RELATIONSHIPS AMONG MATERNAL SERUM URIC ACID IN MID-PREGNANCY, MATERNAL BLOOD PRESSURE, FETAL GROWTH, AND PLACENTAL PATHOLOGY

By

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ABSTRACT

RELATIONSHIPS AMONG MATERNAL SERUM URIC ACID IN MID-PREGNANCY, MATERNAL BLOOD PRESSURE, FETAL GROWTH, AND PLACENTAL PATHOLOGY

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Gestational hypertension in mother and atypical fetal growth (very small or very large infant) contribute to substantial adverse health and cost burden in society. In pregnant women, high maternal serum uric acid has been related to increased risk of gestational hypertension and poor fetal growth, but the association between low maternal serum uric acid and these health outcomes has been ignored. In addition, there are no studies on the relationship between maternal serum uric acid and placental pathology, a problem known to affect both maternal blood pressure and fetal growth. In this study, we investigated whether there is a J-shaped association between maternal serum uric acid in mid-pregnancy and three outcomes, mothers' blood pressure, birth weight for gestational age (Z-score), and placental pathology.

Our study data came from the Pregnancy Outcomes and Community Health (POUCH) Study cohort, which consisted of 3,019 pregnant women enrolled in the 16th-27th week of pregnancy from 52 clinics in Michigan during the period from August 1998 through June 2004. We considered maternal serum uric acid level measured in blood collected at enrollment as a continuous exposure variable and applied a linear spline with a multiple linear regression model or a restricted cubic spline with a multinomial logistic regression model. The robustness of our results was evaluated and assured by using bootstrap estimation of variance, sensitivity analysis, and 10- or 5-fold cross-validation.

Our results demonstrated that there was a J-shaped relationship between maternal serum uric acid in mid-pregnancy and gestational diastolic blood pressure (DBP) or mean arterial

pressure (MAP) in pregnant women. The breakpoints were 2.6 mg/dL (for DBP) and 2.7 mg/dL (for MAP) of uric acid, respectively. By contrast, maternal systolic blood pressure (SBP) followed a positive linear trend with uric acid level increase. We also found a J-shaped relationship between birth weight Z-score and maternal serum uric acid in mid-pregnancy among small-for-gestational age (SGA) infants (birth weight less than 10^{th} percentile for gestational age); the breakpoint was 4.10 mg/dL. By contrast, in large-for-gestational age (LGA) infants (birth weight more than 90^{th} percentile for gestational age) we observed a positive linear relationship between maternal serum uric acid and birth weight Z-score. Birth weight Z-score was not associated with maternal serum uric acid in the appropriate-for-gestational age (AGA) group (birth weight between 10^{th} and 90^{th} percentile for gestational age). Finally, we found that maternal serum uric acid concentration was associated with maternal vascular lesions in the placenta; the relationship was non-linear. Uric acid levels were not associated with fetal vascular lesions in the placenta.

We proposed that a common mechanism underlying our findings may be related to oxidative stress that follows exceptionally low or high serum uric acid concentration. Our findings may provide clues: 1) to guide the study of biological mechanisms underlying the non-linear relationship between maternal serum uric acid and maternal blood pressure, atypical fetal growth, and placental pathology; and 2) to allow researchers to consider maternal serum uric acid in pregnancy as a marker along with other indicators to predict the progression and/or severity of pregnancy-related health conditions or as a target for early intervention.

This dissertation is dedicated to my family and friends for their support and belief in me.

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KEY TO ABBREVIATIONS

8OHdG	8-Hydroxy-2'-Deoxyguanosine
AD	Alzheimer's Disease
AFP	Alpha-Fetoprotein
AGA	Appropriate-For-Gestational-Age
AMP	Adenosine Monophosphate
AUC	Area Under the Curve
BMI	Body Mass Index
BP	Blood Pressure
BW_ZS	Birth Weight Z Score
CI	Confidence Interval
CKD	Chronic Kidney Disease
CVD	Cardiovascular Disease
DAG	Directed Acyclic Graph
DBP	Diastolic Blood Pressure
FV	Fetal Vascular Lesions in Placenta
FV-I	Fetal Vascular-Disturbance of Integrity in Placenta
FV-O	Fetal Vascular-Obstructive in Placenta
GA	Gestational Age
GDM	Gestational Diabetes Mellitus
GFR	Glomerular Filtration Rate
GH	Gestational Hypertension
GMP	Guanine Monophosphate

GWG	Gestational Weight Gain
Hb	Hemoglobin
HDP	Hypertensive Disorders of Pregnancy
H_2O_2	Hydrogen Peroxide
HTN	Hypertension
IMP	Inosine Monophosphate
IRB	Institutional Review Board
IUGR	Intrauterine Growth Restriction
LBW	Low Birth Weight
LGA	Large-For-Gestational-Age
LMP	Last Menstrual Period
LOWESS	Locally Weighted Scatterplot Smoothing
MAP	Mean Arterial Pressure
MnSOD	Manganese Superoxide Dismutase
MS	Multiple Sclerosis
mTOR	Mammalian Target of Rapamycin
MV	Maternal Vascular Lesions in Placenta
MV-D	Maternal Vascular-Development in Placenta
MV-I	Maternal Vascular-Disturbance of Integrity in Placenta
MV-O	Maternal Vascular-Obstructive in Placenta
NEC	Necrotizing Enterocolitis
OR	Odds-Ratio
OS	Oxidative Stress

PD	Parkinson's Disease
PE	Pre-eclampsia
PIH	Pregnancy-Induced Hypertension
POUCH	Pregnancy Outcomes and Community Health Study
r	Correlation Efficient
RCS	Restricted Cubic Spline
RMSE	Root Mean Square Error
ROC	Receiver Operation Characteristic
ROS	Reactive Oxygen Species
RR	Relative Risk or Risk Ratio
SBP	Systolic Blood Pressure
SD	Standard Deviation
SE	Standard Error
SGA	Small-For-Gestational-Age
SOD	Superoxide Dismutase
SQRT	Square Root
TAC	Total Antioxidant Activity
TNF-α	Tumor Necrosis Factor-Alpha
TPR	Total Peripheral Resistance
UA	Uric Acid
URAT1	Urate Transporter1 Gene

CHAPTER 1: HYPOTHESIS AND SPECIFIC AIMS

Uric acid is an end product of purine metabolism and can be easily measured in serum or urine in humans. Both high and low uric acid concentrations in blood have been associated with many human diseases, as either a marker of disease progression or a potential etiological factor. In pregnant women, high maternal serum uric acid has been related to gestational hypertension and poor fetal growth, but the association between low maternal serum uric acid and these health outcomes has been ignored. In addition, there are no published studies on the relationship between maternal serum uric acid in pregnancy and placental vascular pathology. Our study addresses these gaps.

We hypothesize that maternal serum uric acid in mid-pregnancy has a J-shaped relationship with maternal blood pressure, atypical fetal growth (too small or too large), and placental vascular pathology. Our long-term goal is to understand whether maternal serum uric acid levels in pregnancy serve as an informative marker for predicting short-term (e.g., perinatal) and long-term (e.g., later in life) health conditions in mothers and children, or as a possible etiologic factor for targeted intervention. As a first step, we conducted analyses using data from the Pregnancy Outcomes and Community Health (POUCH) Study in Michigan to examine associations among maternal serum uric acid, maternal blood pressure, birthweight for gestational age, and placental pathology.

Our Specific Aims are as follows:

1) To investigate whether there is a J-shaped relationship between maternal serum uric acid in mid-pregnancy and maternal blood pressure. Our working hypothesis is that both low and high maternal serum uric acid in pregnancy are related to the increased blood pressure in pregnant women.

2) To explore whether there is a J-shaped relationship between maternal serum uric acid in mid-pregnancy and birth weight Z-score. Our working hypothesis is that both low and high maternal serum uric acid in pregnancy are related to extremes in birth weight Z-score.

3) To study whether maternal serum uric acid in pregnancy has a J-shaped relationship with the risk of placental pathology. Our working hypothesis is that both low and high maternal serum uric acid contribute to the increased risk of vascular lesions in placenta.

Completion of our aims would: 1) raise awareness for researchers about risks associated with both high and low serum uric acid in pregnant women; 2) potentially help in reducing the risks of pregnancy complications by using maternal serum uric acid in pregnancy as a predictor or a target to guide an early intervention; and 3) provide epidemiological evidence to guide and/or support the study of the complex uric acid-related mechanisms underlying adverse pregnancy outcomes.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE

2.1 Physical and chemical properties of uric acid as well as its measurement in human serum or urine

Uric acid is a small and weak organic acid molecule with a pKa of 5.75, a molecular weight of 168 Daltons, and a molecular formula of $C_5H_4N_4O_3$. Figure 2.1 shows its chemical structure, a heterocyclic chemical compound.

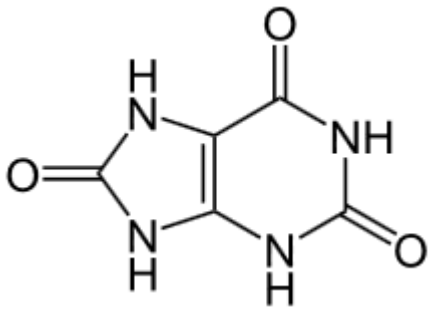


Figure 2.1 Chemical structure of uric acid (UA).

Uric acid usually exists as monosodium urate at physiological pH value in blood and urine (Musso et al., 2012). It can be clinically measured using a colorimetric method based on a specific oxidization of uric acid by uricase into hydrogen peroxide and allantoin (Sanders et al., 1980; Moss, 1980) with an analytical range of 0.5-12 mg/dL uric acid (CDC, 2001).

2.2 Significance of uric acid in human evolution

In most mammals (except humans and apes), uric acid is an intermediate product of purine metabolism and further metabolized into allantoin by uricase (So & Thorens, 2010). In contrast, in humans and apes, uric acid is an end product of purine metabolism due to the mutation-induced silence of uricase during evolution (Oda et al., 2002). Consequently, humans have an elevated uric acid in blood (typically 3.5-7.0 mg/dL), compared to other animals such as mice (0.5-1.5 mg/dL) (Feig et al., 2006). It has been proposed that the elevated uric acid in blood

might have evolutionary significance for humans in developing higher intelligence, maintaining blood pressure in the age of low salt ingestion of human society, and increasing life expectancy (Alvarez-Lario & Macarron-Vicente, 2010).

2.3 Uric acid metabolism and regulations

In humans, serum uric acid is generated in the catabolism of purine including adenosine monophosphate (AMP), guanine monophosphate (GMP), and inosine monophosphate (IMP), which are derived from diet (e.g., meat) and internal nucleotide turnover (Alvarez-Lario & Macarron-Vicente, 2010). All purines can be converted into xanthine via a series of biochemical reactions, followed by an oxidization to generate uric acid under the action of a key enzyme - xanthine oxidase (Fang et al., 2013). There are two fates of serum uric acid: up to 90% of the filtered urate is reabsorbed in nephrons and the rest excreted in urine (Alvarez-Lario & Macarron-Vicente, 2011). Thus, diet, regulators of purine metabolic pathway (e.g., inhibitors of xanthine oxidase), and kidney function are major factors to control the balance of uric acid in blood in humans. In addition, genetics is also involved in changing serum uric acid levels. For instance, URAT1 gene (a human urate transporter 1 gene) mutations (Takahashi et al., 2005; Ichida et al., 2008) and polymorphism (Iwai et al., 2004; Sebesta & Stiburkova, 2014) as well as mutations of xanthine oxidoreductase (an enzyme catalyzing the conversion of hypoxanthine to xanthine) (Ichida et al., 2012) were associated with hypouricemia.

2.4 High and low serum uric acid are separately associated with the risks of different health outcomes

High serum uric acid, also called hyperuricemia, which is typically defined as a serum uric acid concentration of > 7 mg/dL for men and > 5.7 mg/dL for women (CDC, 1996), has been linked to gout (Lin et al., 2000; Choi et al., 2005; MacFarlane & Kim, 2014; Dalbeth &

Palmano, 2011; Levy et al., 2014), hypertension (Agarwal et al., 2013; Beattie et al., 2014), cardiovascular disease (CVD) (Takayama et al., 2012; Gazi et al., 2014; Goicoechea et al., 2015), metabolic syndrome (Oda, 2014), renal disease (Yen et al., 2009; Bakan et al., 2015; Goicoechea et al., 2015), and pre-eclampsia (Williams and Galerneau, 2002; Wu et al., 2012; van der Tuuk et al., 2015). The reported risk factors for hyperuricemia include: age, gender, race, purine-rick foods and high protein intake, consumption of fructose and sugar sweetened soft drinks, alcohol consumption, adiposity, and some medications such as diuretics and postmenopausal hormone therapy (reviewed by Rho et al., 2011).

In contrast, low serum uric acid, also called hypouricemia, is usually defined as a serum uric acid concentration of < 2 mg/dL (Sebesta & Stiburkova, 2014). Evidence has shown that low serum uric acid is associated with multiple sclerosis (MS) (Spitsin et al., 2001; Toncev et al., 2002; Rentzos et al., 2006; Liu et al., 2012; Moccia et al., 2015a,b), Parkinson's disease (PD) (de Lau et al., 2005; Schlesinger & Schlesinger, 2008; Shen & Ji, 2013; Simon et al., 2014; Lolekha et al., 2015), and Alzheimer's disease (AD) (Kim et al., 2006; Kutzing, & Firestein, 2008; Lu et al., 2016; Du et al., 2016). Hypouricemia can be caused by decreasing consumption of protein, purines, and alcohol, reducing obesity, taking medications such as xanthine oxidase inhibitor and URAT1 transporter inhibitor (Kutzing & Firestein, 2008), as well as genetics (Takahashi et al., 2005; Ichida et al., 2012; Sebesta & Stiburkova, 2014).

2.5 High and low serum uric acid are associated with the risk of a single health outcome, i.e., a J-shaped relationship between serum uric acid and a health outcome:

Investigations have found that serum uric acid has a J-shaped association with age- and gender-adjusted rates of CVD (myocardial infarction, stroke, unstable angina, congestive heart failure, and deaths from all other CVD causes) in patients with mild-to-moderate hypertension in

New York (Alderman et al., 1999); with systolic blood pressure in a general population in Italy (Verdecchia et al., 2000); with coronary heart disease mortality in non-insulin-dependent diabetic elderly people in Italy (Mazza et al., 2007); and with stroke outcomes in Asian patients with ischemic stroke (Seet et al., 2010).

A J-shaped association also exists between serum uric acid and kidney conditions. For instance, in a J-shaped manner, serum uric acid has been associated with the all-cause mortality in patients (18-70 years old) with chronic kidney disease (CKD) stage 5 starting renal replacement therapy after adjusting for age, sex, glomerular filtration rate (GFR), cholesterol level, phosphate level, inflammation, CVD, diabetes mellitus, diuretic use, and allopurinol use in Sweden (Suliman et al., 2006); with the loss of kidney function in healthy males in Japan (Kanda et al., 2015); and with the mortality in hemodialysis patients in Taiwan (Hsu et al., 2004).

2.6 Anti-oxidant and pro-oxidant properties of uric acid

Uric acid is an important antioxidant in blood. Studies have shown that there is an inverse association between blood urate and PD risk (Davis et al., 1996; De Lau et al., 2005; Weisskopf et al., 2007; Chen et al., 2009) whereas PD patients have a decreased antioxidant enzyme activity (Fahn & Cohen, 1992) and increased oxidative stress (OS) biomarkers (Yoritaka et al., 1996; Danielson & Andersen, 2008), suggesting that there might be connections among blood urate, PD risk, and oxidative stress. A direct link between plasma urate and plasma antioxidant capacity can be found in healthy lowland individuals who were exposed to high altitude hypoxia (Baillie et al., 2007). Studies also demonstrated that thioredoxin-1 (an antioxidant) and serum uric acid correlated significantly and positively whereas thioredoxin-1 and oxidative stress index correlated significantly and negatively (Nakatsukasa et al., 2013). A more recent study has shown that obese individuals with high serum uric acid had 20-90% greater systemic

nonenzymatic antioxidant capacity and 30% lower oxidative stress markers than those individuals with normal serum uric acid; furthermore, an acute reduction of serum uric acid contributed a 45-95% decrease in nonenzymatic antioxidant capacity and a 25-40% increase in the levels of systemic oxidative stress markers in these obese individuals (Fabbrini et al., 2014).

