point in pregnancy.

In this ARCH study population, the average cholesterol for the first and second specimen, for LDL-C, HDL-C, and TC, was higher in women who delivered preterm compared to those who delivered term and post-term. Average first LDL-C levels and first TC levels were significantly higher in women who delivered preterm term. Referencing table 2.6 from chapter two of this dissertation, although results are inconclusive, the significant LDL-C and TC results from the ARCH population support findings reported in other studies.

Corrected gestational age was not significantly associated with change in LDL-C or change in TC as a continuous variable when looking at the univariate analyses. Although not statistically significant in this study population, the unadjusted relationships between change in maternal HDL-C, as a continuous variable, and corrected gestational age trended towards a significant relationship. Given that the change in HDL-C during pregnancy is small, a larger sample size may be needed to identify a statically significant relationship. The unadjusted relationship between change in TC as a categorical variable and corrected gestational age was also trending towards significance. In the exploratory analyses, the unadjusted relationship between the first LDL-C levels and corrected gestational age was statistically significant at p-value= 0.05. The unadjusted relationship between the first HDL-C levels and corrected gestational age was statistically significant, p-value= 0.02. The unadjusted relationship between the first TC levels and corrected gestational age was also statistically significant, p-value= 0.01. For each of these three univariate models, as the first cholesterol levels increased, the corrected gestational age at birth decreased indicating higher levels of cholesterol early in pregnancy were associated with a shorter gestation. In addition, the unadjusted relationship between the unit

change in HDL-C per gestational week and corrected gestational age was statistically significant, p-value= 0.04, suggesting that those women with a larger increase in HDL-C per gestational week had longer gestations. These results should be interpreted with caution as HDL-C may not linearly increase during gestation, but rather tends to peak in the second trimester and decrease in the third trimester. Studying these relationships that were trending towards statistical significance and that were statistically significant in greater detail in a study population with a greater incidence of preterm birth is warranted.

In adjusted models, the change in LDL-C was not significantly associated with corrected gestational age. Both the percent change in LDL-C per gestational week and the unit change in LDL-C per gestational week in women with a history of preterm birth were statistically significant compared to women with no history of preterm birth. In women with a history of preterm birth, as the change per gestational week increased, the gestational age at birth also increased. This finding suggests that in women with a history of preterm birth, larger rates of increase in LDL-C are protective against preterm birth. When looking at first and second LDL-C levels in the adjusted model, interaction terms between the first LDL-C and previous preterm birth and the second LDL-C and gestational diabetes were included. As first LDL-C levels increased, again a protective effect against preterm birth was found. For women with gestational diabetes, as second LDL-C levels increased the corrected gestational age at birth decreased. The seemingly protective effect of increased LDL-C during pregnancy against a repeat preterm birth is an interesting finding within this population. Current research does not report any significant interactions with a previous preterm birth, but some do report an overall protective effect of high LDL-C, see table 2.6 in chapter two of this dissertation.

When looking at change in HDL-C, both the continuous and the categorical change in HDL-C models had interaction terms to adjust for. In the continuous model, interactions between the change in HDL-C and previous preterm birth and between the change in HDL-C and gestational diabetes were adjusted for. These two interaction terms were also included in the final adjusted models looking at the percent change in HDL-C per gestational week and the unit change in HDL-C per gestational week. As the HDL-C measurements increased, there was no protective effect as women with a history of preterm birth had significantly smaller gestational ages at birth when compared to women with no history of preterm birth. Of note, these findings are opposite of what was found for change in LDL-C. As the HDL-C levels increased, women with gestational diabetes and no previous preterm birth had increased gestational ages at birth. Although elevated HDL-C levels are not protective against a repeat preterm birth in this study population, increased changes in HDL-C seem to be protective against preterm birth in women with gestational diabetes. No women in this study population had gestational diabetes with a history of a previous preterm birth. In addition, the adjusted model for first and second HDL-C levels found a significant interaction between the second HDL-C levels and previous preterm birth. As with the other models, this model found in women with a history of preterm birth, as the second HDL-C levels increased, the corrected gestational age at the birth of the baby decreased. Future research should attempt to replicate these findings in a population with a greater prevalence of previous preterm births.

Although the relationship between the categorical change in HDL-C and corrected gestational age was not statistically significant when interacting with a previous preterm birth, it is important to point out the relationship between change in HDL-C and corrected gestational age in women with a history of preterm birth. Women in the lowest 25th percentile for change in

HDL-C were estimated to have infants at larger gestational ages and women in the highest 25th percentile were estimated to have infants at smaller gestational ages when compared to women with change of HDL-C in the reference group. This finding is consistent with the results from change in HDL-C as a continuous variable, where women with a history of preterm birth and higher rates of change in HDL-C had smaller gestational ages at birth. The current literature looking at maternal HDL-C levels at one time point includes studies that suggest an association between low maternal HDL-C and gestational age at birth, specifically preterm birth [1, 14 – 16], a single study that found an association between high maternal HDL-C and gestational age at birth [17], and studies that report no association [18, 19]. Two studies which looked at the percent change in HDL-C during gestation found no relationship between change in HDL-C and gestational age at birth [12, 13].

For change in TC, the continuous TC model adjusted for an interaction with previous preterm birth and found a significant association with gestational age. The same significant interaction was found in the model looking at the percent change in TC per gestational week. In women with a positive history of preterm birth, as the change in TC increased the corrected gestational age decreased. This finding is similar as to that for the HDL-C models. When looking at the percent change in TC per gestational week, the final model estimated that for each percent increase in TC levels, the gestational age at birth would decrease by six weeks. The confidence interval for this estimate is wide (95% CI: -12.15, -0.17) and these results should be interpreted with caution, but warrant further investigation. The final adjusted model with the first and second TC levels found a significant interaction between the first TC levels and history of preterm birth. Interestingly, this model found a protective effect as first TC levels increased, similar to the results for the LDL-C models. As first TC levels increased so did the corrected gestational age.

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These results suggest elevated TC levels at the start of pregnancy are protective against preterm birth but as TC levels increase the protective effect is lost. One thought as to why these findings were significant has to do with TC levels being the sum of HDL-C, LDL-C and very low density-protein levels. In the first trimester, the ratio of LDL-C to HDL-C in TC levels favors LDL-C. In the second trimester, HDL-C levels peak and play a larger role in TC levels. As the findings for the first TC levels are similar to the findings to the LDL-C models, perhaps this is a result of the LDL-C levels playing a large role in the calculation of the TC levels. As HDL-C levels increase in the calculation of the second TC levels, the results no longer are similar to those from the LDL-C models and the association with corrected gestational age becomes non-significant. This suggests that the significant relationship with TC and corrected gestational age may have more to do with how TC is calculated rather than how TC levels may biologically influence preterm birth.

The adjusted model for the unit change in TC per gestational week did not find a significant interaction between TC and previous preterm birth. Instead, this model found a significant protective interaction between the unit change in TC per gestational week and gestational diabetes. As the TC levels increased the corrected gestational age at the birth of the baby increased. One reason for the differences in significant interaction terms is likely due to the small number of women that had either gestational diabetes (n=5) or a history of preterm birth (n=3) in this sample population. These significant interaction terms warrant additional research to see if they remain statistically significant in a larger sample.

As reviewed in chapter two, maternal cholesterol levels are precursors for sex hormones, including progesterone, which are needed for a healthy pregnancy. Therefore cholesterol levels during pregnancy may have a protective effect for length of gestation. Although this study did

not replicate the protective effects found for HDL-C and TC levels, a protective effect was found for LDL-C levels in women with a history of preterm birth. However, current literature also suggests that when these changes are either too small or too large, women are at increased risks of adverse outcomes including preterm birth. Small changes in maternal cholesterol levels may be a result of acute illness or poor nutritional intake [13, 16, 20]. Large changes in maternal cholesterol levels are associated with inflammation and oxidative stress [13, 16, 20 − 23]. Elevated maternal cholesterol levels may cause increases in inflammation and oxidative stress, may be the result of inflammation and oxidative stress, or may have a synergistic relationship with inflammation and oxidative stress [13]. Research suggests some illnesses, poor nutritional intake, inflammation, and oxidative stress may all be risk factors for preterm birth [6, 13, 16, 20 - 23]. The current study was unable to control for each of these risk factors as the data was unavailable. It is therefore unknown if the lack of change or increased changes in maternal cholesterol in this study population were a result or cause of underlying complications that caused preterm birth. Future research should measure and control for both the social, e.g. nutritional intake, socio-economic status and BMI, and biological, e.g. infection markers, inflammation, and oxidative stress, risk factors for preterm birth.

The findings in this chapter support a protective effect with LDL-C levels against preterm birth. In addition, if there is a history of preterm birth, maternal cholesterol levels, both changes in levels as well as first and second measurements, are significantly associated with the corrected gestational age at birth. Although history of preterm birth is not a modifiable risk factor, maternal cholesterol levels are measurable and modifiable. Future studies should focus on maternal cholesterol levels in women with a history of preterm birth as this study population only had three women in this group. If these findings are replicated in are larger sample of

women with a history of preterm birth, the measurement of maternal cholesterol levels during pregnancy may help physicians identify women at risk of preterm delivery and therefore initiate non-invasive interventions to help reduce the risk of preterm birth.

LIMITATIONS

Utilizing a rich database such as ARCH provided a large amount of data related to pregnancy, maternal health, pregnancy outcomes, and fetal health. Despite the vast amount of data available, there were still some limitations identified with using ARCH for this analysis. The main limitation was the low incidence of preterm birth within the study population. In this ARCH study population the incidence of preterm birth was almost half that of the national average in the United States (4.1% compared to 9.63%, respectively). This may be because women who participate in research studies during pregnancy may start prenatal care earlier in pregnancy and may have healthier lifestyles. The lack of preterm births, although clinically a highly favorable statistic, limits the ability to statistically detect a relationship between changes in maternal cholesterol levels and preterm birth. A larger sample size of women with a higher incidence of preterm birth and cholesterol measurements from multiple time points during pregnancy may have addressed this limitation.

Looking at maternal cholesterol levels two time points pregnancy presents a limitation when looking at preterm birth. Some women may go into labor prior to the collection of their second serum sample. The lack of a second specimen excluded these women from the analysis, even though their cholesterol levels might provide valuable information on this topic. To avoid this limitation, maternal cholesterol levels could be collected at the time of delivery for all those delivering prematurely and have not already had a second specimen collected. Thirdly, in this

study there was variability in gestational age at the time of specimen collection. This study grouped the first specimens together and grouped the second specimens together, regardless of gestational age at the time of specimen collection. As maternal cholesterol levels consistently change during pregnancy, the rate of change in those women with 15 weeks between specimens may not be comparable to the rate of change in women with 10 weeks between specimens. Future research should limit the variability in the gestational age at the time of specimen collections.

The exploratory regression models looked at the percent change per gestational week, the unit change per gestational week, and the raw first and second cholesterol levels. The percent change per gestational week and the unit change per gestational week were calculated under the assumption that the specific maternal cholesterol increased linearly throughout gestation. This assumption seems to hold true for both LDL-C and TC levels in this study population, see figures 3.5a and 3.6a in chapter three of this dissertation. With HDL-C levels, both the literature and this study population suggest a non-linear change in HDL-C levels through gestation, see figure 3.4a in chapter three of this dissertation. The results for the percent change in HDL-C per gestational week and the unit change in HDL-C per gestational week should be interpreted with caution since there is large variation in when serum specimens were collected from each ARCH participant. As future research reduces the variability in when serum specimens are collected, the percent change and unit change in HDL-C per gestational week should be reevaluated.

Lastly, for this analysis, there was no information available on hereditary hypercholesterolemia, diet and nutritional intake during pregnancy. Data was collected on pre-pregnancy hypercholesterolemia, however only 66% of the study participants answered this question on the questionnaire. Of the 66% that answered the question, only two women answered

yes. The remaining 127 women answered no. The lack of variability in this data point caused us to exclude the question from analyses. Maternal BMI, pre-pregnancy, postpartum, and the change between the two, was investigated as a proxy to maternal nutrition, but this method has its limitations. Because maternal diet can have an impact on the changes seen in maternal cholesterol [24], future studies should gather information on maternal diet and appropriately control for this variable in analyses.

APPENDICES

APPENDIX A

Tables

Table 5.1: Demographics of ARCH study population with a singleton pregnancy, cholesterol at two time points during pregnancy, and corresponding birth certificates

	N (%)
NUMBER OF PARTICIPANTS	195
MATERNAL RACE	
Black or African American	37 (19)
White	127 (65.1)
Other (American Indian, Alaska Native, Native Hawaiian, Pacific Islander, Asian, or Multiracial)	17 (8.7)
Missing Data	14 (7.2)
MATERNAL ETHNICITY	
Hispanic or Latino	26 (13.3)
Not Hispanic or Latino	169 (86.7)
Missing	0 (0)
MATERNAL AGE AT BIRTH OF BABY	
18-24 years	91 (46.7)
25-30 years	69 (35.4)
31-40 years	34 (17.4)
>40 years	1 (0.5)
Missing Data	0 (0)
MATERNAL EDUCATION	
Did not finish high school	25 (12.8)
High school graduate or GED	61 (31.3)
Some College	58 (29.7)
College graduate or more	47 (24.1)
Missing Data	4 (2.1)
HOUSEHOLD INCOME	
Under $25,000	130 (66.7)
$25,000 to $49,999	34 (17.4)
$50,000 to $74,999	15 (7.7)
$75,000 or above	11 (5.6)
Missing Data	5 (2.6)
MARITAL STATUS	
Married, living with the baby's father	60 (30.8)
Married	10 (5.1)
Unmarried, living with the baby's father	62 (31.8)
Unmarried	63 (32.3)
Missing Data	0 (0)

ARCH: Archive for Research on Child Health

Table 5.2: Unadjusted univariate analysis between corrected gestational age, continuous variable, and each covariate being evaluated for inclusion in the multiple regression model

	Mean gestational age (weeks)	R-Square	Type 3 P-value
LDL-C Change	-	0.0029	0.46
HDL-C Change	-	0.009	0.19*
HDL-C Change quartiles		0.013	0.29
Lowest 25th percentile	39.3		
Middle 50%	39.7		
Highest 25th percentile	39.7		
TC Change	-	0.006	0.26
TC Change 2 groups		0.009	0.18*
Lowest 25th percentile	39.3		
Other 75%	39.7		
Race		0.004	0.39
White	39.6		
Non-white	39.4		
Missing data (n=14)	39.7		
Ethnicity		0.001	0.69
Not Hispanic or Latino	39.6		
Hispanic or Latino	39.7		
Maternal age	-	0.017	0.07*
Maternal education		0.009	0.65
Did not finish high school	39.8		
High school graduate/GED	39.6		
Some college	39.5		
College graduate or more	39.3		
Missing data (n=4)	40.3		
Household income		0.016	0.39
< $25, 000	39.6		
$25,000 - $49,999	39.1		
$50,000 - $74,999	39.6		
≥ $75,000	39.7		
Missing data (n=5)	41.0		
Marital status		0.017	0.35
Married	39.2		
Married, living with baby's father	39.5		
Unmarried, living with baby's father	39.9		
Unmarried	39.4		
First pregnancy		0.017	0.07*
No	39.4		
Yes	39.8		

Table 5.2 (cont'd)

Tobacco use			0.0008	0.70
	No	39.5		
	Yes	39.7		
Gestational diabetes			0.02	0.03*
	No	39.6		
	Yes	38.0		
Gestational hypertension			0.07	0.0001*
	No	39.7		
	Yes	37.7		
Previous preterm birth			0.09	<0.0001*
	No	39.6		
	Yes	35.7		
Pre-pregnancy BMI (con)		-	0.0016	0.58
Pre-pregnancy BMI (cat)			0.001	0.98
	<18.5	39.6		
	18.5 -<25	39.5		
	25- <30	39.6		
	>= 30	39.5		
	Missing data (n=2)	39.0		
Postpartum BMI (con)		-	0.001	0.62
Postpartum BMI (cat)			0.009	0.62
	<18.5	38.0		
	18.5 -<25	39.6		
	25- <30	39.6		
	>= 30	39.6		
	Missing data (n=1)	40.0		
Sex of baby			0.008	0.22*
	Male	39.4		
	Female	39.7		
Net maternal weight gain		-	0.00006	0.92

*Type 3 Analysis of Effects p-value < 0.25, used in multivariate model.

TC: total cholesterol; LDL-C: low density-lipoprotein cholesterol; HDL-C: high density-lipoprotein cholesterol; BMI: body mass index; con: continuous variable; cat: categorical variable

Table 5.3a: Multiple linear regression model, full model, for corrected gestational age as a continuous outcome variable including change in maternal LDL-C as a continuous variable and the significant covariates from step one of purposeful selection

	Estimate	95% CI		P-value
LDL-C change	-0.001	-0.007	0.005	0.77
Maternal race				
Non-white	-0.36	-0.87	0.15	0.16
Maternal age	-0.02	-0.07	0.02	0.34
First pregnancy	0.30	-0.18	0.78	0.23
Gestational diabetes	-2.15	-3.72	-0.58	0.007*
Gestational hypertension	-1.92	-2.91	-0.94	0.0001*
Previous preterm birth	-3.22	-4.97	-1.46	0.0003*

*Type 3 Analysis p-value $\leq$ 0.10, used in reduced multivariate model

CI: confidence interval; LDL-C: low density-lipoprotein cholesterol

Table 5.3b: Multiple linear regression model, full model, for corrected gestational age as a continuous outcome variable including change in maternal HDL-C as a continuous variable and the significant covariates from step one of purposeful selection

	Estimate	95% CI		P-value
HDL-C change	0.008	-0.004	0.02	0.19
Maternal age	-0.008	-0.05	0.04	0.73
First pregnancy	0.32	-0.15	0.80	0.19
Gestational diabetes	-1.94	-3.54	-0.35	0.02*
Gestational hypertension	-1.78	-2.73	-0.82	0.0003*
Previous preterm birth	-3.33	-5.13	-1.53	0.0003*

*Type 3 Analysis p-value ≤ 0.10, used in reduced multivariate model

CI: confidence interval; HDL-C: high density-lipoprotein cholesterol

Table 5.3c: Multiple linear regression model, full model, for corrected gestational age as a continuous outcome variable including change in maternal HDL-C as a categorical variable and the significant covariates from step one of purposeful selection