On the other hand, in vitro studies have shown that uric acid can induce oxidative stress, i.e., functioning as a pro-oxidant, in adipocytes (Sautin et al., 2007), vascular smooth muscle cells (Corry et al., 2008), and hepatocytes (Lanaspa et al., 2012). Intake of fructose induced intracellular uric acid generation and further caused mitochondrial oxidative stress in hepatocytes (Lanaspa, et al., 2012; Johnson et al., 2013). In pre-eclamptic (PE) women (n=30), serum uric acid (6.1 versus 2.8 mg/dL) as well as endogenous O_2^- (2.2 versus 1.6 nM), H_2O_2 (1.8 versus 1.4 nM) and tumor necrosis factor-alpha (TNF-α) (91.6 versus 40.4 pg/mL) released from monocytes were significantly higher than in normotensive pregnant women (n=30) in the last trimester of pregnancy ($P < 0.05$). Superoxide dismutase (SOD) activity in erythrocytes was also significantly elevated in the PE group (5969.2 versus 4834.7 U/g Hemoglobin (Hb)) (Peracoli et al., 2011). A community-based study in Colorado with 107 healthy adult participants (20~78 years old) revealed that higher serum uric acid is inversely associated with the expression of manganese superoxide dismutase (MnSOD), an enzyme located in mitochondria and protecting mitochondria from oxidative damage (Li & Zhou, 2011), in endothelial cells ($r = -0.5$, $P = 0.01$, $n = 25$) (Jalal et al., 2012).

The anti-oxidant and pro-oxidant properties of uric acid might be related to extracellular or intracellular action site of uric acid, acute or chronic change of uric acid level, tissue or cell types, as well as certain subgroups of population such as individuals exposed to hypoxia, obese people, PD patients, PE women, or people with the intake of dietary fructose.

2.7 Health burdens of gestational hypertension (GH), atypical fetal growth (infant too small or too large for gestational age), and placental pathology

The age-adjusted incidence rate of GH was from 10.8 in 1988 to 29.7 in 2004 per 1,000 deliveries in the US (Wallis et al., 2008). GH comprising hypertension with and without proteinuria increased from 3.0% in 1990 to 3.9% in 2004 in the US and has been associated with increased risk of stillbirth, pre-eclampsia, preterm delivery, small-for-gestational-age birth, and maternal and/or neonatal mortality (Ananth et al., 1995; Zhang et al., 2003; Xiong et al., 2007; Ananth and Basso, 2010; Backes et al., 2011; Seyom et al., 2015). High blood pressure during pregnancy has been related to increased risk of women's later chronic kidney disease and diabetes mellitus (Mannisto et al., 2013) as well as cardiovascular disease (Mannisto et al., 2013; Tooher et al., 2013).

The prevalence of fetal macrosomia (defined as a neonate with a birth weight above 4.0 kg) was estimated as 7% of all births in developed countries (Campbell, 2014). Large-for-gestational age (LGA) birth has been associated with increased risk of caesarean sections (Ng et al., 2010). Intrauterine growth restriction (IUGR) (the estimated fetal weight is below the 10th percentile) is a major contributor to low birth weight (LBW) when LBW incidence is higher than 10% (Villar and Belizan, 1982). In developing countries, IUGR prevalence was about 24%; in developed countries including the US, about one-third of small-for-gestational age (SGA) births were the result of IUGR (Saleem et al., 2011). IUGR has been related to increased risk of stillbirth, premature birth, neonatal morbidity (e.g., necrotizing enterocolitis (NEC)), low Apgar score, hypoxic brain injury, and long-term sequelae (Cosmi et al., 2011).

The percentages of maternal and fetal vascular lesions in placenta with the gestational hypertension (n=64) were 60.9% and 9.4%, respectively, in Israel (Kovo et al., 2010). Placental

lesions are not only one of the main causes of fetal death, but also associated with neonatal morbidity including low Apgar score, neonatal infection, NEC, respiratory distress, asphyxia, and neurological impairments such as hearing loss and neonatal encephalopathy (Roescher et al., 2014).

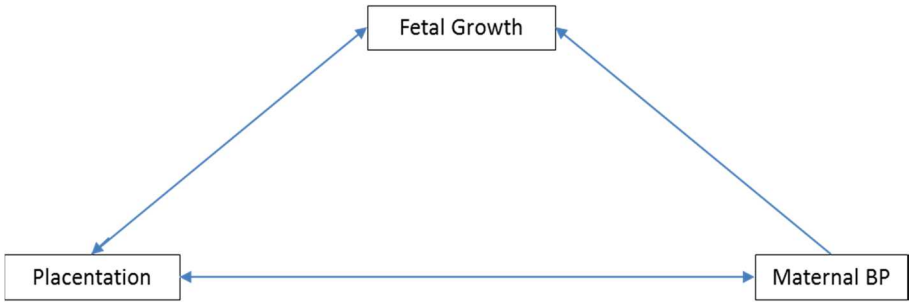


Figure 2.2 A directed acyclic graph (DAG) to indicate associations among gestational hypertension, fetal growth, and placentation. Note: BP=Blood Pressure.

Figure 2.2 is a directed acyclic graph (DAG) to indicate associations among gestational hypertension, fetal growth, and placentation, which have been well established. In this DAG, these three outcomes are represented in an interactive triad. Gestational hypertension is associated with placental vascular pathology (Jain et al., 2007; Furuya et al., 2008; Krielessi et al., 2012; Salmani et al., 2014; Nahar et al., 2015) and fetal growth restriction (Xiong and Fraser, 2004; Jain et al., 2007; Nahar et al., 2015). Placentation influences fetal growth by mediating the effects of maternal pre-pregnancy obesity, gestational diabetes (GDM), and excessive gestational weight gain (GWG) (Ouyang et al., 2013) and interrupting nutrient transfer from mother to fetus through placental vasculopathy (Krishna and Bhalerao, 2011; Vedmedovska et al., 2011; Mifsud and Sebire, 2014). Maternal vascular lesions in the placenta are found more frequently in placentae of SGA infants (Althabe et al., 1985) and in women with pre-eclampsia (Ghidini et al., 1997). In addition, fetal overgrowth has been associated with the increased risk of maternal morbidity and mortality (Koyanagi et al., 2013) and abnormal placental growth and transport function (Jansson et al., 2006).

2.8 Literature review on relationships among maternal serum uric acid in pregnancy, maternal blood pressure, fetal growth, and placental pathology as well as research gaps

In general population, high serum uric acid has been consistently associated with hypertension. For instance, a systematic review and random-effects meta-analysis demonstrated that hyperuricemia was associated with a higher risk of incident hypertension (unadjusted: Relative Risk (RR)=1.73, 95% Confidence Interval (CI) 1.46~2.06 for categorical data, RR=1.22, 95% CI 1.03~1.45 for a 1 SD increase; adjusted: RR=1.48, 95% CI 1.33~1.65 for categorical data, RR=1.15, 95% CI 1.06~1.26 for a 1 mg/dl increase), and the risk is consistent in subgroup analyses and have a dose-response relationship (Wang et al., 2014).

However, a consistent association between maternal serum uric acid and gestational hypertension has not been well established. A small observational study showed that maternal diastolic blood pressure (DBP) was significantly higher in pregnant women (n=58) with high serum uric acid (≥357 micromol/L) than in the counterparts with normal serum uric acid (<357 micromol/L) (Paula et al., 2008). More evidence seems indirectly to come from the studies of the concurrence of higher serum uric acid and gestational hypertension with some perinatal outcomes such as preterm birth, pre-eclampsia, and cesarean section. For example, an observational study on 259 women with prior preeclampsia showed that compared to women with normal blood pressure, normal urinary protein, and normal uric acid, women with hypertension and hyperuricemia had greater risk of preterm birth (relative risk [RR] = 3.8, P < .01) (Schmella et al., 2015); a multivariable logistic regression analysis on women with gestational hypertension or mild preeclampsia at term revealed that serum uric acid is a significant intrapartum predictor variable (Odds Ratio (OR)=1.4) for the risk of cesarean section (van der Tuuk et al., 2015); and in a retrospective cohort study of 249 women with a singleton

pregnancy, those who later progressed to preeclampsia had a significantly higher mean serum uric acid level at the initial presentation of gestational hypertension than those who did not develop PE (5.06 vs. 4.59 mg/dl, $P < 0.01$) (Wu et al., 2012). The above-mentioned studies suggest that there be a relationship between hyperuricemia and gestational hypertension, and elevated serum uric acid may be useful for identifying perinatal risk in high-risk women with gestational hypertension.

With respect to relationship between maternal serum UA and birth weight and/or fetal growth, a retrospective study of women with preeclampsia (study group) and women with gestational hypertension who delivered at term (control group) (N=40) demonstrated that severity of retinopathy was inversely related to fetal birth weight ($P = 0.044$) and positively related to serum uric acid level ($P = 0.022$) in the preeclampsia group (Gupta et al., 2008). It also has been documented that maternal serum thioredoxin-1 and redox potential significantly correlated not only with serum uric acid but also with neonatal birth weight in 60 pregnant women at the early third trimester (gestational age =27-29 weeks) (Nakatsukasa et al., 2013). In a study of 259 women with prior preeclampsia, compared to women with normal blood pressure, normal urinary protein, and normal uric acid, women with hypertension and hyperuricemia had an increased risk of delivering a SGA infant ($<5^{th}$ centile) ($RR = 8.2$, $p = 0.01$) (Schmella et al., 2015).

More direct evidence to indicate the relationship between maternal serum UA and birth weight and/or fetal growth comes from the following studies although these studies are often limited by issues of small sample size and/or potential confounding effects. High maternal serum uric acid measured in the 34^{th} gestational week of 206 singleton pregnant women with gestational hypertension was associated with the delivery of a SGA infant (unadjusted OR=1.7

with 95% CI=1.4 to 2.2; p<0.001, and adjusted OR=1.6 with 95% CI=1.1 to 2.4; p=0.02) (Bellomo et al., 2011). In this same study, a receiver operating characteristic (ROC) analysis showed that serum uric acid at a 309 μmol/L cutoff may predict a SGA infant (area under the curve (AUC): 0.784) with 83.7% sensitivity and 71.7% specificity. A case-control study of 40 women who delivered SGA infants and 80 participants who delivered appropriate-for-gestational-age (AGA) infants had serum UA measured in the third trimester (Akahori et al., 2012). Investigation found a strong negative correlation between serum uric acid levels and birth weights (r = -0.59; p = 0.006) in cases of severe SGA (<5th percentile) (Akahori et al., 2012). More recently, a prospective cohort study of 247 pregnant women between 20-22 weeks gestational age indicated that higher mid-gestation serum uric acid concentration is associated with lower birth weight in non-insulin resistance women (Nasri et al., 2015).

To date, no studies of the relationship between maternal serum uric acid and placenta have been found. Only one in vitro trophoblast cell model study revealed that elevations in circulating uric acid in preeclamptic women may contribute to the pathogenesis of the disorder, in part, through attenuation of normal trophoblast invasion and spiral artery vascular remodeling (Bainbridge et al., 2009).

However, all above-mentioned studies exclusively focused on high maternal serum uric acid only whereas the importance of low maternal serum uric acid has been ignored. In Section 2.6, we have discussed that both high (Peracoli et al., 2011; Li & Zhou, 2011; Jalal et al., 2012) and low (Weisskopf et al., 2007; Danielson & Andersen, 2008; Chen et al., 2009; Fabbrini et al., 2014) serum uric acid levels are related to increased oxidative stress. Thus, it is speculated that all of those above-mentioned outcomes that were associated with high uric acid might also be

related to low levels of uric acid; in other words, there might be a J-shaped relationship between serum uric acid and those outcomes.

In summary, there are two major research gaps in the current literatures of relationships among maternal complications, perinatal outcomes, and maternal serum uric acid: 1) the lack of investigation regarding associations between low maternal serum uric acid during pregnancy and both gestational hypertension in pregnancy and fetal growth; and 2) the lack of research examining the relationship between maternal serum uric acid and placental pathology. We proposed to address these gaps. Figure 2.3 is a DAG to indicate possible associations among maternal serum uric acid, gestational hypertension, fetal growth, and placentation.

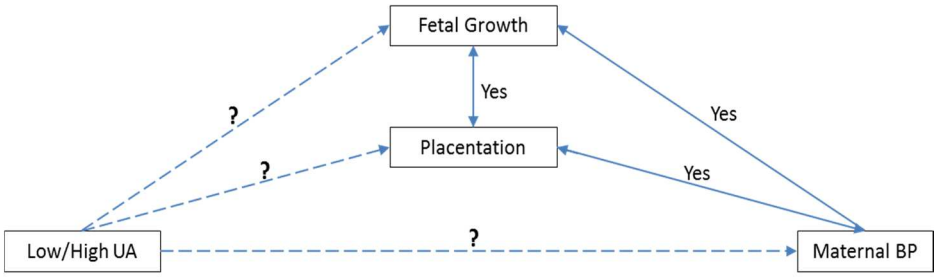


Figure 2.3 A DAG to indicate possible associations among maternal serum uric acid, gestational hypertension, fetal growth, and placentation. Note: BP=Blood Pressure.

Our proposed studies would add knowledge about the potential of a clinically relevant biomarker – maternal serum uric acid in pregnancy, as a predictor for the progression of these three health outcomes or an etiological factor for early intervention.

2.9 Significance and innovations:

To accomplish our specific aims, we conducted a retrospective data analysis using a database of the Pregnancy Outcomes and Community Health (POUCH) Study. Our aims were: 1) to investigate whether there is a J-shaped relationship between maternal serum uric acid in mid-pregnancy and maternal blood pressure; 2) to explore whether there is a J-shaped relationship between maternal serum uric acid in mid-pregnancy and birth weight Z-score; and 3)

to study whether maternal serum uric acid in pregnancy has a J-shaped relationship with the risk of placental pathology.

Completion of our aims would: 1) raise awareness for researchers about risks associated with both high and low serum uric acid in pregnant women, 2) potentially help in reducing the risks of pregnancy complications by using maternal serum uric acid in pregnancy as a predictor or a target to guide an early intervention; and 3) provide epidemiological evidence to guide and/or support the study of the complex uric acid-related mechanisms underlying adverse pregnancy outcomes.

Collectively, our proposed studies are innovative in the following ways: 1) whether maternal serum uric acid in pregnancy has a J-shaped association with blood pressure in pregnant women is unknown; 2) whether both low and high maternal serum uric acid concentrations are associated with extremes in birth weight Z-score is uncertain; and 3) whether there is a J-shaped relationship between vascular lesions in placenta and maternal serum uric acid in pregnancy is unclear.

CHAPTER 3: ASSOCIATION BETWEEN MATERNAL BLOOD PRESSURE DURING PREGNANCY AND MATERNAL SERUM URIC ACID IN MID-PREGNANCY

3.1 Introduction

Hypertension is a common medical problem among pregnant women (Mammaro et al., 2009). It has been associated not only with many adverse perinatal health outcomes such as stillbirth, pre-eclampsia, preterm delivery, small-for-gestational-age birth, and maternal and/or neonatal mortality (Ananth et al., 1995; Zhang et al., 2003; Xiong et al., 2007; Ananth and Basso, 2010; Backes et al., 2011; Seyom et al., 2015) but also with a major economic burden to human society, for instance, the total cost of deliveries complicated by hypertensive disorders of pregnancy (HDP) in 2011 in California was estimated to reach $106.9 million for the Medi-Cal program (Pourat et al., 2013).

Clinically relevant biomarkers related to HDP may provide clues to understanding etiology and/or serve as a marker for progression of complications. One such biomarker is uric acid. Evidence has shown that serum uric acid may be used either for clinically differential diagnosis between preeclampsia and gestational hypertension (Johnson et al., 2011) or as a biochemical indicator for predicting the prognosis of gestational hypertension (Williams and Galerneau, 2002; Bellomo et al., 2011; Andrew and Patel, 2016) and of preeclampsia (Kang et al., 2004; Bainbridge and Roberts, 2008; Martin and Brown, 2010). Although serum uric acid has been used as a modifiable target for clinical treatment of hypertension with allopurinol (a purine analogue that inhibits xanthine oxidase, and thus, reduces blood uric acid) in adolescents (Feig and Johnson, 2003; Kanbay et al., 2007, 2011), similar applications of targeting serum uric acid with allopurinol for reducing gestational hypertension have not yet been found due to the possible teratogenicity of allopurinol (Kozenko et al., 2011; Hoeltzenbein et al., 2013). However,

it may still be reasonable and promising to use serum uric acid as a modifiable factor for controlling gestational hypertension by modifying diet or using other safe medications to control serum uric acid concentration based on the metabolic pathways of uric acid and its regulation, as reviewed in the chapter 2. On the other hand, uric acid could also serve as a biomarker related to BP not just as a causal factor but also as an indicator of shared underlying pathology and severity of complications; for example, serum uric acid was used to predict severe gestational hypertension in women who had pre-eclampsia (Thangaratinam et al., 2006). Measuring serum uric acid concentration is a simple, inexpensive, and reproducible method, which is a required feature for a biomarker being used for large-scale epidemiological studies.

To date, all relevant studies have exclusively focused on the relationship between hyperuricemia (high blood uric acid) and blood pressure during pregnancy, whereas, the association between hypouricemia (low blood uric acid) and maternal blood pressure has been ignored. Such information is important for developing more comprehensive and safer methods (e.g., changing maternal serum uric acid concentration via diets or safe medications) to control maternal blood pressure during pregnancy. The present study tests whether maternal blood pressure during pregnancy is associated with serum uric acid concentration during mid-pregnancy in a non-linear manner by using data from the Pregnancy Outcomes and Community Health (POUCH) Study.

3.2 Materials and Methods

3.2.1 Study population and sampling

The POUCH project was a prospective cohort study designed to examine pathways to preterm delivery in 3,019 pregnant women who were recruited from 52 clinics in 5 Michigan

communities between August 1998 and June 2004 in the 16th-27th week of pregnancy. Inclusion criteria included no known congenital anomaly, maternal age≥15 years, prenatal screening of maternal serum alpha-fetoprotein (AFP) at the 15th-22nd week of pregnancy, no prepregnancy history of diabetes mellitus, and competency in English (Holzman et al., 2013).

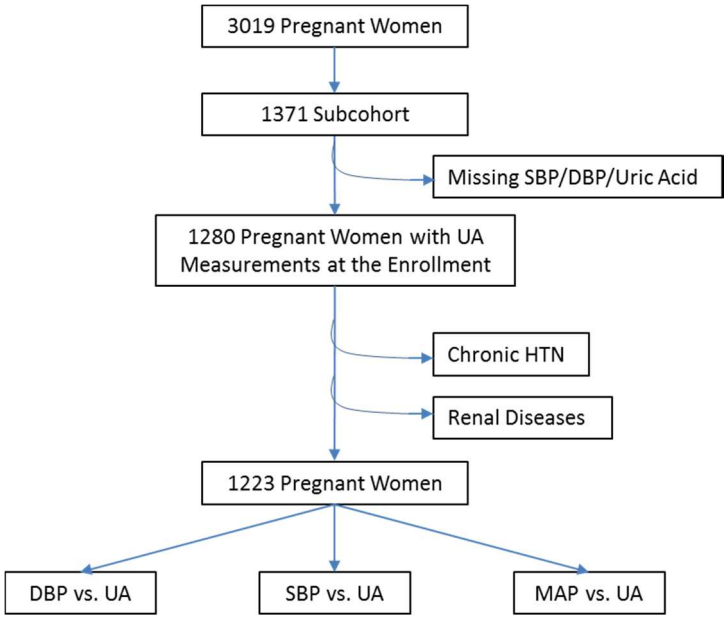


Figure 3.1 A flowchart to indicate the selection of the participants in the POUCH cohort for the current study in Chapter 3. Note: UA=uric acid, SBP=systolic blood pressure, DBP=diastolic blood pressure, MAP=mean arterial pressure, and HTN=hypertension.

As shown in Figure 3.1, among 3,019 enrolled pregnant women who were successfully followed through delivery, a sub-cohort of about 1371 participants was sampled with the inclusion of all pre-term deliveries (<37 gestational weeks), all term deliveries with high maternal serum AFP, and a race-stratified random sample of term deliveries with normal maternal serum AFP including oversampling of African-Americans (Holzman et al., 2013). Within the subcohort, we excluded women who had no abstracted measurements of outcomes (systolic blood pressure (SBP) and diastolic blood pressure (DBP), n=30), no blood collected to measure exposure (maternal serum uric acid concentration during pregnancy, n=61), a diagnosis

of chronic hypertension (defined as having a preexisting diagnosis of chronic hypertension or having a systolic BP 140 mm Hg or diastolic 90 mm Hg before 20 weeks of gestation, n=42), or a diagnosis of renal disease including pyelonephritis, glomerulonephritis, and renal diseases secondary to systemic disease (n=15). The remaining 1223 pregnant women from the subcohort were included in the final analysis. Sampling weights were calculated to adjust for oversampling of women with high AFP levels and with a race of African-American and for selecting a subcohort and/or missing measurements of biological specimens (Holzman et al., 2013). The POUCH Cohort Studies were approved by the institutional review board (IRB) of the Michigan State University. All data used in the current studies were de-identified based on the HIPAA Security Guidelines.

3.2.2 Measurements of outcomes – DBP, SBP, and MAP

Both DBP and SBP were abstracted from medical records. The highest DBP value was used as a major outcome in the current study. In order to study the relationship between maternal serum uric acid concentration and mean arterial blood pressure (MAP), which was calculated with the formula MAP = (2*DBP + SBP)/3, the recorded SBP at the time when the highest diastolic blood pressure was measured was used for the analysis of the association between maternal serum uric acid and SBP or MAP.

3.2.3 Measurements of exposure variable – maternal serum uric acid concentration

Maternal serum uric acid (mg/dL) was measured at the time of the enrollment, i.e., in the 16th-27th week of pregnancy, using the method developed by Fossati et al (1980), which involves a uricase-mediated uric acid oxidation followed by a colorimetric analysis at 520 nm.

Standards, control sera, and serum calibrators were included in each run and the coefficient of variation between runs was 3.6%.

3.2.4 Measurements of potential confounding variables

Covariates including maternal race, maternal age at the enrollment, maternal education, maternal medical insurance (i.e., Medicaid vs. non-Medicaid), maternal body mass index (BMI) before pregnancy, parity, maternal smoking during pregnancy, maternal alcohol use during pregnancy, and gestational age at enrollment were considered as potential confounding variables in the current study. Maternal race was self-reported and classified into black and non-black groups. Maternal age was determined as the woman's age on the date of the POUCH Study enrollment and dichotomized with a cut-off age of 30 years. Maternal education was defined as the highest grade completed at time of the POUCH Cohort Enrollment. Maternal medical insurance coverage was based on self-reported Medicaid Insurance status (yes vs. no) at the time of the POUCH Study Enrollment. Maternal BMI before pregnancy was calculated based on either participants' self-report or medical record abstraction of height and pre-pregnancy weight and then dichotomized as obese (BMI≥30) and non-obese (BMI<30). Parity was expressed as the number of pregnancies prior to the POUCH pregnancy that ended in a livebirth (multiple pregnancies counted as one event). Both maternal smoking and alcohol use during pregnancy were recorded based on participants' self-report at enrollment. Gestational age at enrollment was calculated using the date of the last menstrual period (LMP). If a gestational age estimate from early ultrasound differed from the LMP-based estimate by more than two weeks, then the ultrasound estimated gestational age was used.

3.2.5 Data management and statistical analysis

Outcomes (DBP, SBP, and MAP) and exposure (maternal serum uric acid concentration) were treated as continuous variables with a mean and standard error (SE) based on their approximate normal distributions. In order to simplify the analysis of non-linear relationships between outcomes and exposure, all covariates were categorized as binary and are presented as percentages (Table 1). For univariate analyses, a t-test was used to examine whether the outcome and/or exposure are associated with each potential confounding variable.

To help choose the functional form for the relationship between outcome and exposure, a locally weighted smoothing curve of regression of the outcome on the exposure (also called lowess) was first used. To fit a possible piecewise linear regression with two segments, a breakpoint was visualized by lowess and the corresponding cut-off value of maternal serum uric acid concentration was chosen as an initial location parameter for the linear spline regression analysis. Three models were compared: an unadjusted linear spline model with only the exposure variable (maternal serum uric acid) (model 1); an adjusted model with all potential confounding covariates (i.e., model 2 = model 1 + covariates); and finally we conducted a reduced linear spline regression model (model 3) in which only significant covariates were included (i.e., model 1 + significant covariates). To find an optimal breakpoint value to split the piecewise function for the reduced linear spline regression model, a nonlinear least-squares estimation was applied by using the model 3 as a specific function (Bruin, 2006). The reduced model was re-fitted with the optimal breakpoint value of exposure to generate the final linear spline regression model. For the multiple linear regression, the process of model selection was similar to that used for the linear spline regression except for the application of the nonlinear least-squares estimation for the optimal breakpoint value. The assumptions of normality and homoscedasticity were examined by

visualization with the P-P plot and Q-Q plot of residuals vs. inverse normal as well as residuals vs. fitted values, respectively.

To evaluate a possible overfitting problem for the final model, a 10-fold cross-validation procedure (Hastie et al., 2001) was used. Briefly, the data were randomly split into 10 roughly equal-sized subsets; then the final model was fitted to the 9 subsets of the data and the prediction error of the fitted model to predict the 10th subset of the data was calculated; and finally the procedure was repeated 10 times such that each subset was used for testing exactly once and an average root-mean-square error (RMSE) of 10-fold cross-validation was calculated by using the formula: $SQRT((RMSE_1^2 + RMSE_2^2 + \ldots + RMSE_{10}^2)/10)$. The average RMSE of 10-fold cross-validation was compared with that from the model-fitting using the full sample to determine if there was an overfitting issue.

To obtain a robust variance estimation for the parameters in the final model, a bootstrap procedure, a non-parametric method of resampling with replacement (Efron and Tibshirani, 1994), with sampling weights and 1000 bootstrap replications for complex survey data (Kolenikov, 2010) was carried out.

To further examine the robustness of the final model, a sensitivity analysis was performed by re-fitting the final model after excluding the women who were diagnosed to have gestational hypertension (GH) or preeclampsia (PE) from the studied subcohort followed by a robust bootstrap estimation of variance for the re-fitted final model.

The statistical significance level, α, was set as 0.05 for a two-sided test. Data management was conducted with SAS v9.4 (SAS Institute, Cary, North Carolina) and all statistical analyses were weighted with the POUCH sampling weights and carried out with STATA v13.0 (StataCorp LP, College Station, Texas).

3.3 Results

3.3.1 Study population and maternal characteristics

As shown in Table 3.1, based on the weighted percentage distributions, 24.6% were African-American, 27.6% were ≥30 years of age, 18.7% had ≤11 years of education, 25.8% were obesity before pregnancy, 48.6% were insured by Medicaid, 58.4% had parity≥1, 17.0% were smokers, 17.5% reported alcohol during pregnancy, and 15.1% were <20 weeks gestational age at enrollment. Univariate analysis indicated that there was a significant ($p<0.05$) or marginally significant ($p<0.1$) associations between outcomes (i.e., SBP, DBP, and MAP) and all individual covariates except for alcohol drinking during pregnancy and gestational age at the enrollment, respectively (Table 3.1). In contrast, only three covariates, maternal age, maternal obesity before pregnancy, and maternal Medicaid insurance status, had a significant ($p<0.05$) or marginally significant ($p<0.1$) relationship with maternal serum uric acid concentration (Table 3.1). These three covariates would not be considered as mediators or collider variables but could serve as potential confounders.

Table 3.1 Maternal characteristics and distributions of maternal blood pressures and maternal serum uric acid (UA) in the subcohort (N=1223, weighted).

		SBP		DBP		MAP		Uric Acid	
Variable	**N (%)**	**Mean (SE)**	**p[a]**	**Mean (SE)**	**p**	**Mean (SE)**	**p**	**Mean (SE)**	**p**
Maternal race:									
Non-Black	727 (75.4)	122.10 (0.55)	<0.01	78.48 (0.38)	<0.01	93.02 (0.40)	<0.01	3.22 (0.03)	0.80
Black	496 (24.6)	118.33 (0.70)		73.67 (0.54)		88.55 (0.52)		3.21 (0.03)	
Maternal age:									
<30	910 (72.4)	120.56 (0.53)	0.03	76.57 (0.38)	<0.01	91.23 (0.39)	<0.01	3.25 (0.03)	0.09
≥30	313 (27.6)	122.77 (0.85)		79.19 (0.53)		93.72 (0.58)		3.16 (0.04)	
Education:									
≥12th	952 (81.3)	121.76 (0.52)	<0.01	77.97 (0.35)	<0.01	92.56 (0.37)	<0.01	3.22 (0.03)	0.69
7-11th	271 (18.7)	118.64 (0.83)		74.35 (0.71)		89.11 (0.67)		3.24 (0.05)	
Obesity before pregnancy:									
No	885 (74.2)	119.76 (0.52)	<0.01	76.43 (0.35)	<0.01	90.87 (0.37)	<0.01	3.11 (0.03)	<0.01
Yes	338 (25.8)	125.25 (0.86)		79.79 (0.63)		94.94 (0.64)		3.54 (0.05)	
Medicaid:									
No	547 (51.4)	122.61 (0.63)	<0.01	79.15 (0.43)	<0.01	93.64 (0.45)	<0.01	3.14 (0.03)	<0.01
Yes	676 (48.6)	119.65 (0.65)		75.33 (0.45)		90.10 (0.46)		3.30 (0.04)	
Parity:									
=0	520 (41.6)	122.56 (0.76)	0.01	78.71 (0.52)	<0.01	93.33 (0.55)	<0.01	3.19 (0.04)	0.20
≥1	703 (58.4)	120.19 (0.55)		76.28 (0.38)		90.92 (0.39)		3.25 (0.03)	
Smoking during pregnancy:									
No	1005 (83.0)	121.51 (0.50)	0.09	78.03 (0.34)	<0.01	92.52 (0.36)	<0.01	3.22 (0.03)	0.59
Yes	218 (17.0)	119.53 (1.04)		73.70 (0.75)		88.98 (0.74)		3.25 (0.06)	
Alcohol during pregnancy:									
No	1002 (82.5)	121.20 (0.50)	0.93	77.44 (0.34)	0.34	92.02 (0.36)	0.50	3.24 (0.03)	0.24
Yes	209 (17.5)	121.10 (1.14)		76.60 (0.78)		91.43 (0.80)		3.16 (0.06)	
GA at enrollment									
≥20 wk	1031 (84.9)	121.26 (0.49)	0.66	77.39 (0.34)	0.50	92.01 (0.35)	0.53	3.24 (0.03)	0.10
<20 wk	192 (15.1)	120.70 (1.16)		76.76 (0.86)		91.41 (0.88)		3.14 (0.05)	

3.3.2 J-shaped relationship between maternal diastolic blood pressure (DBP) and maternal serum uric acid concentration

Figure 3.2A visualized a lowess curve with the raw data of maternal DBP and serum uric acid concentration. It showed that there is a breakpoint (the lowest point on the curve) at about 2.4 mg/dL of uric acid concentration and about 72 mmHg of DBP (the lowest DBP). On the left side of the breakpoint, there was a negative linear trend starting from the lowest uric acid concentration to the breakpoint (2.4 mg/dL of uric acid). In contrast, on the right side of the breakpoint, there appeared a positive linear trend starting from the breakpoint (2.4 mg/dL of uric acid) to the highest uric acid concentration. The lowess curve indicated that a linear spline regression should be applied to study the relationship between maternal DBP and serum uric acid concentration.

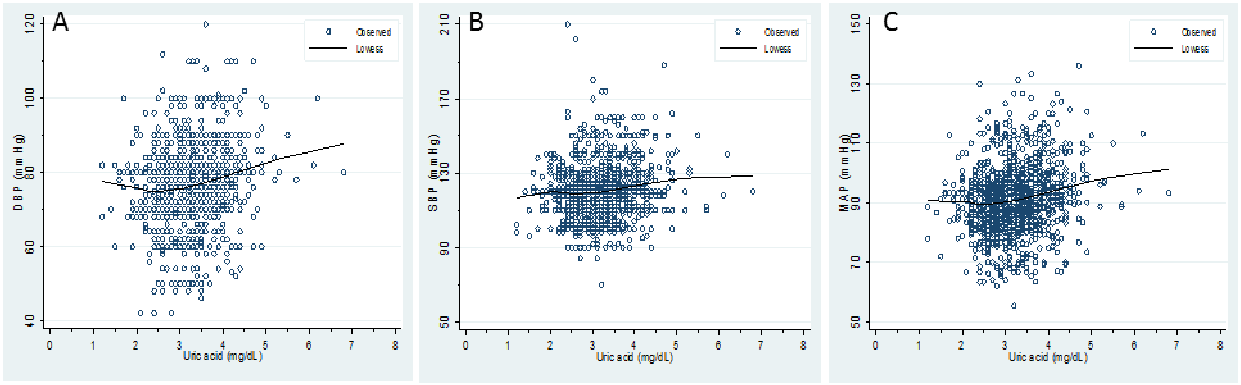


Figure 3.2 Lowess smoothing plots for maternal DBP/SBP/MAP vs. maternal serum UA. Note: panel A: DBP vs. UA; panel B: SBP vs. UA; panel C: MAP vs. UA. The solid lines represent lowess curves and the dots indicate the observed raw data points.

The top section in Table 3.2 summarized a process of model selection for the linear spline regression of maternal DBP on maternal serum uric acid concentration. In the unadjusted model, the first segment on the left side of the breakpoint (i.e., UA<2.40 mg/dL) presented a negative

linear trend (β=-5.13, SE=2.97) with a marginal significance (p = 0.08), whereas the second segment on the right side of the breakpoint (i.e., UA>2.40 mg/dL) had a significant positive linear trend (β=2.84, SE=0.52, p<0.01). In the full model adjusted with all considered possible confounders, both trends were statistically significant (β=-8.00, SE=3.03, p=0.01 for the left and β=2.72, SE=0.54, p<0.01 for the right); at the same time, all of the included covariates significantly contributed to the model at the significance level α=0.05 except for the variables maternal education, maternal alcohol drinking during pregnancy, and gestational age at the enrollment. In the reduced model, after removing the non-significant covariates, an equation was drawn as: DBP = 96.58 – 7.94*uric_acid + 2.73*max[(uric_acid – 2.40), 0] – 3.82*race + 1.70 *age – 2.23*medicaid + 3.76*obesity – 2.75*parity – 3.20*smk + ε.