	Estimate	95% CI		P-value
HDL-C change quartile				
Lowest 25th percentile	-0.47	-1.03	0.08	0.10*
Highest 25th percentile	-0.05	-0.61	0.52	0.87
Maternal age	-0.01	-0.05	0.04	0.73
First pregnancy	0.33	-0.14	0.81	0.17
Gestational diabetes	-1.98	-3.57	-0.40	0.01*
Gestational hypertension	-1.79	-2.74	-0.84	0.0002*
Previous preterm birth	-3.24	-5.03	-1.44	0.0004*

*Type 3 Analysis p-value ≤ 0.10, used in reduced multivariate model

CI: confidence interval; HDL-C: high density-lipoprotein cholesterol

Table 5.3d: Multiple linear regression model, full model, for corrected gestational age as a continuous outcome variable including change in maternal TC as a continuous variable and the significant covariates from step one of purposeful selection

	Estimate	95% CI		P-value
TC Change	0.003	-.007	0.01	0.59
Maternal Age	-0.009	-0.06	0.04	0.70
First Pregnancy	0.30	-0.18	0.78	0.22
Gestational Diabetes	-2.02	-3.63	0.41	0.01*
Gestational Hypertension	-1.70	2.67	-0.74	0.0006*
Previous Preterm Birth	-3.32	-5.13	-1.52	0.0003*

* p-value ≤ 0.10, used in reduced multivariate model

CI: confidence interval; TC: total cholesterol

Table 5.3e: Multiple linear regression model, full model, for corrected gestational age as a continuous outcome variable including change in maternal TC as a categorical variable and the significant covariates from step one of purposeful selection

	Estimate	95% CI		P-value
TC Change				
Lowest 25th percentile	-0.17	-0.72	0.37	0.53
Maternal Age	-0.009	-0.06	0.04	0.71
First Pregnancy	0.29	-0.19	0.78	0.23
Gestational Diabetes	-2.00	-3.61	-0.39	0.01*
Gestational Hypertension	-1.69	-2.66	-0.72	0.0007*
Previous Preterm Birth	-3.37	-5.18	-1.55	0.0003*

* p-value ≤ 0.10, used in reduced multivariate model

CI: confidence interval; TC: total cholesterol

Table 5.4a: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and change in maternal LDL-C as a continuous variable and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
LDL-C change	-0.001	-0.007	0.005	0.74
Maternal race				
Non-white	-0.41	-0.90	0.08	0.10
Maternal age	-0.04	-0.08	0.008	0.11
Gestational diabetes	-2.14	-3.71	-0.57	0.008*
Gestational hypertension	-1.93	-2.92	-0.95	0.0001*
Previous preterm birth	-3.32	-5.07	-1.57	0.0002*

* p-value ≤ 0.05

CI: confidence interval; LDL-C: low density-lipoprotein cholesterol

Table 5.4b: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and change in maternal HDL-C as a continuous variable and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
HDL-C change	0.01	-0.002	0.02	0.09
HDL-C change and gestational diabetes interaction	0.20	0.09	0.30	0.0002*
HDL-C change and previous preterm birth interaction	-0.10	-0.16	-0.03	0.004*
Gestational diabetes	-1.14	-2.45	0.16	0.09
Gestational hypertension	-1.44	-2.35	-0.53	0.002*
Previous preterm birth	-1.92	-3.91	0.06	0.06*

* p-value ≤ 0.05

CI: confidence interval; HDL-C: high density-lipoprotein cholesterol

Table 5.4c: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and change in maternal HDL-C as a categorical variable and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
HDL-C change quartile				
Lowest 25th percentile	-0.51	-1.02	-0.01	0.05*
Highest 25th percentile	-0.0007	-0.51	0.51	1.0
HDL-C change and previous preterm **birth interaction**				
Lowest 25th percentile	3.51	-0.59	7.61	0.09
Highest 25th percentile	-2.47	-6.68	1.74	0.25
First pregnancy	0.36	-0.05	0.78	0.09
Gestational diabetes	-1.65	-2.96	-0.34	0.01*
Gestational hypertension	-1.53	-2.46	-0.59	0.001*
Previous preterm birth	-3.71	-6.61	-0.81	0.01*

* p-value ≤ 0.05

CI: confidence interval; HDL-C: high density-lipoprotein cholesterol

Table 5.4d: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and change in maternal TC as a continuous variable and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
TC Change	0.003	-0.005	0.01	0.47
TC Change and previous preterm birth interaction	-0.12	-0.23	0.001	0.05*
Gestational Diabetes	-1.71	-3.05	-0.36	0.01*
Gestational Hypertension	-1.61	-2.54	-0.67	0.0008*
Previous Preterm Birth	0.98	-3.91	5.87	0.69

* p-value ≤ 0.05

CI: confidence interval; TC: total cholesterol

Table 5.4e: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and change in maternal TC as a categorical variable and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
TC Change				
Lowest 25th percentile	-0.18	-0.68	0.33	0.50
First Pregnancy	0.34	-0.09	0.76	0.13
Gestational Diabetes	-1.67	-3.03	-0.32	0.02*
Gestational Hypertension	-1.69	-2.64	-0.75	0.0005*
Previous Preterm Birth	-3.41	-5.18	-1.65	0.0001*

* p-value ≤ 0.05

CI: confidence interval; TC: total cholesterol

Table 5.5a: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and percent change in maternal LDL-C per gestational week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
LDL-C percent change per week	-0.03	-0.12	0.05	0.42
LDL-C percent change per week and previous preterm birth interaction	3.85	0.56	7.15	0.02*
Gestational diabetes	-1.85	-3.15	-0.54	0.006*
Gestational hypertension	-1.62	-2.61	-0.62	0.001*
Previous preterm birth	-10.43	-16.56	-.30	0.001*

* p-value ≤ 0.05

CI: confidence interval; LDL-C: low density-lipoprotein cholesterol

Table 5.5b: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and percent change in maternal HDL-C per gestational week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
HDL-C percent change per week	0.17	0.02	0.33	0.03*
HDL-C percent change per week and gestational diabetes interaction	2.72	1.24	4.20	0.003*
HDL-C percent change per week and previous preterm birth interaction	-1.71	-2.84	-0.57	0.003*
Gestational diabetes	-1.15	-2.44	0.15	0.08
Gestational hypertension	-1.48	-2.38	-0.58	0.001*
Previous preterm birth	-2.39	-4.20	-0.59	0.01*

* p-value ≤ 0.05

CI: confidence interval; HDL-C: high density-lipoprotein cholesterol

Table 5.5c: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and percent change in maternal TC per gestational week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
TC percent change per week	0.03	-0.11	0.16	0.69
TC percent change per week and previous preterm birth interaction	-6.16	-12.15	-0.17	0.04*
Gestational diabetes	-1.74	-3.08	-0.40	0.01*
Gestational hypertension	-1.61	-2.55	-0.66	0.0008*
Previous preterm birth	9.96	-3.30	22.02	0.15

* p-value ≤ 0.05

CI: confidence interval; TC: total cholesterol

Table 5.6a: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and unit (mg/dL) change in maternal LDL-C per gestational week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
LDL-C unit change per week	-0.05	-0.14	0.04	0.29
LDL-C unit change per week and previous preterm birth interaction	2.42	0.56	4.28	0.01*
Maternal race				
Non-white	-0.41	-0.89	0.08	0.10
Gestational diabetes	-2.54	-4.03	-1.05	0.001*
Gestational hypertension	-1.62	-2.62	-0.62	0.001*
Previous preterm birth	-9.08	-13.69	-4.47	0.0001*

* p-value ≤ 0.05

CI: confidence interval; LDL-C: low density-lipoprotein cholesterol

Table 5.6b: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and unit (mg/dL) change in maternal HDL-C per gestational week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
HDL-C unit change per week	0.26	0.01	0.52	0.04*
HDL-C unit change per week and gestational diabetes interaction	2.84	1.25	4.42	0.0004*
HDL-C unit change per week and previous preterm birth interaction	-2.91	-4.86	-0.95	0.004*
Gestational diabetes	-0.83	-2.17	0.50	0.22
Gestational hypertension	-1.47	-2.37	-0.57	0.001*
Previous preterm birth	-2.63	-4.38	-0.88	0.003*

* p-value ≤ 0.05

CI: confidence interval; HDL-C: high density-lipoprotein cholesterol

Table 5.6c: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and unit (mg/dL) change in maternal TC per gestational week and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
TC unit change per week	-0.005	-0.08	0.07	0.88
TC unit change per week and gestational diabetes interaction	0.65	0.12	1.18	0.02*
First pregnancy	0.35	-0.07	0.77	0.10
Gestational diabetes	-3.01	-4.70	-1.32	0.0005*
Gestational hypertension	-1.77	-2.70	-0.84	0.0002*
Previous preterm birth	-3.34	-5.07	-1.60	0.0002*