Using a nonlinear least-squares estimation in STATA with a specific function of DBP = {cons=`cons'} + {b1=`b1'}*uric_acid + {b2=`b2'}*max(uric_acid – {k1=2.40},0) – {b3=`b3'}*race + {b4=`b4'}*age – {b5=`b5'}*medicaid + {b6=`b6'}*obesity – {b7=`b7'}*parity – {b8=`b8'}*smk, the breakpoint was optimized to 2.60 mg/dL (95% CI: 2.35 ~ 2.86 mg/dL) of maternal serum uric acid concentration. With this optimal breakpoint value (2.60 mg/dL), an equation for the final model was adapted as: DBP = 90.88 – 5.05*uric_acid + 2.99*max[(uric_acid – 2.60), 0] – 3.81*race + 1.72*age – 2.24*medicaid + 3.76*obesity – 2.71*parity – 3.21*smk + ε (Table 3.3, Figure 3.3A). The final model with the optimal breakpoint (2.60 mg/dL of uric acid) had a better fit due to the resulted smaller RMSE, compared to the reduced model with the initial breakpoint (2.40 mg/dL of uric acid) (data not shown).

Table 3.2 Model selections for maternal DBP/SBP/MAP vs. maternal serum UA.

Parameter	Unadjusted Model (Model 1)		Full Model (Model 2)		Reduced Model (Model 3)	
	Coef. (SE)	p	Coef. (SE)	p	Coef. (SE)	p
DBP vs. UA:						
Intercept	87.08 (6.94)	<0.01	96.95 (7.13)	<0.01	96.58 (7.00)	<0.01
UA<2.4 mg/dL	-5.13 (2.97)	0.08	-8.00 (3.03)	0.01	-7.94 (2.97)	0.01
UA>2.4 mg/dL	2.84 (0.52)	<0.01	2.72 (0.54)	<0.01	2.73 (0.54)	<0.01
Race (Black vs. Non-Black)	na	na	-3.77 (0.68)	<0.01	-3.82 (0.68)	<0.01
Age (≥30 vs. <30)	na	na	1.70 (0.71)	0.02	1.70 (0.69)	0.02
Education (7-11th vs. ≥12th)	na	na	-0.53 (0.82)	0.52	na	na
Medicaid (Yes vs. No)	na	na	-2.12 (0.70)	<0.01	-2.23 (0.69)	<0.01
Obesity before pregnancy (Yes vs. No)	na	na	3.68 (0.75)	<0.01	3.76 (0.74)	<0.01
Parity (≥1 vs. 0)	na	na	-2.82 (0.62)	<0.01	-2.75 (0.62)	<0.01
Smoke during pregnancy (Yes vs. No)	na	na	-3.01 (0.82)	<0.01	-3.20 (0.81)	<0.01
Alcohol during pregnancy (Yes vs. No)	na	na	-0.95 (0.85)	0.26	na	na
GA at enrollment	na	na	0.19 (0.86)	0.82	na	na
SBP vs. UA:						
Intercept	111.72 (2.30)	<0.01	115.73 (2.44)	<0.01	116.19 (2.41)	<0.01
Uric acid (mg/dL)	2.93 (0.71)	<0.01	2.26 (0.77)	<0.01	2.27 (0.78)	<0.01
Race (Black vs. Non-Black)	na	na	-2.86 (1.01)	<0.01	-3.08 (0.99)	<0.01
Age (≥30 vs. <30)	na	na	1.56 (1.10)	0.16	na	na
Education (7-11th vs. ≥12th)	na	na	-0.71 (1.19)	0.55	na	na
Medicaid (Yes vs. No)	na	na	-2.09 (1.13)	0.06	-2.87 (0.99)	<0.01
Obesity before pregnancy (Yes vs. No)	na	na	5.72 (1.13)	<0.01	5.65 (1.11)	<0.01
Parity (≥1 vs. 0)	na	na	-2.96 (0.93)	<0.01	-2.77 (0.91)	<0.01
Smoke during pregnancy (Yes vs. No)	na	na	-0.80 (1.22)	0.51	na	na
Alcohol during pregnancy (Yes vs. No)	na	na	-0.18 (1.24)	0.89	na	na
GA at enrollment	na	na	-0.05 (1.25)	0.97	na	na
MAP vs. UA:						
Intercept	97.86 (7.22)	<0.01	107.20 (7.50)	<0.01	106.93 (7.37)	<0.01
UA<2.4 mg/dL	-3.55 (3.09)	0.25	-6.29 (3.19)	0.05	-6.25 (3.13)	0.05
UA>2.4 mg/dL	2.95 (0.56)	<0.01	2.70 (0.60)	<0.01	2.71 (0.60)	<0.01
Race (Black vs. Non-Black)	na	na	-3.47 (0.71)	<0.01	-3.55 (0.70)	<0.01
Age (≥30 vs. <30)	na	na	1.67 (0.76)	0.03	1.69 (0.74)	0.02
Education (7-11th vs. ≥12th)	na	na	-0.60 (0.84)	0.48	na	na
Medicaid (Yes vs. No)	na	na	-2.13 (0.76)	<0.01	-2.24 (0.75)	<0.01
Obesity before pregnancy (Yes vs. No)	na	na	4.35 (0.80)	<0.01	4.39 (0.78)	<0.01
Parity (≥1 vs. 0)	na	na	-2.88 (0.65)	<0.01	-2.83 (0.65)	<0.01
Smoke during pregnancy (Yes vs. No)	na	na	-2.28 (0.84)	0.01	-2.45 (0.81)	<0.01
Alcohol during pregnancy (Yes vs. No)	na	na	-0.70 (0.87)	0.42	na	na
GA at enrollment	na	na	0.15 (0.90)	0.87	na	na

Table 3.3 Final models for maternal DBP/SBP/MAP vs. maternal serum UA.

Parameter	Coef.	Linearized		Bootstrapped	
		SE	p	SE	p
DBP vs. UA:					
Intercept	90.88	5.20	<0.01	5.34	<0.01
UA<2.6 mg/dL	-5.05	2.07	0.02	2.11	0.02
UA>2.6 mg/dL	2.99	0.58	<0.01	0.56	<0.01
Race (Black vs. Non-Black)	-3.81	0.68	<0.01	0.64	<0.01
Age (≥30 vs. <30)	1.72	0.69	<0.01	0.66	<0.01
Medicaid (Yes vs. No)	-2.24	0.69	<0.01	0.67	<0.01
Obesity before pregnancy (Yes vs. No)	3.76	0.74	<0.01	0.70	<0.01
Parity (≥1 vs. 0)	-2.71	0.62	<0.01	0.61	<0.01
Smoke during pregnancy (Yes vs. No)	-3.21	0.81	<0.01	0.78	<0.01
SBP vs. UA:					
Intercept	116.19	2.41	<0.01	2.38	<0.01
Uric acid (mg/dL)	2.27	0.78	<0.01	0.75	0.02
Race (Black vs. Non-Black)	-3.08	0.99	<0.01	0.94	<0.01
Medicaid (Yes vs. No)	-2.87	0.99	<0.01	0.77	<0.01
Obesity before pregnancy (Yes vs. No)	5.65	1.11	<0.01	0.97	<0.01
Parity (≥1 vs. 0)	-2.77	0.91	<0.01	0.78	<0.01
MAP vs. UA:					
Intercept	101.29	4.71	<0.01	4.81	<0.01
UA<2.7 mg/dL	-3.35	1.83	0.07	1.85	0.07
UA>2.7 mg/dL	3.15	0.69	<0.01	0.66	<0.01
Race (Black vs. Non-Black)	-3.53	0.70	<0.01	0.66	<0.01
Age (≥30 vs. <30)	1.72	0.74	0.02	0.70	0.02
Medicaid (Yes vs. No)	-2.28	0.75	<0.01	0.73	<0.01
Obesity before pregnancy (Yes vs. No)	4.39	0.78	<0.01	0.73	<0.01
Parity (≥1 vs. 0)	-2.80	0.65	<0.01	0.64	<0.01
Smoke during pregnancy (Yes vs. No)	-2.46	0.81	<0.01	0.76	<0.01

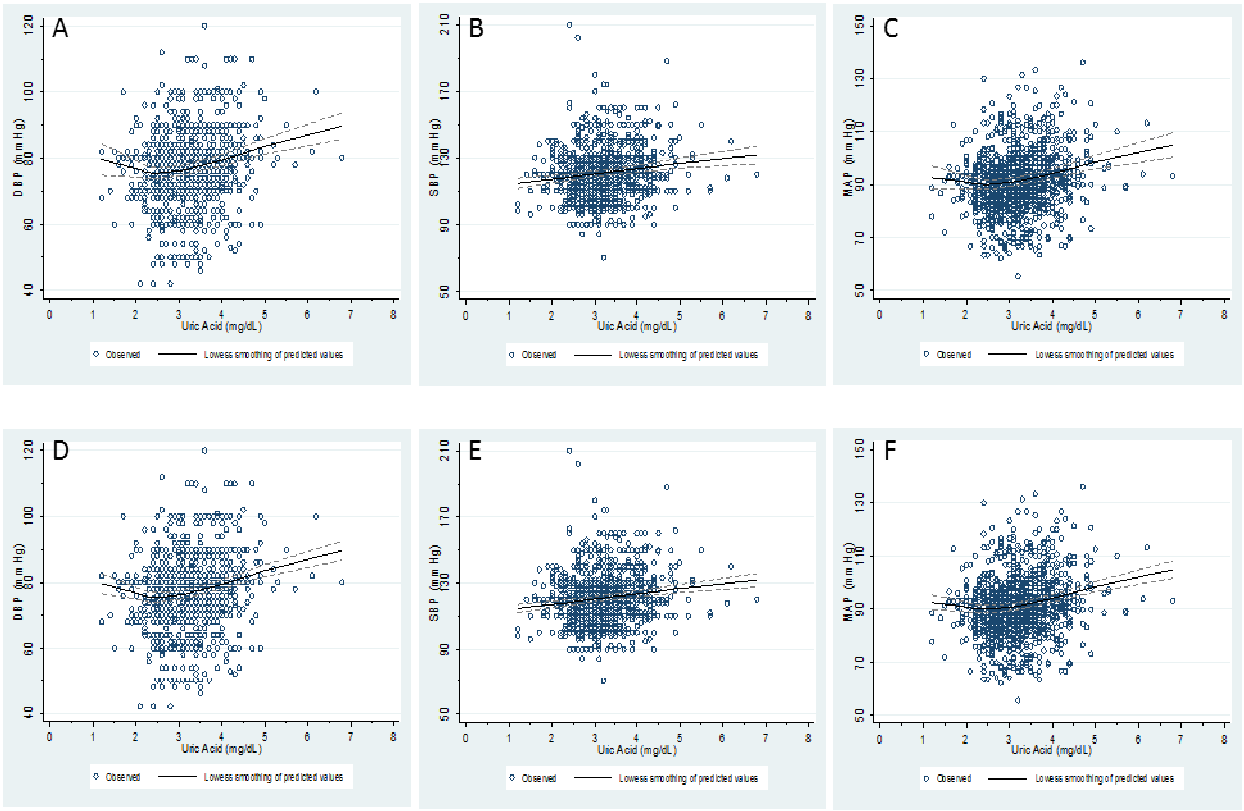


Figure 3.3 Visualization of the final models for DBP/SBP/MAP vs. maternal serum UA. Note: panels A, B, C: linearized estimates of variance; panels D, E, F: Bootstrapped estimates of variance. Panel A & D: DBP vs. UA; panel B & E: SBP vs. UA; panel C & F: MAP vs. UA. The solid lines represent point estimates and the upper and lower dashed lines indicate 95% confidence bands by nonparametric smoothing with locally weighted regression (lowess).

3.3.3 Positive linear relationship between maternal systolic blood pressure (SBP) and maternal serum uric acid concentration

Lowess smoothing curve for the raw data of maternal SBP and serum uric acid concentration showed a simple positive linear relationship between maternal SBP and serum uric acid concentration (Figure 3.2B). As shown in the middle section in Table 3.2, the unadjusted simple linear regression model had a significant slope of independent variable (i.e., maternal serum uric acid) (β=2.93, SE=0.71, and $p<0.01$); inclusion of all possible confounders reduced the slope of exposure to 2.26 with a SE=0.77 and $p<0.01$; and among all of the included

covariates, only maternal race, maternal Medicaid insurance status, maternal obesity before pregnancy, and maternal parity played a (marginally) significant role in the relationship between maternal SBP and maternal serum uric acid concentration ($p=0.06$ for Medicaid and $p<0.01$ for other three). In the reduced model (also the final model), removing the non-significant covariates resulted in an equation as follows: SBP = 116.19 + 2.27*uric_acid – 3.08*race – 2.87*medicaid + 5.65*obesity – 2.77*parity + ε (Table 3.3, Figure 3.3B).

3.3.4 J-shaped relationship between maternal mean arterial pressure (MAP) and maternal serum uric acid concentration

MAP is an average blood pressure indicator from the formula MAP = (2*DBP + SBP)/3. The lowess smoothing for the raw data of MAP vs. uric acid still presented a potential J-shaped curve (Figure 3.2C). As shown in the bottom section in Table 3.2, in the unadjusted model, the first segment on the left side of the breakpoint (i.e., UA<2.40 mg/dL) presented a negative linear trend ($\beta=-3.55$, SE=3.09) but with no statistical significance ($p = 0.25$), whereas the second segment on the right side of the breakpoint (i.e., UA>2.40 mg/dL) had a significant positive linear trend ($\beta=2.95$, SE=0.56, $p<0.01$). In the full model adjusted with all possible confounders, the negative linear trend for the left segment was marginally significant ($\beta=-6.29$, SE=3.19, $p=0.05$) whereas the positive linear trend for the right segment was extremely significant ($\beta=2.70$, SE=0.60, $p<0.01$); among all covariates, only three variables, maternal education, maternal alcohol drinking during pregnancy, and gestational age at the enrollment were not statistically significant (Table 3.2). In the reduced model, after removing the non-significant covariates, an equation was drawn as: MAP = 106.93 – 6.25*uric_acid + 2.71*max[(uric_acid – 2.40), 0] – 3.55*race + 1.69 *age – 2.24*medicaid + 4.39*obesity – 2.83*parity – 2.45*smk + ε (Table 3.2). The optimal breakpoint value was determined as 2.70 mg/dL (95% CI: 2.25 ~ 3.15

mg/dL) of maternal serum uric acid concentration by using a nonlinear least-squares estimation and an equation for the final model was adapted as: MAP = 101.29 – 3.35*uric_acid + 3.15*max[(uric_acid – 2.70), 0] – 3.53*race + 1.72*age – 2.28*medicaid + 4.39*obesity – 2.80*parity – 2.46*smk + ε (Table 3.3, Figure 3.3C).

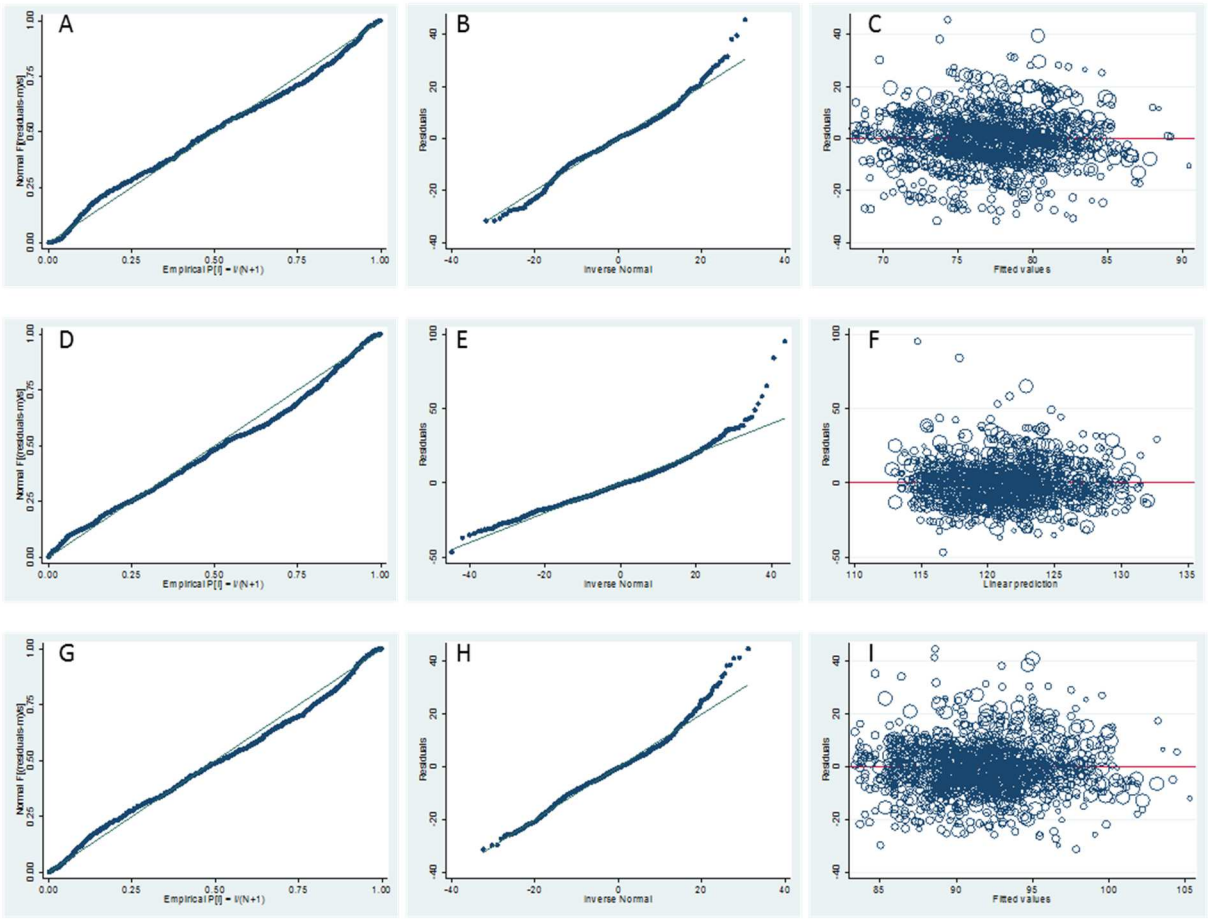


Figure 3.4 Examinations of the assumptions – normality and homoscedasticity for the final models: DBP/SBP/MAP vs. maternal serum UA. Note: panels A, B, C: for DBP vs. UA; panels D, E, F: for SBP vs. UA; panels G, H, I: for MAP vs. UA. Panels A, D, G: for normality of the data in the middle range of uric acid; Panels B, E, H: for normality of the data near the tails of uric acid. Panels C, F, I: for homoscedasticity (residuals vs. fitted values).

Table 3.4 Ten-fold cross-validation for the final models: maternal DBP/SBP/MAP vs. maternal serum UA.

RMSE	$Model_{(DBP\ vs.\ UA)}$	$Model_{(SBP\ vs.\ UA)}$	$Model_{(MAP\ vs.\ UA)}$
10-fold cross-validation:			
RMSE1	11.09	18.03	12.38
RMSE2	10.34	12.46	10.05
RMSE3	9.06	12.85	9.12
RMSE4	8.59	13.32	8.93
RMSE5	7.78	12.83	8.08
RMSE6	10.67	12.68	10.03
RMSE7	9.18	12.39	9.12
RMSE8	10.15	13.55	10.00
RMSE9	9.55	13.60	9.90
RMSE10	10.90	17.25	11.95
Average RMSE (cross_validation)	9.73	13.90	9.96
$RMSE_{(full\ sample)}$	9.72	13.95	9.96
ΔRMSE	0.01	0.05	0.00
% change in RMSE	0.10	0.36	0.00

Table 3.5 Sensitivity analysis for the final models: maternal DBP/SBP/MAP vs. maternal serum UA after removing the women who had the diagnosed GH and PE.

Parameter	Coef.	Linearized		Bootstrapped	
		SE	p	SE	p
DBP vs. UA:					
Intercept	85.99	3.83	<0.01	3.78	<0.01
UA<2.6 mg/dL	-3.34	1.53	0.03	1.50	0.03
UA>2.6 mg/dL	2.23	0.49	<0.01	0.46	<0.01
Race (Black vs. Non-Black)	-3.72	0.63	<0.01	0.62	<0.01
Age (≥30 vs. <30)	1.12	0.65	0.08	0.64	0.08
Medicaid (Yes vs. No)	-2.54	0.64	<0.01	0.64	<0.01
Obesity before pregnancy (Yes vs. No)	3.26	0.71	<0.01	0.67	<0.01
Parity (≥1 vs. 0)	-2.08	0.58	<0.01	0.58	<0.01
Smoke during pregnancy (Yes vs. No)	-2.73	0.77	<0.01	0.72	<0.01
SBP vs. UA:					
Intercept	115.99	2.10	<0.01	2.07	<0.01
Uric acid (mg/dL)	1.76	0.65	0.01	0.63	<0.01
Race (Black vs. Non-Black)	-2.59	0.89	<0.01	0.91	<0.01
Medicaid (Yes vs. No)	-3.20	0.87	<0.01	0.87	<0.01
Obesity before pregnancy (Yes vs. No)	5.00	1.02	<0.01	0.98	<0.01
Parity (≥1 vs. 0)	-2.00	0.83	0.02	0.82	0.02
MAP vs. UA:					
Intercept	97.77	3.64	<0.01	3.61	<0.01
UA<2.7 mg/dL	-2.29	1.41	0.11	1.39	0.10
UA>2.7 mg/dL	2.39	0.54	<0.01	0.50	<0.01
Race (Black vs. Non-Black)	-3.33	0.62	<0.01	0.63	<0.01
Age (≥30 vs. <30)	0.91	0.66	0.17	0.66	0.16
Medicaid (Yes vs. No)	-2.72	0.66	<0.01	0.67	<0.01
Obesity before pregnancy (Yes vs. No)	3.82	0.72	<0.01	0.68	<0.01
Parity (≥1 vs. 0)	-2.07	0.59	<0.01	0.59	<0.01
Smoke during pregnancy (Yes vs. No)	-2.00	0.73	0.01	0.72	<0.01

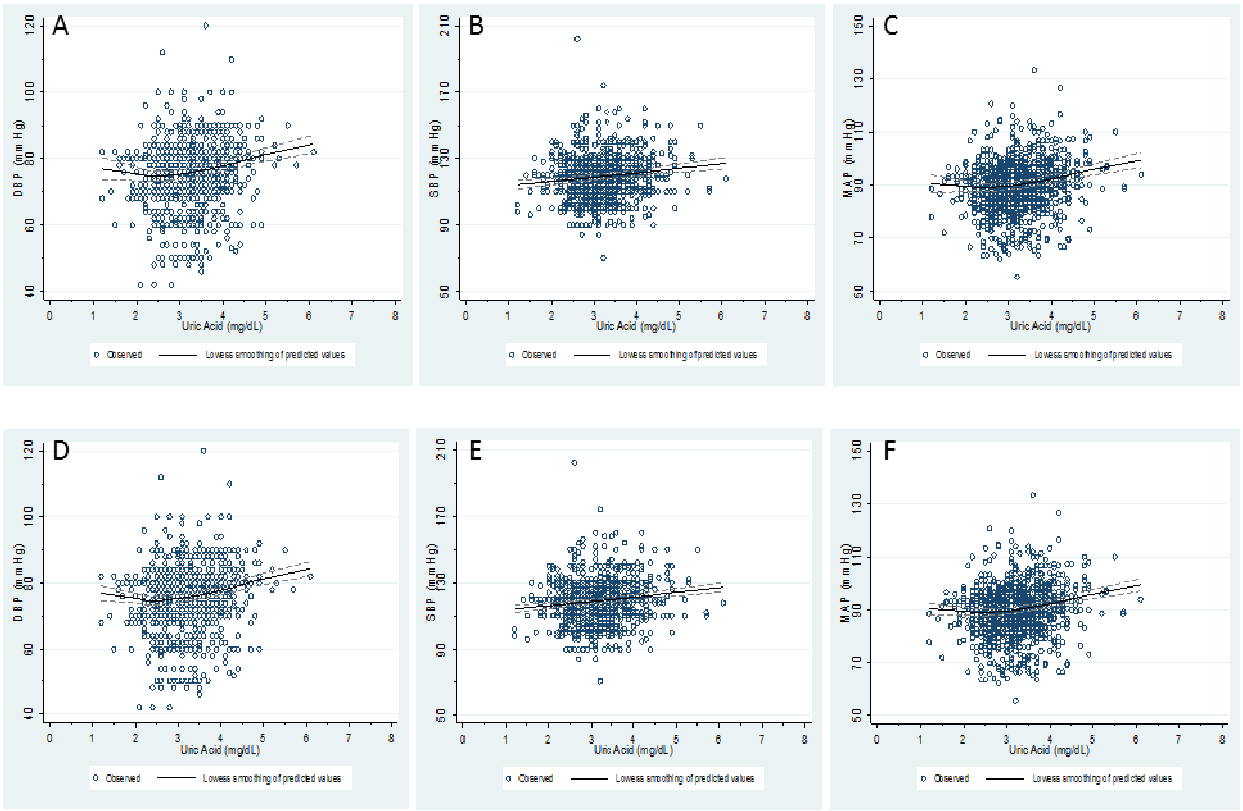


Figure 3.5 Visualization of sensitivity analysis for DBP/SBP/MAP vs. maternal serum UA after removing the women who had diagnosed GH and PE. Note: panels A, B, C: linearized estimates of variance; panels D, E, F: Bootstrapped estimates of variance. Panel A & D: DBP vs. UA; panel B & E: SBP vs. UA; panel C & F: MAP vs. UA. The solid lines represent point estimates and the upper and lower dashed lines indicate 95% confidence bands by nonparametric smoothing with locally weighted regression (lowess).

3.3.5 Examinations of assumptions, over-fitting issues, and sensitivity analysis of the final models

For all three final models, there was an approximate normality in the middle range of uric acid (Figure 3.4-A, D, G), but a slight deviation from normality was found near the upper tail (Figure 3.4-B, E, H), suggesting that a robust estimation of variance such as bootstrap is needed for the parameters in the final models. The data points in Figure 3.4-C, F, I, representing the corresponding residuals vs. fitted values, were roughly even-distributed, which is an indication of no heteroscedasticity for all three modellings. Bootstrap estimation of variance for each

parameter in the three final models demonstrated an improved statistical power, i.e., narrowing the parameters' standard errors and confidence intervals (Table 3.3, Figure 3.3A and 3.3D for DBP; 3.3B and 3.3E for SBP; 3.3C and 3.3F for MAP). The results from 10-fold cross-validations demonstrated that the over-fitting percentage in the overall model fitting only accounts for 0.10%, 0.36%, and 0.00% for DBP, SBP, and MAP, respectively (Table 3.4), indicating that there is no over-fitting issue in all three model-fitting processes. Sensitivity analysis revealed that the absolute slope sizes for all parameters were reduced after removing pregnant women with GH and PE but the directions and statistical significance of all parameters were still unaffected; exception was maternal Medicaid insurance status, of which the absolute slope size was increased in all three modellings (Table 3.5, Figure 3.5). These results suggest that the fitted final models are relatively robust regardless of the inclusion of the women with GH and PE.

3.4 Discussion

We found there was a J-shaped relationship between maternal DBP or MAP and maternal serum uric acid in mid-pregnancy with a breakpoint of 2.6 mg/dL (for DBP) or 2.7 mg/dL (for MAP), respectively, after adjusting for potential confounding covariates maternal race, maternal age, maternal Medicaid insurance status, maternal obesity before pregnancy, parity, and maternal smoking during pregnancy. In contrast, the regression of maternal SBP on maternal serum uric acid only followed a simple positive linear trend with an adjustment for confounders.

To our knowledge, the current study is the first to report a non-linear relationship between maternal DBP or MAP and maternal serum uric acid concentration in pregnant women. Paula et al (2008) studied the relationship between categorized maternal serum uric acid and blood pressure in pregnant women (n=58) at a hospital setting with a univariate statistical method and

found that higher serum uric acid was associated with higher maternal DBP, but not SBP. Hawkins et al (2012) conducted a retrospective cohort study (n=1880) with categorized maternal serum uric acid levels measured near delivery. They found that women with gestation-corrected hyperuricemia had higher SBP and DBP. In these two studies, serum uric acid concentration was categorized and only univariate analysis or linear regression models were applied without the consideration of nonlinear relationships.

The mechanism underlying our findings is, at this point, open for speculation. MAP reflects Cardiac Output × Total Peripheral Resistance (TPR) (Klabunde, 2011); thus, for a given cardiac output, MAP is positively correlated with TPR. A recent cross-sectional study using multiple linear regression models reported a significant positive relationship between uric acid and TPR in women (standardized coefficient=0.158 and $p<0.001$), but not in men (Hsu et al., 2013). The underlying mechanism connecting uric acid, TPR, and MAP may be related to blood viscosity, based on the Hagen-Poiseuille Equation, i.e., Resistance = 8*Vessel Length*Blood Viscosity / (π*Vessel Radius^4) (Thurston, 1976). Blood viscosity is a sensitive marker for oxidative stress (Richards and Nwose, 2010) while oxidative stress is associated with high uric acid levels (Baillie et al., 2007; Nakatsukasa et al., 2013; Fabbrini et al., 2014). In addition, reactive oxygen species (ROS, a series of indicators of oxidative stress) may increase peripheral vascular resistance by directly impairing endothelial function (Taddei et al., 1998; Schiffrin, 1999; Wong et al., 2010). At physiological levels, uric acid is an important anti-oxidant in blood (Fahn and Cohen, 1992; Davis et al., 1996; De Lau et al., 2005; Weisskopf et al., 2007; Chen et al., 2009; Fabbrini et al., 2014). Thus, low serum uric acid may reduce antioxidant activity in blood and increase ROS, and finally, increase MAP via an oxidative stress-induced increase of blood viscosity. On the other hand, high serum uric acid might increase oxidative stress by at

least two pathways: 1) high uric acid functions as pro-oxidant to increase oxidative stress (Corry et al., 2008); and 2) high uric acid may reflect a high level of xanthine oxidase activity (Dawson and Walters, 2006) whereas increased xanthine oxidase activity can increase oxidative stress (Romagnoli et al., 2010; Ryan et al., 2011).

Because MAP equals ⅔ × DBP + ⅓ × SBP, MAP is more sensitive to changes in DBP, compared to that in SBP. As discussed above, MAP is positively correlated with TPR. Thus, low serum uric acid-induced oxidative stress may increase DBP via the oxidative-stress-induced TPR, whereas high uric acid-related increasing DBP would have the same underlying mechanisms as that for high uric acid-related increasing MAP.

Arterial stiffness is a major contributor to high SBP (O'Rourke, 1989). Studies have shown that high uric acid is positively correlated with arterial stiffness (Gomez-Marcos et al., 2013; Vlachopoulos et al., 2011; Kuo et al., 2010). Thus, SBP rises with the increased uric acid. Arterial stiffness involves a complex and dynamic interaction among structural proteins, extracellular matrix, inflammatory molecules, and ROS in vessel wall (Zieman et al., 2005). Low uric acid-related ROS might contribute less to the arterial stiffness, and consequently did not significantly influence the pattern of SBP increase with uric acid-related arterial stiffness.

Figure 3.6 summarizes the possible mechanisms underlying the relationships between maternal DBP/SBP/MAP and maternal serum uric acid concentration. However, more vigorous efforts will be needed to clarify these connections in pregnant women.

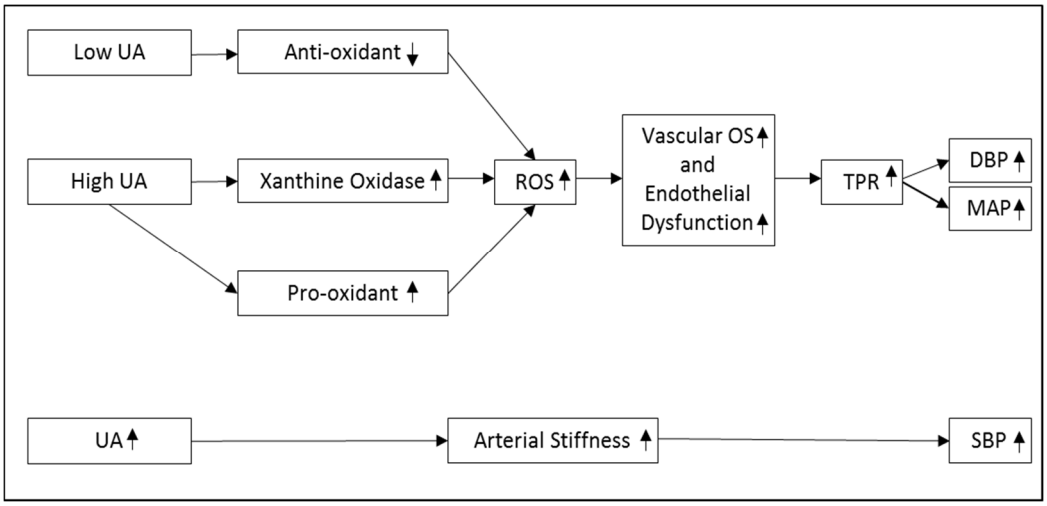


Figure 3.6 Possible mechanisms underlying the relationships between maternal DBP/SBP/MAP and maternal serum UA concentration. Note: UA=uric acid, ROS=reactive oxygen species, TPR=total peripheral resistance, DBP=diastolic blood pressure, MAP=mean arterial pressure, SBP=systolic blood pressure.