* p-value ≤ 0.05

CI: confidence interval; TC: total cholesterol

Table 5.7a: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and first and second maternal LDL-C levels and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
First LDL-C	-0.0004	-0.01	0.01	0.95
Second LDL-C	-0.002	-0.01	0.01	0.53
First LDL-C and previous preterm birth interaction	0.11	0.02	0.19	0.01*
Second LDL-C and gestational diabetes interaction	-0.17	-0.28	-0.06	0.003*
Maternal race				
Non-white	-0.36	-0.84	0.13	0.15
Maternal age	-0.02	-0.07	0.02	0.30
First pregnancy	-0.39	-0.85	0.07	0.10
Gestational diabetes	16.56	4.36	28.76	0.008*
Gestational hypertension	-2.03	-2.99	-1.07	<0.0001*
Previous preterm birth	-3.04	-4.79	-1.28	0.0007*

* p-value ≤ 0.05

CI: confidence interval; LDL-C: low density-lipoprotein cholesterol

Table 5.7b: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and first and second maternal HDL-C levels and the covariates determined to be significant through purposeful selection

	Estimate	95% CI		P-value
First HDL-C	-0.04	-0.06	-0.01	0.003*
Second HDL-C	0.01	-0.006	0.04	0.17
Second HDL-C and previous preterm birth interaction	-0.14	-0.28	-0.007	0.04*
Gestational diabetes	-1.37	-2.70	-0.04	0.04*
Gestational hypertension	-1.64	-2.58	-0.69	.0007*
Previous preterm birth	5.65	-3.26	14.56	0.21

* p-value ≤ 0.05

CI: confidence interval; HDL-C: high density-lipoprotein cholesterol

Table 5.7c: Final multiple linear regression model for corrected gestational age as a continuous outcome variable and first and second maternal TC levels and the covariates determined to be significant through purposeful selection

|	Estimate	95% CI		P-value
First TC	-0.008	-0.02	0.0003	0.06
Second TC	-0.0004	-0.006	0.005	0.89
First TC and previous preterm birth interaction	0.05	0.003	0.09	0.04*
First pregnancy	0.35	-0.07	0.77	0.11
Gestational diabetes	-1.44	-2.79	-0.10	0.03*
Gestational hypertension	-1.81	-2.74	-0.87	0.0001*
Previous preterm birth	-13.39	-23.26	-3.52	0.008*

* p-value ≤ 0.05

CI: confidence interval; TC: total cholesterol

APPENDIX B

Figures

Figure 5.1: Distribution of corrected gestational age for the 195 ARCH participants included in study population
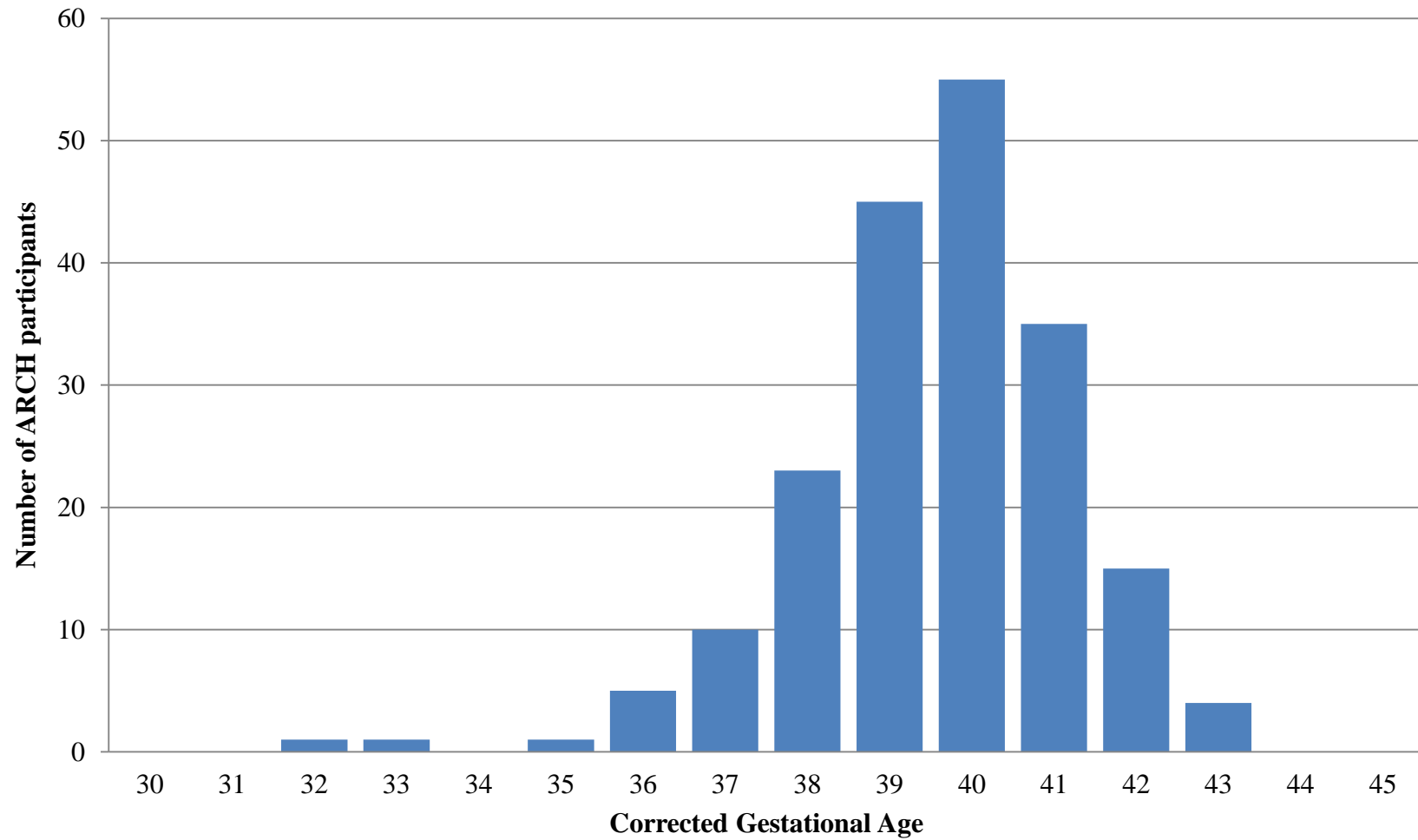
ARCH: Archive for Research on Child Health

Figure 5.2a: Average LDL-C for the first and second specimens for the 189 study subjects stratified by preterm (less than 37 weeks gestation), term (37 – 40 weeks gestation), and post-term (greater than 40 weeks gestation) births