Our results are consistent with clinical reference levels for UA and with previous literatures in UA. First, clinically, a normal range of serum uric acid concentration in the second trimester in pregnant women is from 2.4 mg/dL to 4.9 mg/dL (Abbassi-Ghanavati et al., 2009). The breakpoint values of maternal serum uric acid concentration in the final models of DBP and MAP are 2.6 mg/dL and 2.7 mg/dL, respectively. Thus, our results are consistent with existing clinical reference. Second, the positive linear relationships between DBP/MAP and uric acid after the breakpoints as well as between SBP and uric acid are consistent with the report by Hawkins et al (2012), in which the gestation-corrected hyperuricemia was associated with both SBP and DBP in pregnant women.

Our current study has several strengths. First, the POUCH Cohort is a large, diverse pregnancy cohort enrolled from multi-communities with detailed placental measures and many biomarker measurements. Second, our results have biological plausibility regarding the interpretations of the models. Third, the application of 10-fold cross-validation technique against

model overfitting issue improves the internal validity of our studies. Fourth, the application of non-linear regression models increases the statistical power and reduces the loss of information that occurs when an exposure variable is categorized. Finally, the use of sensitivity analysis assures the robustness of our analyses, and thus, improves the generalizability of our results. There are also some limitations in our study. We abstracted DBP based on one single highest measurement, which may have measurement error and/or large variance. Both exposure (maternal serum uric acid) and outcomes (maternal DBP/SBP/MAP) were measured at the POUCH enrollment. Therefore, the time order between exposure and outcomes cannot be determined and an inverse causal relationship is possible. We could not rule out some common underlying pathologies in pregnancy as an explanation for the association between UA and maternal blood pressure. Maternal serum uric acid concentration was measured at onetime point only, and consequently a specific dynamic pattern of serum uric acid during the entire pregnancy period cannot be studied. In our models, two continuous covariates – maternal age and maternal BMI were dichotomized, which may have resulted in some residual confounding effects. Diet may influence both maternal serum UA levels and pregnancy complications, thus there might be unmeasured confounding that is not addressed in our analysis. Finally, the proportion of the women who had low serum uric acid was still relatively small.

We conclude that maternal serum uric acid concentration is associated non-linearly with maternal DBP/MAP and linearly with maternal SBP in pregnant women.

CHAPTER 4: BIRTH WEIGHT Z-SCORE IS ASSOCIATED WITH MATERNAL SERUM URIC ACID IN MID-PREGNANCY NON-LINEARLY IN SMALL FOR GESTATIONAL AGE (SGA) AND LINEARLY IN LARGE FOR GESTATIONAL AGE (LGA) INFANTS

4.1 Introduction

Atypical fetal growth is frequently measured as small-for-gestational age (SGA, fetal weight below the 10th percentile for gestational age) and large-for-gestational age (LGA, fetal weight greater than the 90th percentile for gestational age).

SGA infants have an increased risk of cardiovascular diseases and metabolic syndrome (Barker et al., 1993), higher adult BMI (Meas et al., 2008), lower intelligence, poor academic performance, low social competence, and behavioral problems later in life (Lundgren & Tuvemo, 2008). On the other hand, LGA birth is an important risk factor for the subsequent obstetric complications including the increased need for delivery by caesarean sections or instrumental procedures, resuscitation, and transfer to intensive/special care nursery (Ng et al., 2010).

Studies have shown that maternal serum uric acid is negatively associated with birth weight. Bellomo et al (2011) reported that elevated maternal serum uric acid increased the risk of SGA infant (adjusted OR=1.6 with 95% CI=1.1 to 2.4; p=0.02; n=206) or decreased the birth weight centile in pregnant women with gestational hypertension using simple logistic and multiple linear regression models. A similar negative relationship between maternal serum uric acid and birth weight also was found in normotensive pregnant women (β=-0.51, p=0.004) with a multiple linear regression model in a case-control study (cases=40, controls=80) (Akahori et al., 2012). However, these studies suffer from some major problems such as small sample size, selection bias, and loss of information due to the inappropriate use of statistical models. The

possible association between low maternal serum uric acid and extremes in birth weight has been ignored, though biologically too low and too high blood uric acid concentrations may be related to oxidative stress (discussed in chapter 3). High maternal serum uric acid during the first 20 weeks of pregnancy has been associated with higher risk for gestational diabetes mellitus (GDM) (Wolak et al., 2012; Laughon et al., 2009). Although GDM is a major risk factor for developing macrosomia (Walsh and McAuliffe, 2012; Chiavaroli et al., 2016; Koyanagi et al., 2013; Sridhar et al., 2013; Kim et al., 2015; Lenoir-Wijnkoop et al., 2015), to date, no published studies have assessed the relationship between maternal serum uric acid and birth weight in macrosomia or LGA infants. Associations between low and/or high maternal serum uric acid levels and fetal growth may be important to predict trajectory of a fetus with inappropriate growth and/or provide insights into developing early intervention measure(s).

Thus, in the present study, based on the dual properties of uric acid in the aspects of anti-oxidant and pro-oxidant that has been reviewed in the chapter 2, we hypothesized that both unusually high and low maternal serum uric acid concentrations may be linked to extremes in birth weight Z Score. We tested this hypothesis with non-linear regression models by using data from the POUCH cohort study.

4.2 Materials and Methods

4.2.1 Study population and sampling

As described in chapter 3, the study sample was from the POUCH cohort study, which consisted of 3,019 pregnant women enrolled in the 16th-27th week of pregnancy from 52 clinics in Michigan during the period from August 1998 through June 2004 (Holzman et al., 2013). Based on Figure 2.3, the DAG of the current studies, a possible mediation analysis might be

applied for the relationships among maternal serum uric acid, placental vascular pathology, and birth weight Z-score. Thus, the POUCH Study subcohort sample that is used for both chapters 4 and 5 is based on the inclusion and exclusion criteria described in chapter 3 with modifications. Briefly, women with missing data of infant birth weight (n=2), serum uric acid levels during pregnancy (n=48), or placental pathology examined (n=198), or who had a diagnosis of chronic hypertension (n=38), or renal diseases (n=14) were excluded. That left 1071 pregnant women for the final analysis of the current studies (Figure 4.1). Sampling weights were re-calculated based on such specific inclusions. The Michigan State University's IRB had approved the POUCH Cohort Study and the HIPAA Security Guidelines were applied for the de-identification of all data used in the current studies.

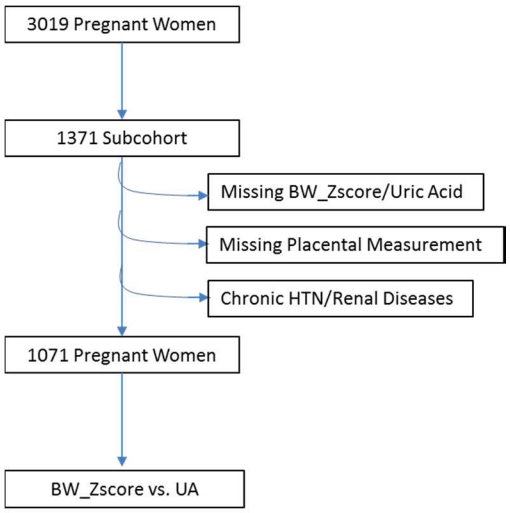


Figure 4.1 A flowchart to indicate the selection of the participants in the POUCH cohort for the current study in Chapter 4. Note: BW_ZScore=birth weight Z score, UA=uric acid, HTN=hypertension.

4.2.2 Measurement of dependent variable – birth weight Z-score

Infant birth weights were abstracted from medical labor and delivery records. Gestational age was calculated based on the last menstrual period (LMP) or the ultrasound results when the LMP estimates disagreed with the ultrasound results by greater than 2 weeks (Shroff et al.,

2013). Birth weight Z-scores were calculated with the formula: gestational age (GA) specific birth weight Z-score = (observed GA specific birth weight – mean US GA specific birth weight)/standard deviation (Kramer et al., 2001). The mean US birth weight and the corresponding standard deviation (SD) in the formula were based on an updated US birth weight for gestational age reference that was generated from the US Singleton Live Birth files maintained by the National Center for Health Statistics (2009–2010) (Talge et al., 2014).

4.2.3 Measurements of independent variables – maternal serum uric acid concentration and potential confounding covariates

As described in chapter 3, maternal serum uric acid (mg/dL) was measured at enrollment using the method developed by Fossati et al (1980). Covariates including maternal age at the enrollment, maternal race/education/Medicaid/obesity before pregnancy, parity, and maternal smoking and alcohol drinking during pregnancy were self-reported at the enrollment interview. Gestational age at enrollment was calculated using the LMP method or estimated by ultrasound when there was a difference of more than two weeks between early ultrasound and LMP-based estimates.

4.2.4 Data management and statistical analysis

Birth weight Z-score and maternal serum uric acid concentration were expressed as mean and standard error for the overall subcohort. Because our preliminary analysis with lowess showed a simple positive linear relationship between birth weight Z-score and maternal serum uric acid in the overall subcohort, which was not consistent with our original hypothesis, i.e., a J-shaped relationship, we speculated that there might be heterogeneity in the relationship between birth weight Z-score and maternal serum uric acid across infant sizes for gestational age. Thus, we split the overall subcohort into three groups, i.e., appropriate for gestational age (AGA, birth

weight between 10th and 90th percentile for gestational age), small for gestational age (SGA, birth weight less than 10th percentile for gestational age), and large for gestational age (LGA, birth weight more than 90th percentile for gestational age). All covariates were categorized as binary and presented as weighted percentage (Table 4.1). A t-test was used to examine whether the outcome and/or exposure were associated with individual potential confounding variable.

Multivariate analyses were based on the methods described in chapter 3. Briefly, lowess was used to determine the use of a linear or non-linear regression model for the next analysis. For a multiple linear regression, a routine procedure of model selection starting from an unadjusted model followed by a full model including all covariates and ending with a reduced model with significant covariates was conducted. For a possible piecewise linear regression with two segments, an initial breakpoint value was chosen for model selection based on lowess smoothing curve. An optimal breakpoint value with the reduced model was determined using a nonlinear least-squares estimation and further used to generate the final linear spline regression model. The assumptions of normality and homoscedasticity were examined by visualization. Ten-fold cross-validation was conducted against the overfitting problem for the final model. A bootstrap procedure with sampling weights and 1000 bootstrap replications for complex survey data was carried out to obtain a robust variance estimation for the parameters in the final model. A sensitivity analysis was performed with the final model by excluding the participants who had diagnosed GH or PE. A p-value less than 0.05 was considered as statistically significant for a two-sided test. SAS v9.4 (SAS Institute, Cary, North Carolina) was used for data management and STATA v13.0 (StataCorp LP, College Station, Texas) for all statistical analyses with the POUCH sampling weights.

4.3 Results

4.3.1 Study population and maternal characteristics

As shown in Figure 4.1, the original POUCH Study cohort consisted of 3019 pregnant women. After applying sampling weights, the population composition was almost the same for both overall subcohort and AGA subset (Table 4.1). Within the SGA group in the study population, 24.6% were African-American, 18.3% were ≥30 years of age, 22.7% had ≤11 years of education, 7.0% were underweight before pregnancy, 58.0% were insured by Medicaid, 44.4% had parity≥1, 33.6% were smokers, 15.7% reported alcohol during pregnancy, and 20.4% were <20 weeks gestational age at enrollment (Table 4.1). Within the LGA group in the study population, 23.9% were African-American, 38.3% were ≥30 years of age, 12.4% had ≤11 years of education, 28.8% were pre-pregnancy obesity, 38.7% were insured by Medicaid, 70.0% had parity≥1, 5.9% were smokers, 19.6% reported alcohol during pregnancy, and 21.9% were <20 weeks gestational age at enrollment (Table 4.2). Considering that SGA infants have been reported to be associated with maternal pre-pregnancy underweight (Yu et al., 2013; Akahoshi et al., 2016) and LGA infants with maternal obesity before pregnancy (Yu et al., 2013), the potential confounding variable – maternal underweight before pregnancy (yes/no) was included into the analyses of relationship between birth weight Z-score and maternal uric acid in overall subcohort as well as the subsets of AGA and SGA. Instead, the potential confounding variable maternal obesity before pregnancy (yes/no) was used in the analysis of LGA-UA relationship. Table 4.1and Table 4.2 showed that 7.0% of pregnant women who were categorized as delivering an SGA infant were underweight before pregnancy and that about 28.8% of the participants who had LGA infants were obesity before pregnancy.

Table 4.1 Maternal characteristics and distributions of birth weight Z-score and maternal serum UA in the subcohort (N=1071) as well as AGA (n=845) and SGA (n=118) groups (weighted).

	Overall					AGA					SGA				
		BW_ZS		Uric Acid			BW_ZS		Uric Acid			BW_ZS		Uric Acid	
	N (%)	Mean (SE)	p	Mean (SE)	p	N (%)	Mean (SE)	p	Mean (SE)	p	N (%)	Mean (SE)	p	Mean (SE)	p
Race:															
Non-black	659 (75.4)	0.25 (0.05)	<0.01	3.22 (0.03)	0.90	528 (75.4)	0.13 (0.04)	<0.01	3.20 (0.04)	0.73	45 (75.4)	-1.61 (0.05)	0.51	3.30 (0.11)	0.51
Black	412 (24.6)	-0.32 (0.05)		3.21 (0.03)		317 (24.6)	-0.17 (0.04)		3.22 (0.04)		73 (24.6)	-1.66 (0.05)		3.21 (0.08)	
Age:															
<30	783 (71.5)	-0.02 (0.04)	<0.01	3.24 (0.03)	0.20	618 (71.9)	-0.01 (0.03)	<0.01	3.23 (0.04)	0.15	101 (81.7)	-1.63 (0.05)	0.54	3.33 (0.10)	0.05
≥30	288 (28.5)	0.44 (0.08)		3.17 (0.04)		227 (28.1)	0.23 (0.05)		3.14 (0.05)		17 (18.3)	-1.57 (0.09)		3.04 (0.10)	
Education:															
12-17	844 (82.2)	0.19 (0.04)	<0.01	3.22 (0.03)	0.85	668 (82.0)	0.11 (0.03)	<0.01	3.20 (0.03)	0.90	81 (77.3)	-1.61 (0.04)	0.78	3.27 (0.10)	0.94
7-11	227 (17.8)	-0.26 (0.07)		3.21 (0.06)		177 (18.0)	-0.17 (0.05)		3.19 (0.07)		37 (22.7)	-1.65 (0.11)		3.28 (0.16)	
Underweight:															
no	1022 (96.3)	0.13 (0.04)	<0.01	3.23 (0.03)	0.02	810 (96.6)	0.07 (0.03)	0.01	3.21 (0.03)	0.09	108 (93.0)	-1.61 (0.04)	0.55	3.30 (0.09)	0.27
yes	49 (3.7)	-0.44 (0.16)		2.99 (0.10)		35 (3.4)	-0.31 (0.13)		3.01 (0.12)		10 (7.0)	-1.74 (0.29)		2.99 (0.36)	
Medicaid:															
no	487 (51.4)	0.32 (0.06)	<0.01	3.14 (0.03)	0.00	386 (50.7)	0.15 (0.04)	<0.01	3.11 (0.03)	<0.01	35 (42.0)	-1.57 (0.06)	0.23	3.17 (0.16)	0.32
yes	583 (48.6)	-0.10 (0.05)		3.30 (0.04)		459 (49.3)	-0.04 (0.04)		3.29 (0.04)		83 (58.0)	-1.66 (0.06)		3.45 (0.09)	

Table 4.1 (cont'd)

	Overall					AGA					SGA				
		BW_ZS		Uric Acid			BW_ZS		Uric Acid			BW_ZS		Uric Acid	
	N (%)	Mean (SE)	p	Mean (SE)	p	N (%)	Mean (SE)	p	Mean (SE)	p	N (%)	Mean (SE)	p	Mean (SE)	p
Parity:															
=0	451 (41.9)	-0.04 (0.06)	<0.01	3.17 (0.04)	0.14	352 (41.9)	0.05 (0.04)	0.89	3.18 (0.05)	0.49	67 (55.6)	-1.68 (0.06)	0.10	3.23 (0.11)	0.02
≥1	619 (58.1)	0.22 (0.05)		3.25 (0.03)		493 (58.1)	0.06 (0.04)		3.22 (0.04)		51 (44.4)	-1.55 (0.04)		3.33 (0.13)	
Smoke:															
no	883 (83.1)	0.21 (0.04)	<0.01	3.21 (0.03)	0.55	692 (83.2)	0.10 (0.03)	<0.01	3.20 (0.03)	0.93	90 (66.4)	-1.61 (0.04)	0.70	3.24 (0.11)	0.51
yes	188 (16.9)	-0.34 (0.08)		3.25 (0.06)		153 (16.8)	-0.17 (0.06)		3.20 (0.07)		28 (33.6)	-1.65 (0.09)		3.35 (0.13)	
Alcohol:															
no	878 (82.6)	0.08 (0.04)	0.05	3.22 (0.03)	0.55	691 (83.4)	0.04 (0.03)	0.06	3.22 (0.03)	0.20	100 (84.3)	-1.65 (0.05)	<0.01	3.28 (0.09)	0.94
yes	183 (17.4)	0.28 (0.09)		3.18 (0.07)		145 (16.6)	0.17 (0.07)		3.12 (0.07)		18 (15.7)	-1.46 (0.02)		3.26 (0.19)	
GA at enrollment:															
≥20wk	909 (85.9)	0.10 (0.04)	0.42	3.23 (0.03)	0.08	723 (87.3)	0.05 (0.03)	0.39	3.22 (0.03)	0.09	101 (79.6)	-1.61 (0.05)	0.65	3.28 (0.10)	0.79
<20wk	162 (14.1)	0.20 (0.11)		3.12 (0.06)		122 (12.7)	0.12 (0.07)		3.09 (0.07)		17 (20.4)	-1.65 (0.08)		3.23 (0.16)	

Table 4.2 Maternal characteristics and distributions of birth weight Z-score and maternal serum UA in the LGA group (n=108, weighted).

	LGA				
	N (%)	BW_ZS		Uric Acid	
		Mean (SE)	p	Mean (SE)	p
Race:					
Non-black	86 (76.1)	1.87 (0.07)	0.84	3.30 (0.09)	0.72
Black	22 (23.9)	1.84 (0.11)		3.24 (0.14)	
Age:					
<30	64 (61.7)	1.76 (0.07)	0.03	3.27 (0.09)	0.72
≥30	44 (38.3)	2.03 (0.10)		3.32 (0.12)	
Education:					
12-17	95 (87.6)	1.87 (0.06)	0.84	3.26 (0.08)	0.24
7-11	13 (12.4)	1.83 (0.19)		3.50 (0.19)	
Pre-Pregnancy Obesity:					
no	76 (71.2)	1.76 (0.06)	0.03	3.18 (0.09)	0.03
yes	32 (28.8)	2.11 (0.14)		3.54 (0.14)	
Medicaid:					
no	66 (61.3)	1.89 (0.08)	0.53	3.28 (0.09)	0.91
yes	41 (38.7)	1.82 (0.08)		3.30 (0.13)	
Parity:					
=0	32 (30.0)	1.75 (0.08)	0.18	3.08 (0.11)	0.06
≥1	75 (70.0)	1.91 (0.08)		3.38 (0.09)	
Smoke:					
no	101 (94.1)	1.88 (0.06)	0.03	3.26 (0.08)	0.10
yes	7 (5.9)	1.62 (0.11)		3.77 (0.33)	
Alcohol:					
no	87 (80.4)	1.89 (0.07)	0.21	3.27 (0.08)	0.67
yes	20 (19.6)	1.77 (0.06)		3.36 (0.22)	
GA at enrollment:					
≥20wk	85 (78.1)	1.85 (0.07)	0.74	3.30 (0.09)	0.75
<20wk	23 (21.9)	1.90 (0.12)		3.25 (0.14)	

Univariate statistics demonstrated that two covariates, maternal pre-pregnancy underweight and maternal Medicaid insurance status were significantly associated with both birth weight Z-score and maternal serum uric acid in the overall sample and in the subset of AGA infants (Table 4.1). Associations of both birth weight Z-score and UA were significant or marginally significant with parity in the SGA group (Table 4.1) and with maternal obesity before pregnancy and maternal smoking during pregnancy in the LGA group (Table 4.2). These

covariates were not likely to be mediators or serve as collider variables but could be potential confounders.

4.3.2 There was a marginally positive linear relationship between birth weight Z-score and maternal serum uric acid concentration in the overall sample

As visualized in Figure 4.2A, there was a marginally positive linear relationship between birth weight Z-score and maternal serum uric acid concentration in overall subcohort. In Table 4.3, the slope of the coefficient for the exposure – maternal serum uric acid was 0.10, but statistically non-significant (p=0.12). Inclusion of all possible confounders increased the slope of the exposure to 0.11 with a SE=0.06 and p=0.06 (Table 4.3). In the final model, after removing the non-significant covariates (i.e., maternal education, maternal Medicaid insurance status, and gestational age at enrollment), an equation for the overall subcohort was drawn as follows: birth weight Z-score = -0.24 + 0.10*uric_acid – 0.47*race + 0.28*age -0.32*underweight + 0.25*parity – 0.54*smk + 0.19*alcohol + ε (Table 4.4, Figure 4.3A).

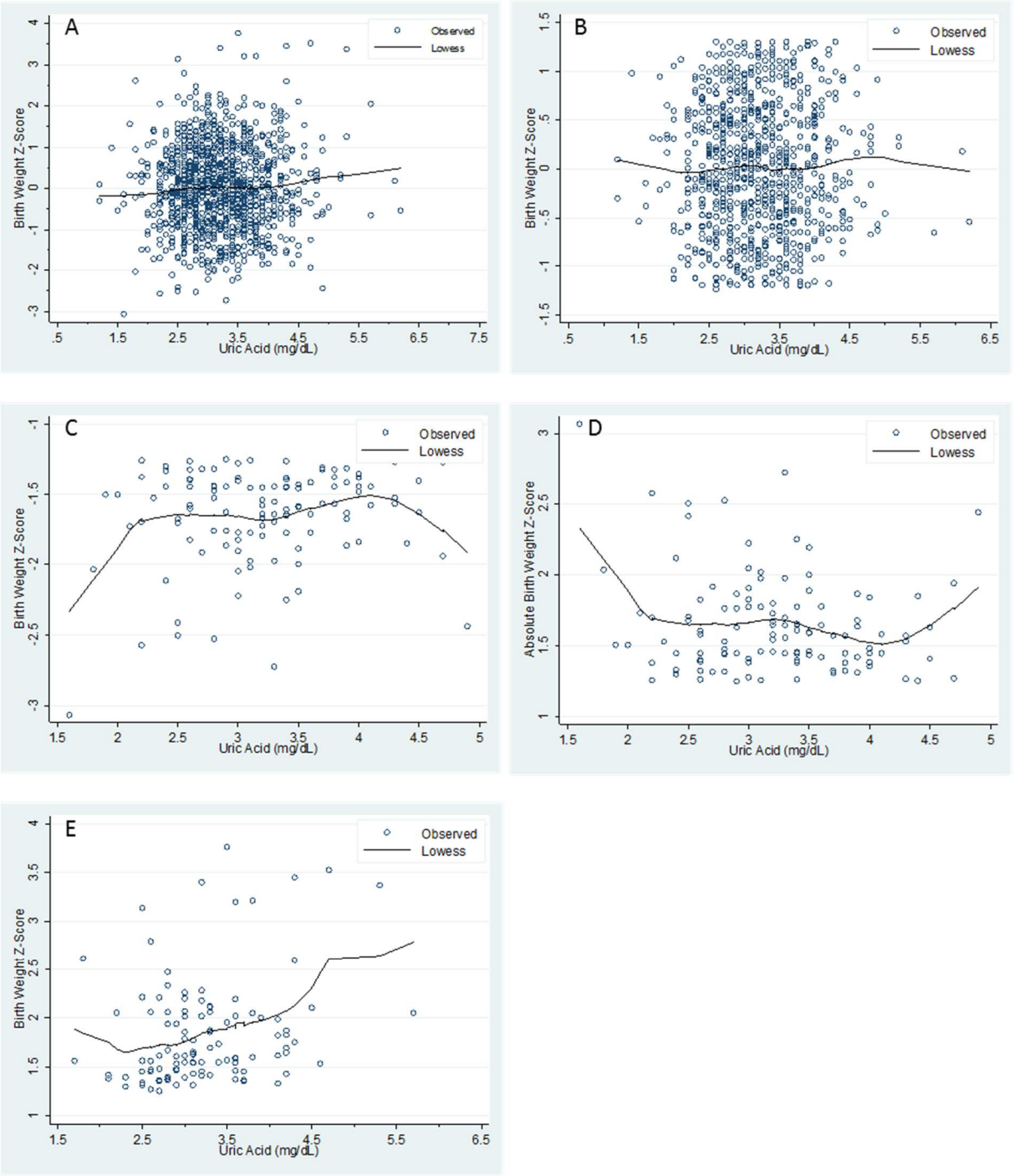


Figure 4.2 Lowess smoothing plots for birth weight Z-score vs. maternal serum UA. Note: panel A: Birth Weight Z-Score vs. UA in overall subcohort; panel B: Birth Weight Z-Score vs. UA in AGA subgroup; pane; C: Birth Weight Z-Score vs. UA in SGA subgroup; and panel D: Birth Weight Z-Score vs. UA in LGA subgroup. The solid lines represented lowess curves. The dots indicate the observed raw data points.

Table 4.3 Model selections for birth weight Z-score vs. maternal serum UA in overall subcohort as well as AGA, SGA, and LGA groups.

	Unadjusted Model		Full Model		Reduced Model	
Parameter	**Coef. (SE)**	**p**	**Coef. (SE)**	**p**	**Coef. (SE)**	**p**
Overall:						
Intercept	-0.20 (0.20)	0.31	-0.23 (0.19)	0.23	-0.24 (0.19)	0.21
Uric acid	0.10 (0.06)	0.12	0.11 (0.06)	0.06	0.10 (0.06)	0.09
Race (Black vs. Non-Black)	na	na	-0.42 (0.07)	<0.01	-0.47 (0.07)	<0.01
Maternal age	na	na	0.23 (0.09)	0.02	0.28 (0.09)	<0.01
Maternal education	na	na	-0.06 (0.09)	0.52	na	na
Underweight before pregnant	na	na	-0.31 (0.19)	0.11	-0.32 (0.19)	0.09
Medicaid	na	na	-0.13 (0.09)	0.14	na	na
Parity	na	na	0.25 (0.08)	<0.01	0.25 (0.07)	<0.01
Smoke during pregnancy	na	na	-0.49 (0.10)	<0.01	-0.54 (0.09)	<0.01
Alcohol during pregnancy	na	na	0.19 (0.09)	0.03	0.19 (0.09)	0.04
Gestational age (GA) at enrollment	na	na	0.12 (0.11)	0.27	na	na
AGA:						
Intercept	-0.10 (0.14)	0.45	-0.08 (0.14)	0.57	-0.08 (0.14)	0.56
Uric acid	0.05 (0.04)	0.23	0.06 (0.04)	0.14	0.06 (0.04)	0.16
Race (Black vs. Non-Black)	na	na	-0.23 (0.06)	<0.01	-0.24 (0.05)	<0.01
Maternal age	na	na	0.14 (0.07)	0.05	0.17 (0.07)	0.01
Maternal education	na	na	-0.10 (0.07)	0.15	na	na
Underweight before pregnant	na	na	-0.29 (0.15)	0.05	-0.31 (0.14)	0.03
Medicaid	na	na	-0.02 (0.07)	0.77	na	na
Parity	na	na	0.01 (0.06)	0.92	na	na
Smoke during pregnancy	na	na	-0.22 (0.07)	<0.01	-0.24 (0.07)	<0.01
Alcohol during pregnancy	na	na	0.13 (0.07)	0.06	0.13 (0.07)	0.06
Gestational age (GA) at enrollment	na	na	0.09 (0.07)	0.19	na	na

Table 4.3 (cont'd)

Parameter	Unadjusted Model		Full Model		Reduced Model	
	Coef. (SE)	p	Coef. (SE)	p	Coef. (SE)	p
SGA:						
Intercept	1.99 (0.22)	<0.01	2.01 (0.21)	<0.01	2.04 (0.20)	<0.01
UA<4.10 mg/dL	-0.12 (0.06)	0.06	-0.15 (0.06)	0.01	-0.16 (0.06)	0.01
UA>4.10 mg/dL	0.90 (0.26)	<0.01	1.07 (0.24)	<0.01	1.05 (0.25)	<0.01
Race (Black vs. Non-Black)	na	na	0.03 (0.07)	0.67	na	na
Maternal age	na	na	0.04 (0.09)	0.63	na	na
Maternal education	na	na	-0.04 (0.09)	0.63	na	na
Underweight before pregnant	na	na	0.04 (0.19)	0.84	na	na
Medicaid	na	na	0.15 (0.07)	0.04	0.17 (0.06)	0.01
Parity	na	na	-0.13 (0.06)	0.04	na	na
Smoke during pregnancy	na	na	-0.15 (0.09)	0.08	na	na
Alcohol during pregnancy	na	na	-0.19 (0.06)	<0.01	-0.18 (0.05)	<0.01
Gestational age (GA) at enrollment	na	na	0.06 (0.06)	0.37	na	na
LGA:						
Intercept	1.08 (0.31)	<0.01	0.99 (0.28)	<0.01	1.00 (0.30)	<0.01
Uric acid (mg/dL)	0.24 (0.31)	<0.01	0.23 (0.09)	0.01	0.22 (0.10)	0.02
Race (Black vs. Non-Black)	na	na	-0.09 (0.13)	0.51	na	na
Maternal age	na	na	0.32 (0.15)	0.04	0.26 (0.11)	0.02
Maternal education	na	na	-0.08 (0.18)	0.68	na	na
Obesity before pregnant	na	na	0.25 (0.13)	0.06	0.27 (0.13)	0.03
Medicaid	na	na	-0.09 (0.14)	0.52	na	na
Parity	na	na	-0.05 (0.11)	0.61	na	na
Smoke during pregnancy	na	na	-0.41 (0.10)	<0.01	-0.41 (0.10)	<0.01
Alcohol during pregnancy	na	na	-0.13 (0.12)	0.28	na	na
Gestational age (GA) at enrollment	na	na	0.99 (0.13)	0.95	na	na

Table 4.4 Final models for birth weight Z-score vs. maternal serum UA in overall subcohort as well as AGA, SGA, and LGA groups.

		Linearized		Bootstrap	
	Coef.	**SE**	**p**	**SE**	**p**
Overall:					
Intercept	-0.24	0.19	0.20	0.19	0.20
Uric acid	0.10	0.06	0.09	0.06	0.08
Maternal race (Black vs. Non-Black)	-0.47	0.07	<0.01	0.07	<0.01
Maternal age	0.28	0.09	<0.01	0.09	<0.01
Underweight before pregnant	-0.32	0.19	0.09	0.18	0.08
Parity	0.25	0.07	<0.01	0.07	<0.01
Smoke during pregnancy	-0.54	0.09	<0.01	0.09	<0.01
Alcohol during pregnancy	0.19	0.09	0.04	0.09	<0.01
AGA:					
Intercept	-0.08	0.14	0.56	0.13	0.55
Uric acid	0.06	0.04	0.16	0.04	0.15
Maternal race (Black vs. Non-Black)	-0.24	0.05	<0.01	0.05	<0.01
Maternal age	0.17	0.07	0.01	0.07	0.01
Underweight before pregnant	-0.31	0.14	0.03	0.13	0.01
Smoke during pregnancy	-0.24	0.07	<0.01	0.07	<0.01
Alcohol during pregnancy	0.13	0.07	0.06	0.07	0.05
SGA:					
Intercept	2.04	0.20	<0.01	0.20	<0.01
UA<4.10 mg/dL	-0.16	0.06	0.01	0.06	0.01
UA>4.10 mg/dL	1.05	0.25	<0.01	0.22	<0.01
Medicaid	0.17	0.06	0.01	0.06	<0.01
Alcohol during pregnancy	-0.18	0.05	<0.01	0.04	<0.01
LGA:					
Intercept	1.00	0.30	<0.01	0.29	<0.01
Uric acid (mg/dL)	0.22	0.10	0.02	0.09	0.02
Maternal age	0.26	0.11	0.02	0.12	0.02
Pre-pregnancy obesity	0.27	0.13	0.03	0.13	0.03
Smoke during pregnancy	-0.41	0.10	<0.01	0.10	<0.01

4.3.3 Birth weight Z-score was not associated with maternal serum uric acid concentration in AGA infants

Figure 4.2B showed a horizontal line, suggesting that birth weight Z-score might not be associated with maternal uric acid in this group. To test this hypothesis, a multiple linear regression model was applied and a routine model selection was conducted. As shown in Table 4.3, the unadjusted simple linear regression model for the AGA subgroup had a non-significant slope of independent variable (i.e., maternal serum uric acid) (β=0.05, SE=0.04, and p=0.23);

inclusion of all possible confounders in the model (full model) increased the slope of exposure to 0.06 with a SE=0.04 and p=0.14; after removing the non-significant covariates, the exposure still had no significant contribution to birth weight Z-score in the AGA group (β=0.06, SE=0.04, and p=0.16), and further using a non-parametric method – bootstrap to estimate the variance of the parameter of exposure did not significantly improve the model-fitting (β=0.06, SE=0.04, and p=0.15) (Table 4.3, Table 4.4, Figure 4.3C).