	1st Average LDL-C *	2nd Average LDL-C
Preterm	111.3	130.6
Term	85.8	118.9
Post-term	87.2	120.9
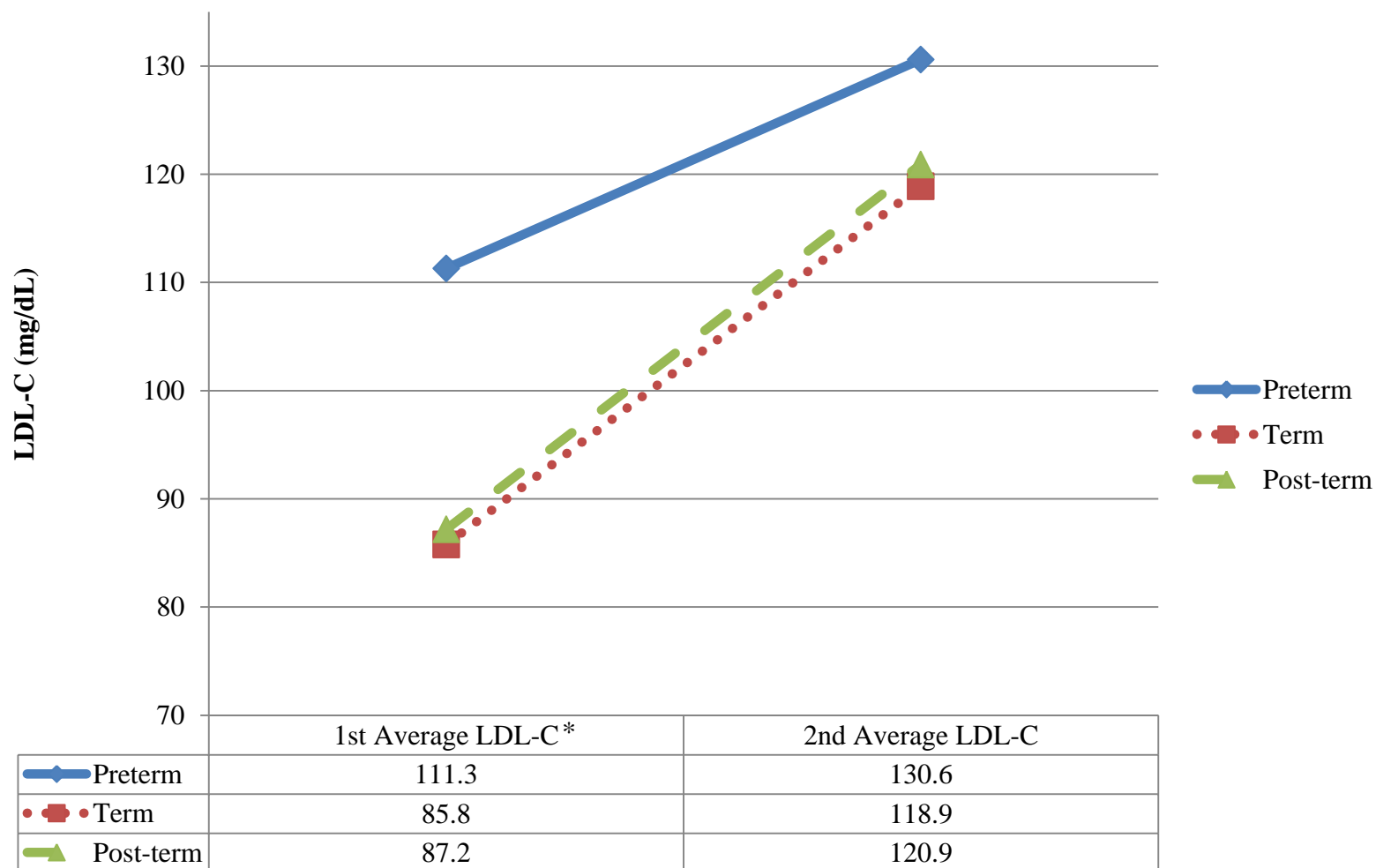
LDL-C: low density-lipoprotein cholesterol
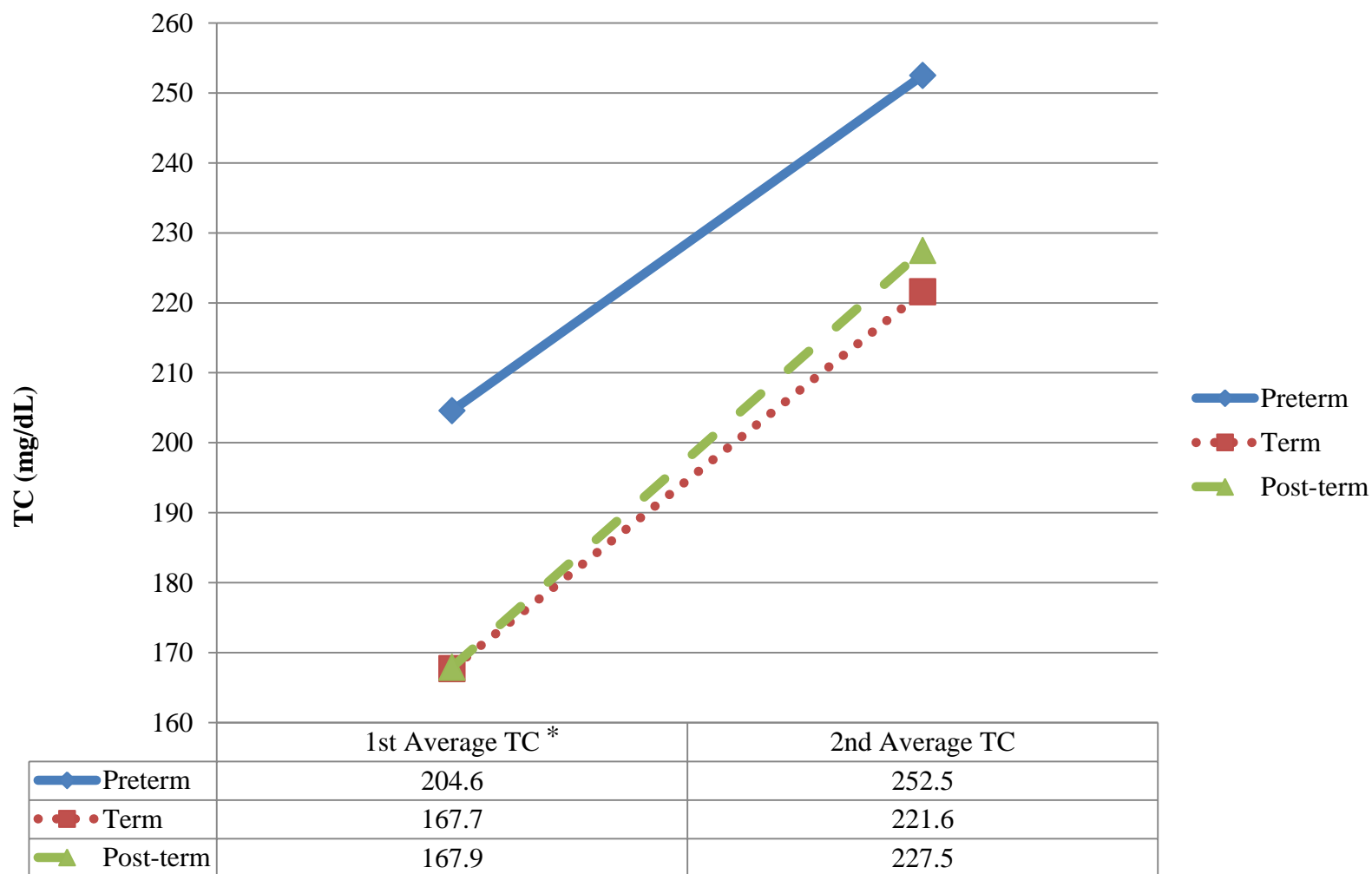LDL-C levels statistically different across gestational age categories
* p-value = 0.02

Figure 5.2b: Average HDL-C for the first and second specimens for the 195 study subjects stratified by preterm (less than 37 weeks gestation), term (37 – 40 weeks gestation), and post-term (greater than 40 weeks gestation) births

	1st Average HDL-C	2nd Average HDL-C
Preterm	67.4	69
Term	58	64.2
Post-term	57.1	65.5
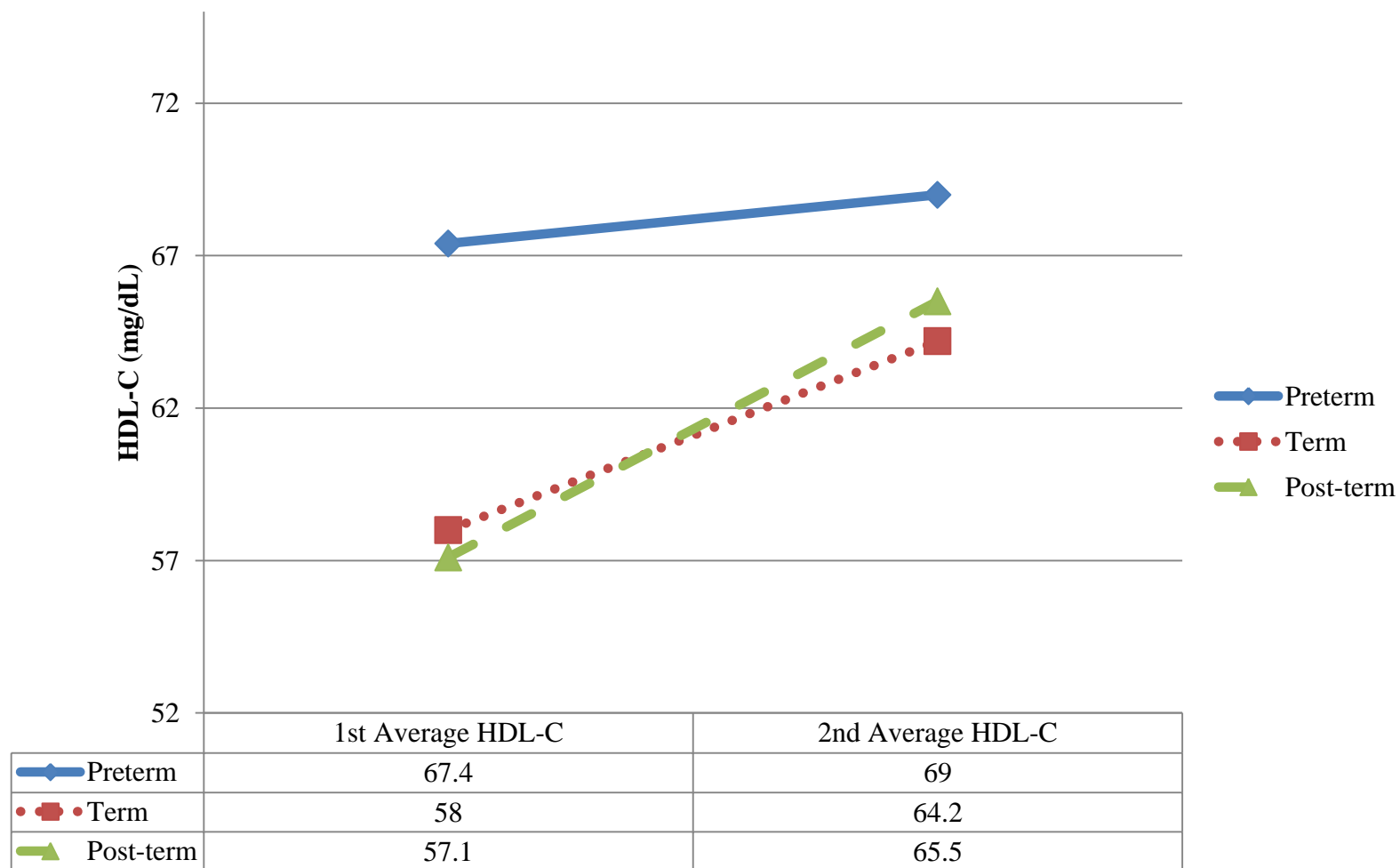
HDL-C: high density-lipoprotein cholesterol

Figure 5.2c: Average TC for the first and second specimens for the 195 study subjects stratified by preterm (less than 37 weeks gestation), term (37 – 40 weeks gestation), and post-term (greater than 40 weeks gestation) births


	1st Average TC *	2nd Average TC
Preterm	204.6	252.5
Term	167.7	221.6
Post-term	167.9	227.5

TC: total cholesterol
TC levels statistically different across gestational age categories
* p-value = 0.01

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Figure 5.3: Distribution of the percent change from first to second specimen in LDL-C by corrected gestational age
Note: Average change in LDL-C not calculated if there were less than four data points for a given corrected gestational age
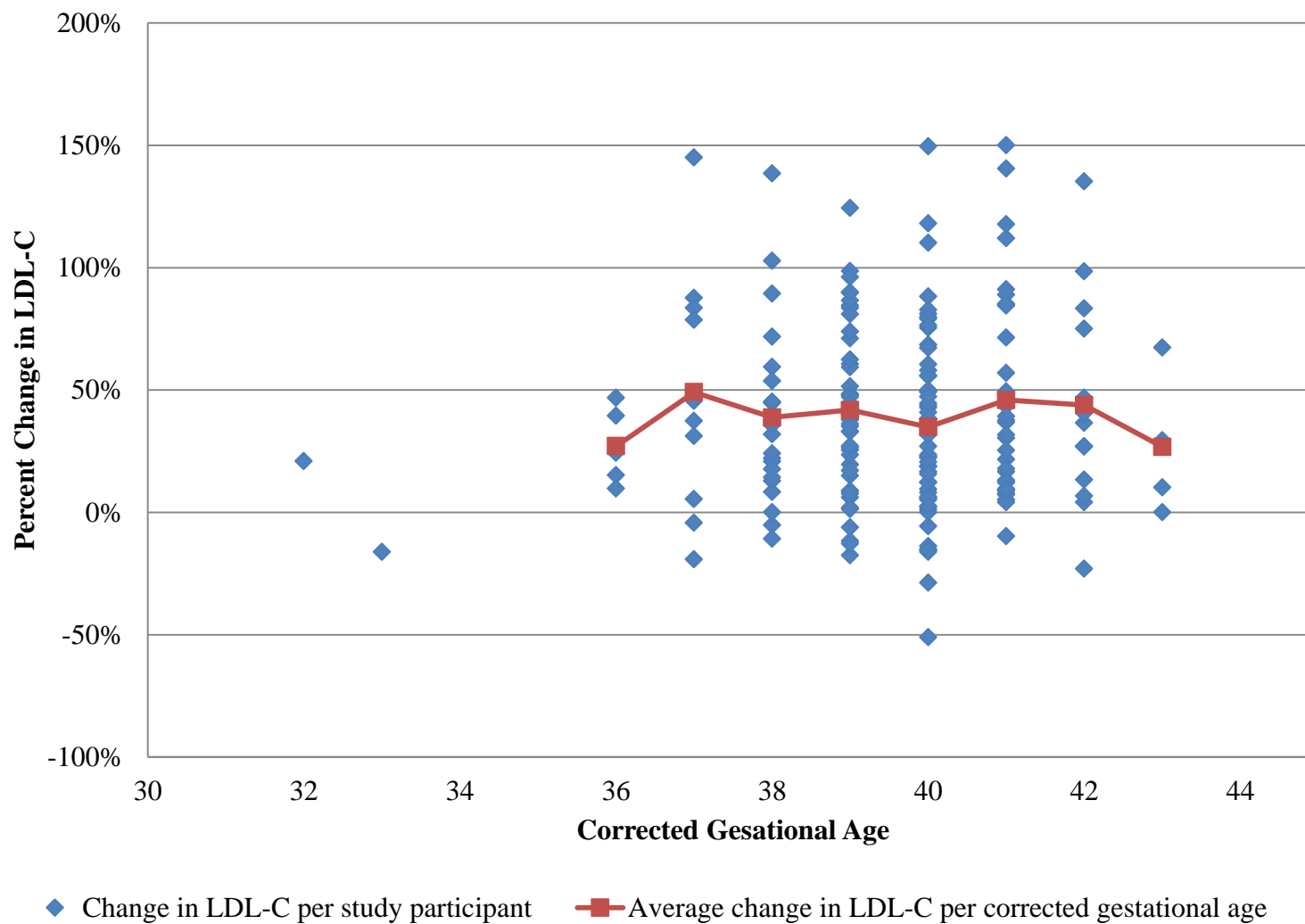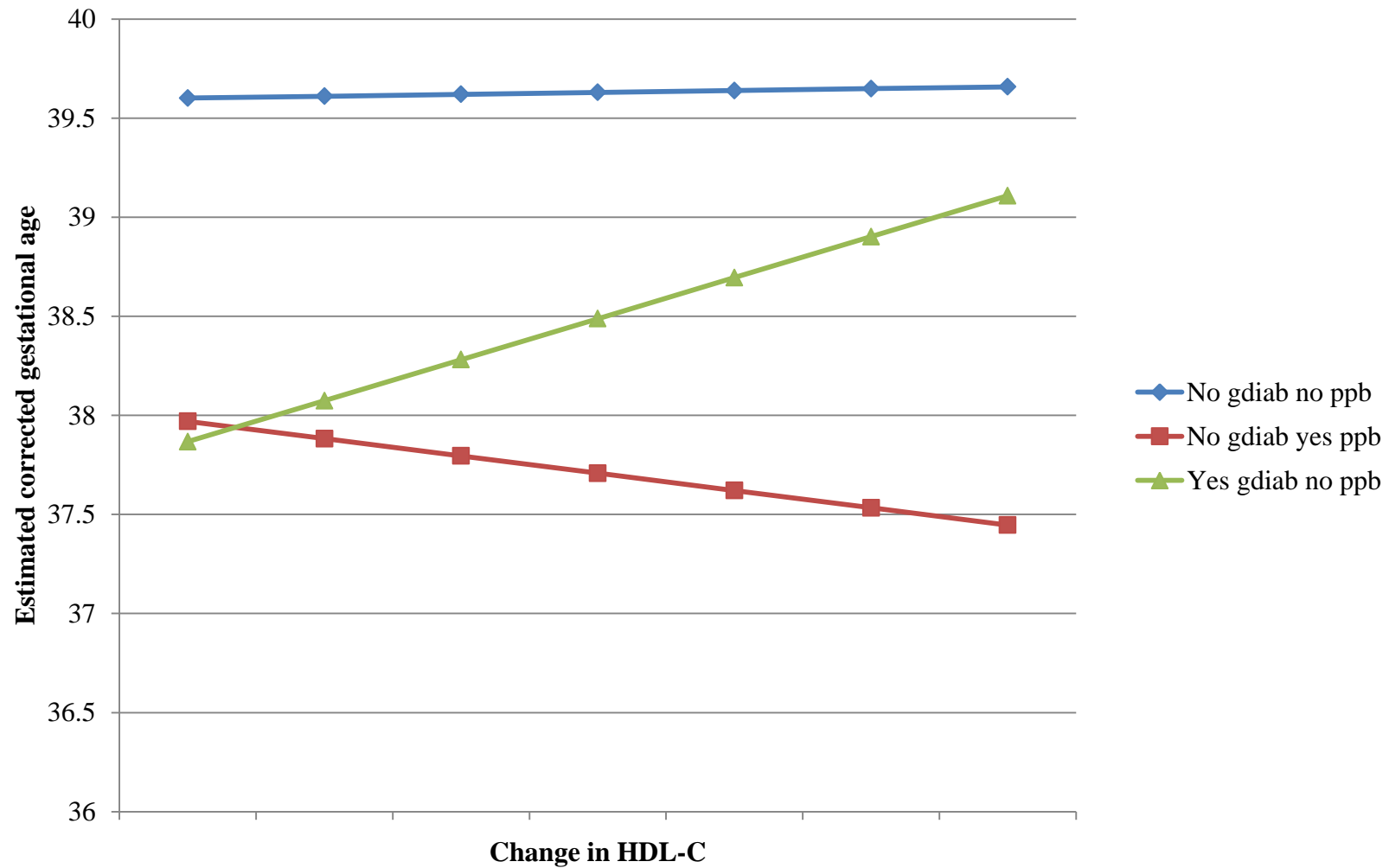

LDL-C: low density-lipoprotein cholesterol

Figure 5.4: Distribution of the percent change from first to second specimen in HDL-C by corrected gestational age
Note: Average change in HDL-C not calculated if there were less than four data points for a given corrected gestational age.
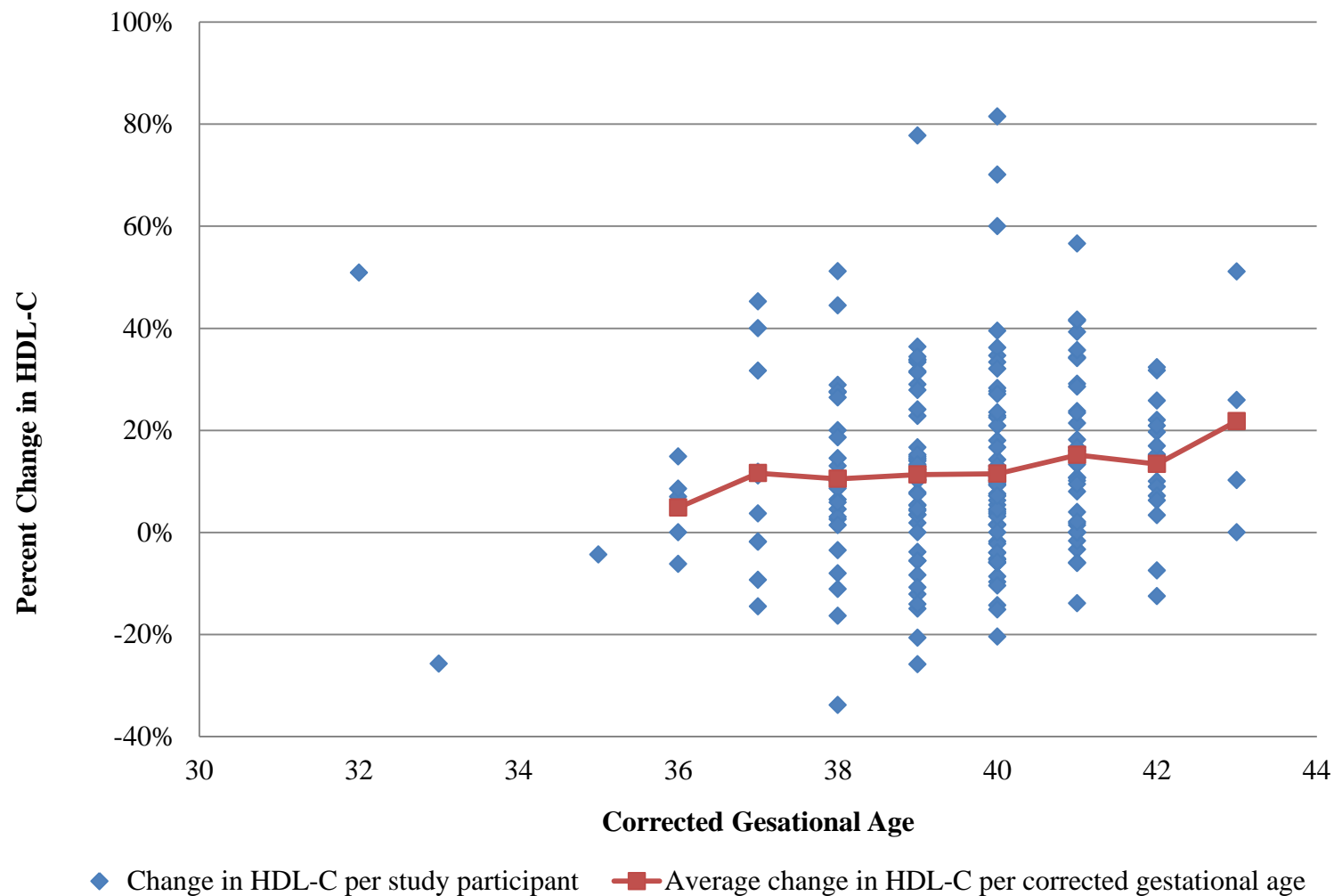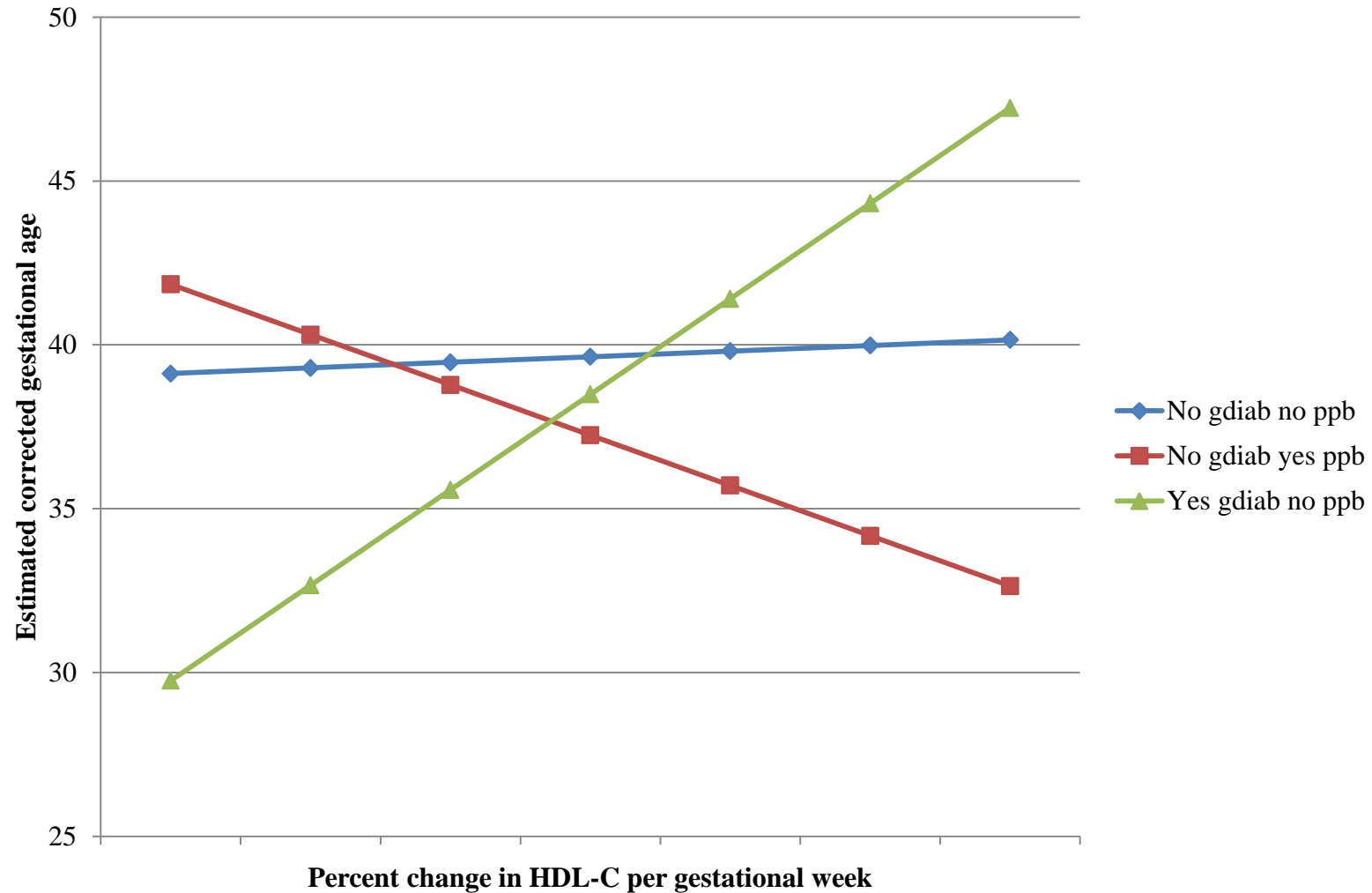

HDL-C: high density-lipoprotein cholesterol

Figure 5.5a: Multivariate linear regression models of the relationship between change in HDL-C as a continuous variable and corrected gestational age at birth, including interactions with gestational diabetes (gdiab) and previous preterm birth (ppb)
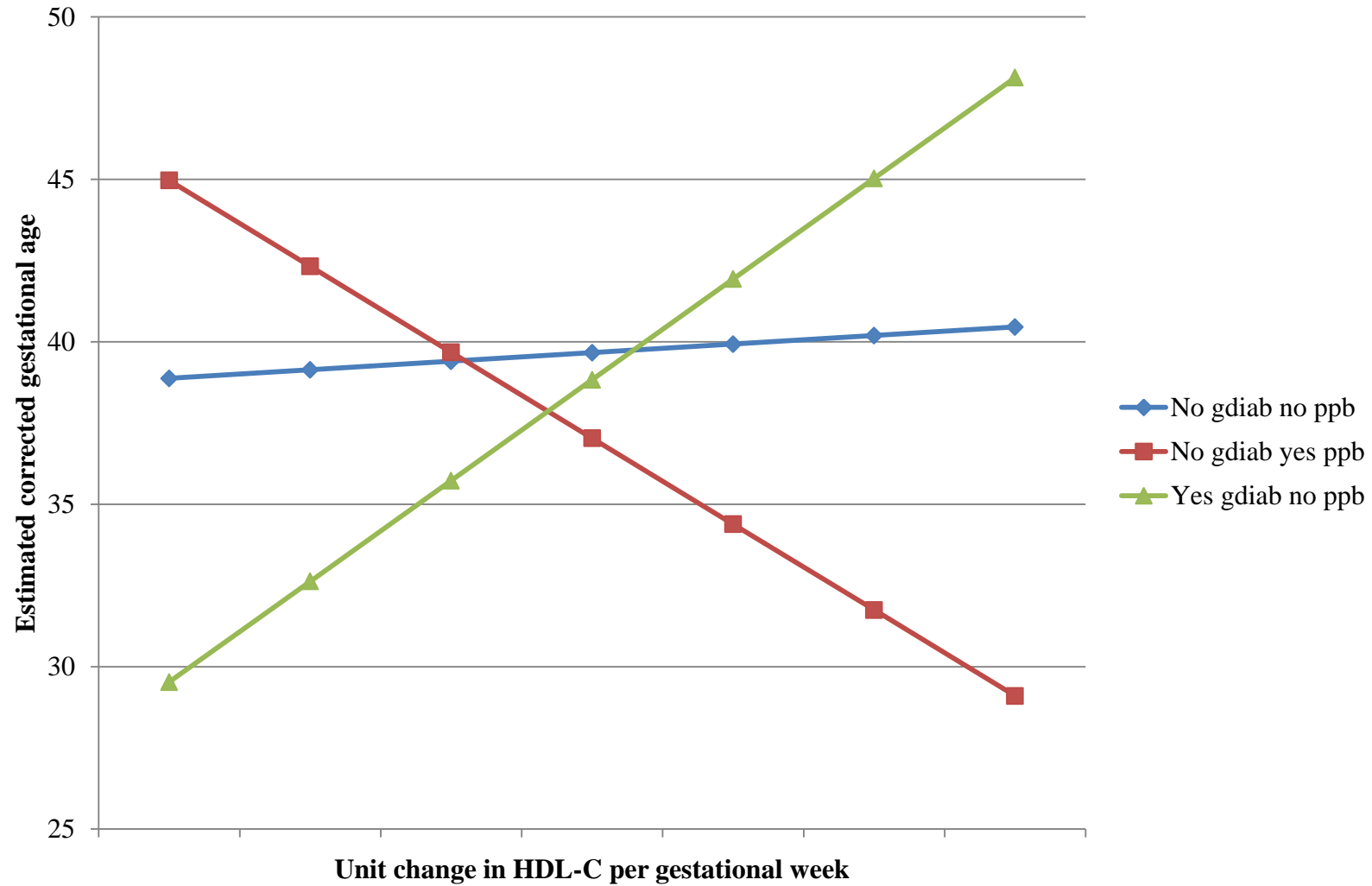

HDL-C: high density-lipoprotein cholesterol

Figure 5.5b: Multivariate linear regression models of the relationship between percent change in HDL-C per gestational week and corrected gestational age at birth, including interactions with gestational diabetes (gdiab) and previous preterm birth (ppb)

HDL-C: high density-lipoprotein cholesterol

Figure 5.5c: Multivariate linear regression models of the relationship between unit change in HDL-C per gestational week and corrected gestational age at birth, including interactions with gestational diabetes (gdiab) and previous preterm birth (ppb)
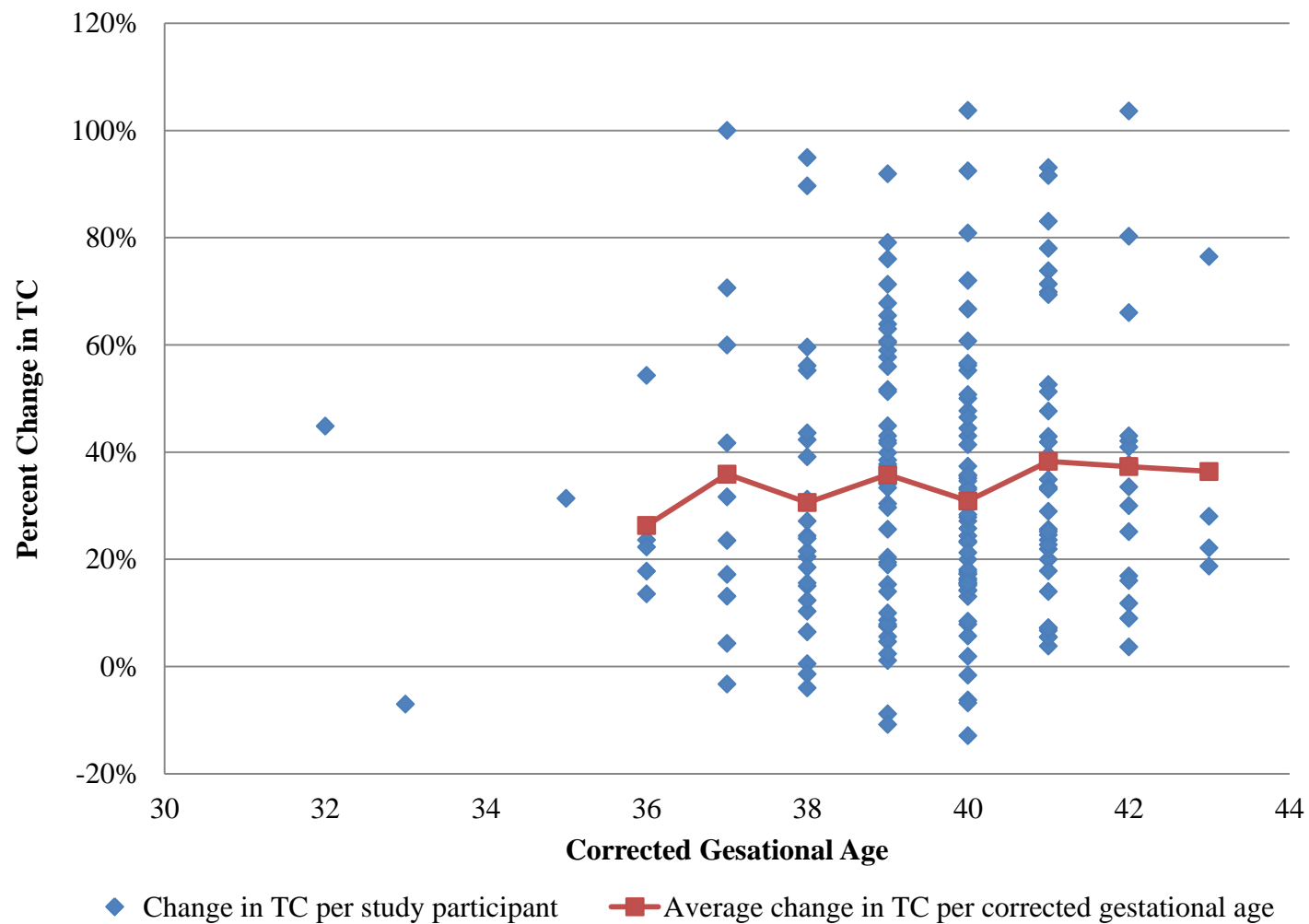

HDL-C: high density-lipoprotein cholesterol

Figure 5.6: Distribution of the percent change from first to second specimen in TC by corrected gestational age

Note: Average change in TC not calculated if there were less than four data points for a given corrected gestational age

TC: total cholesterol

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REFERENCES

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SUMMARY

In summary, maternal cholesterol levels have been shown to increase in pregnancy. This increase is thought to play an important role in pregnancy and fetal development as research suggests that maternal cholesterol is essential during implantation and gestation as it maintains the integrity and structure of cell membranes, activates key patterning proteins and is a precursor for signaling lipids. Fetal growth complications and preterm delivery are among the most common adverse outcomes of pregnancy and are thought to be multifactorial. Research suggests there may be a relationship between maternal cholesterol levels during pregnancy and both fetal growth and preterm birth. The presented research had three objectives. The first objective of this research was to calculate changes in maternal cholesterol levels and study how these changes during pregnancy differ based on various maternal demographics. The second objective of this research was to study the relationship between the changes in maternal cholesterol and fetal growth and the final objective of this research was to analyze the association between the changes in maternal cholesterol levels and gestational age at delivery.

Utilizing the Archive for Research on Child Health (ARCH) database, maternal cholesterol at two time points during pregnancy was analyzed from 195 women. For the first objective, maternal cholesterol levels were stratified on maternal demographics to see if first and second cholesterol levels significantly differed between the maternal demographics of interest. For the second and third objectives, the associations between the change in maternal cholesterol levels and the outcomes of interest, fetal growth and corrected gestational age at delivery, were tested for statistical significance.

In this ARCH population, low density-lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels peaked in the third trimester and high density-lipoprotein cholesterol (HDL-C) peaked in the second trimester. First, second, and third trimester maternal cholesterol levels were higher in women with a pre-pregnancy body mass index less than 25 kg/m^2. There was no difference in maternal cholesterol levels when stratified by ethnicity, age, and parity. No significant associations were found between changes in maternal cholesterol levels and fetal growth in both the unadjusted and adjusted models. Although not statistically significant, exploratory analyses found that maternal cholesterol levels at single time points during gestation were lower in pregnancies resulting in small for gestational age infants. Lastly, in women with a history of a previous preterm birth, changes in maternal cholesterol levels were found to be significantly associated with the corrected gestational age at delivery. The percent change per gestational week in LDL-C was positively associated with the corrected gestational age. The percent change per gestational week in HDL-C and TC was inversely associated with corrected gestational age. Exploratory analyses found LDL-C, HDL-C, and TC levels were higher in pregnancies resulting in preterm birth for all three trimesters.

In conclusion, cholesterol levels in women from the ARCH collaborative increased at rates consistent with what has been previously published in the literature. Changes in maternal cholesterol levels may provide a more complete picture of cholesterol during pregnancy compared to maternal cholesterol levels at a single time point during pregnancy. This research found associations between both low and high maternal cholesterol levels indicating that cholesterol levels that are either too high or too low may increase risk of adverse birth outcomes. Additional research is needed to explore maternal cholesterol levels being higher in pregnancies resulting in preterm birth and lower in pregnancies resulting in small for gestational age babies.

Further research is also needed to study the effect modification of a previous preterm birth on the

association between maternal cholesterol levels and corrected gestational age at birth.