4.3.4 There was a J-shaped relationship between maternal serum uric acid concentration and absolute values of birth weight Z-scores in SGA infants

All birth weight Z-scores were negative in the group of SGA infants (Figure 4.2C) and transformed into positive values with an absolute function for the next analysis. The subsequent lowess curve indicated that there was a potential J-shaped relationship with a breakpoint at about 4.1 mg/dL of uric acid concentration between absolute birth weight Z-scores and maternal serum uric acid in the SGA group (Figure 4.2D). In Table 4.3, the unadjusted model had a negative linear trend (β=-0.12, SE=0.06) but with a marginally statistical significance (p = 0.06) when UA<4.10 mg/dL and a significantly positive linear trend (β=0.90, SE=0.26, p<0.01) when UA>4.10 mg/dL. Inclusion of all possible confounders into the model (i.e., full model), both linear trends on both sides of the breakpoint (i.e., 4.1 mg/dL of UA) were extremely significant (left side: β=-0.15, SE=0.06, p=0.01; right side: β=1.07, SE=0.24, p<0.01). Two covariates including maternal Medicaid insurance status and maternal alcohol drinking during pregnancy were statistically significant (Table 4.3). After removing the non-significant covariates, a functional equation for the non-linear relationship between absolute birth weight Z-score and maternal serum uric acid in the SGA group was drawn as: absolute birth weight Z-score for SGA = 2.04 – 0.16*uric_acid + 1.05*max[(uric_acid – 4.10), 0] + 0.17*medicaid – 0.18*alcohol + ε

(Table 4.3). The optimal breakpoint value was kept at 4.10 mg/dL of maternal serum uric acid concentration by using a nonlinear least-squares estimation and thus, the final model was the same as the reduced model (Table 4.3, Table 4.4, Figure 4.3E).

4.3.5 There was a positive linear relationship between birth weight Z-score and maternal serum uric acid concentration in LGA infants

Figure 4.2E appeared to indicate a J-shaped relationship with a breakpoint at about 2.1 mg/dL of uric acid between birth weight Z-score and maternal serum uric acid in the LGA group. However, data were sparse when UA<2.1 mg/dL. An attempt to fit these data into a linear spline model with a breakpoint=2.1 mg/dL failed, i.e., the negative linear trend on the left of the breakpoint was not statistically significant (data not shown). Thus, a multiple linear regression model was subsequently applied. As shown in Table 4.3, the unadjusted model had a positive linear trend (β=0.24, SE=0.31, $p<0.01$). Inclusion of all possible confounders into the model (i.e., full model) slightly reduced the effect of uric acid, but still statistically significant (β=0.23, SE=0.09, p=0.01). Of the included covariates, maternal age, maternal obesity before pregnancy, and maternal smoking during pregnancy significantly contributed to the model (Table 4.3). After excluding the non-significant covariates, the effect of UA was still statistically significant (β=0.22, SE=1.20, p=0.02) and the final equation for the linear relationship between birth weight Z-score and maternal serum uric acid in the LGA group was obtained as: birth weight Z-score for LGA = 1.00 + 0.22*uric_acid + 0.26*age + 0.27*obesity – 0.41*smk + ε (Table 4.3, Table 4.4, Figure 4.3G).

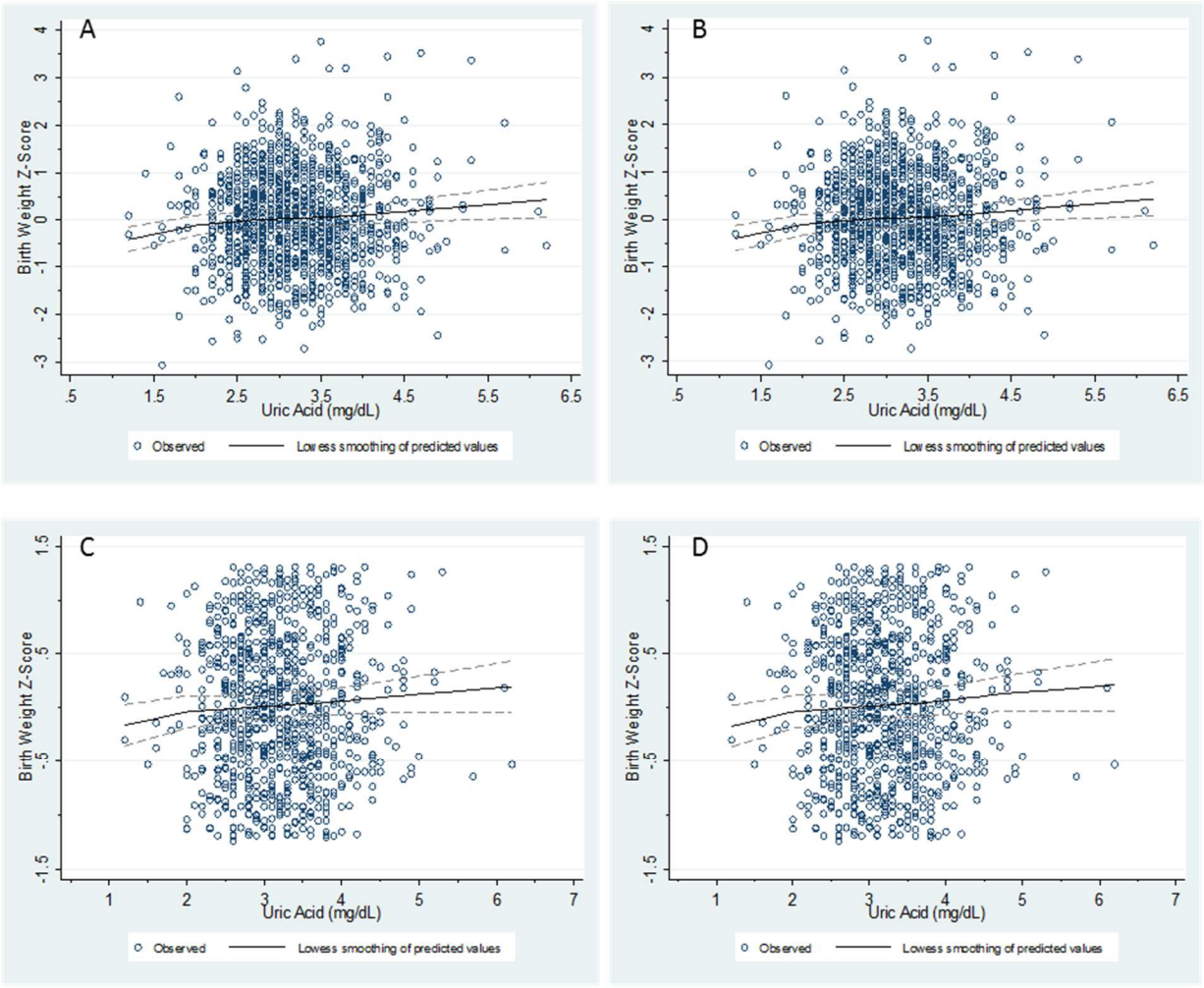
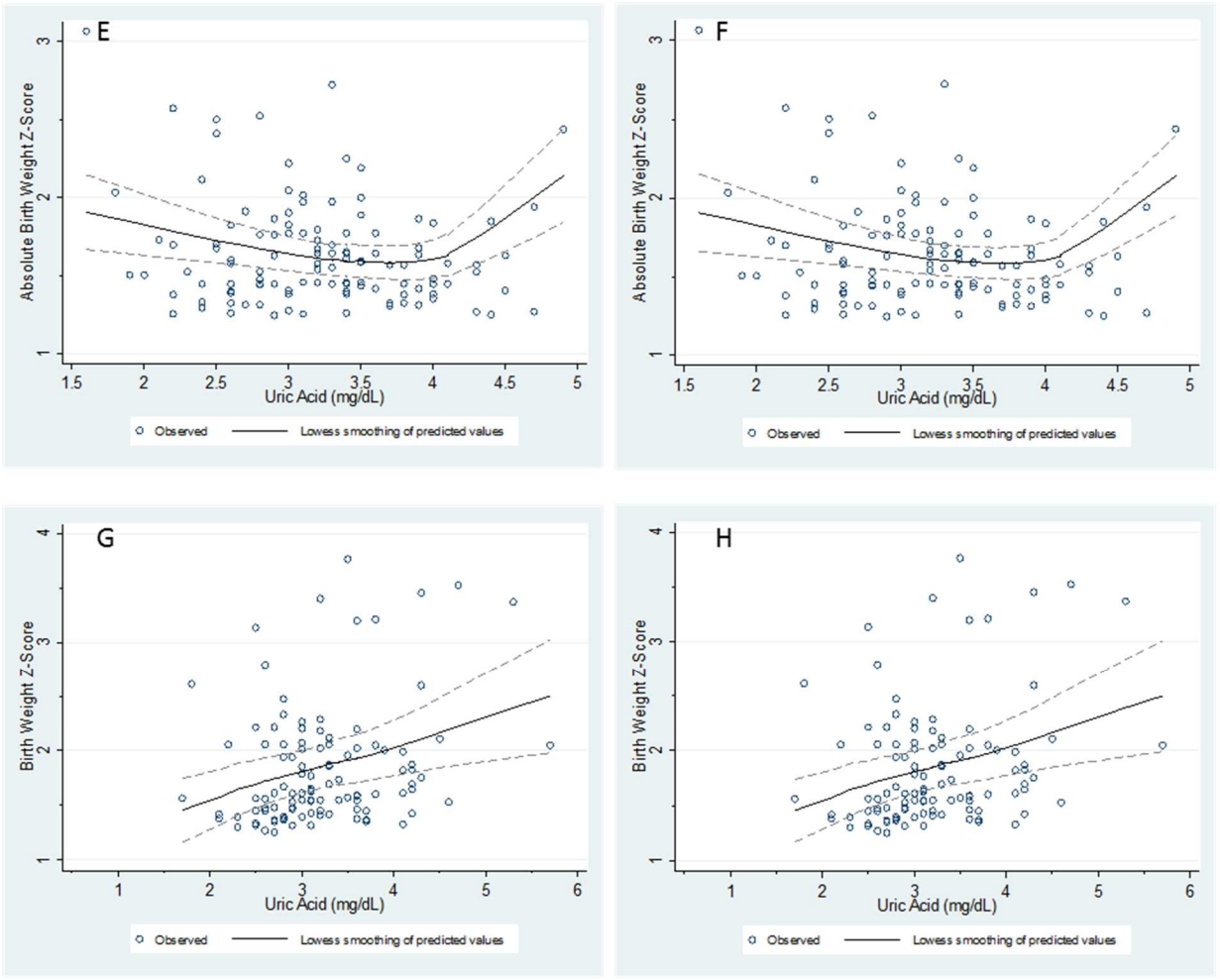


Figure 4.3 Visualization of the final models for birth weight Z-score vs. maternal serum UA. Note: panels A, C, E, G: linearized estimates of variance; panels B, D, F, H: Bootstrapped estimates of variance. Panel A & B: for overall subcohort; panel C & D: for AGA subgroup; panel E & F: for SGA subgroup; panel G & H: for LGA subgroup. The solid lines represent point estimates and the upper and lower dashed lines indicate 95% confidence bands by nonparametric smoothing with locally weighted regression (lowess).

Figure 4.3 (cont'd)



4.3.6 Examinations of assumptions, over-fitting issue, bootstrap estimation of variance, and sensitivity analysis of the final models

Normality assumption in the middle range of data in all four final models was not violated (Figure 4.4-A, D, G, J), a slight deviation from normality was found near the tails in the models for overall subcohort and AGA group, and the violation of the normality assumption near the tails was more severe in the models for both SGA and LGA groups (Figure 4.4-B, E, H, K).

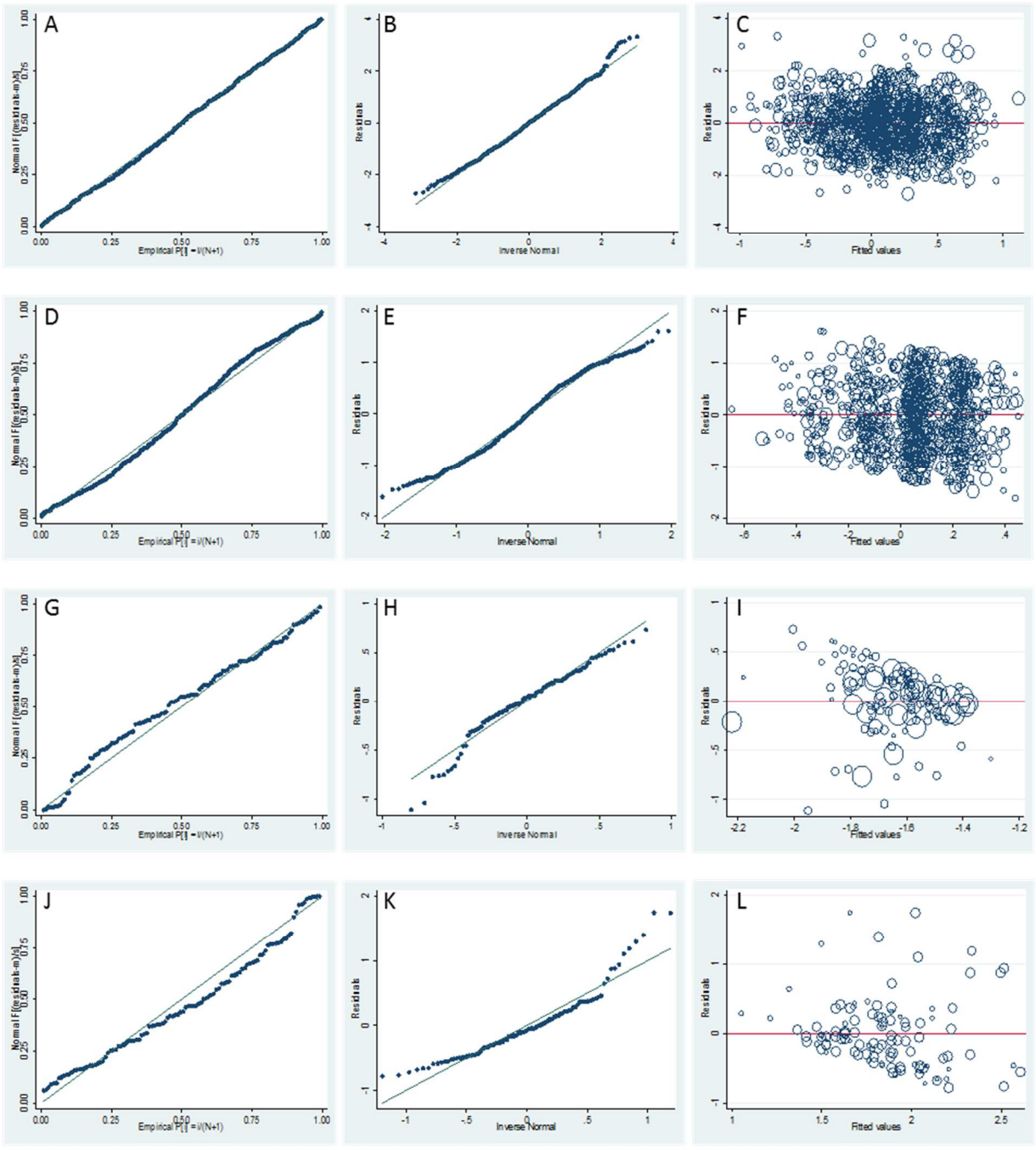


Figure 4.4 Examinations of the assumptions – normality and homoscedasticity for the final models: birth weight Z-score vs. maternal serum UA in overall subcohort as well as AGA, SGA, and LGA groups. Note: panels A, B, C: for overall subcohort; panels D, E, F: for AGA subgroup; panels G, H, I: for SGA subgroup; panels J, K, L: for LGA subgroup. Panels A, D, G, J: for normality of the data in the middle range; Panels B, E, H, K: for normality of the data near the tails. Panels C, F, I, L: for homoscedasticity (residuals vs. fitted values).

Similarly, the variance was almost equal in the final models for overall subcohort and AGA group, but both SGA and LGA models had a significant heteroscedasticity (Figure 4.4-C, F, I, L). The linearized parameters' standard errors in all four final models were not significantly affected with the robust bootstrap estimation of variance (Table 4.4, Figure 4.3B, D, F, H). In addition, the effect of uric acid on birth weight Z-score in the AGA subgroup was still non-significant after the bootstrap variance estimation (SE=0.04 and p=0.15 for linearized variance estimation; SE=0.04 and p=0.16 for bootstrap variance estimation) (Table 4.4), further suggesting that maternal serum uric acid was not associated with birth weight Z-score in this subgroup.

Table 4.5 Ten-fold cross-validation for the final models: birth weight Z-score vs. maternal serum UA in overall subcohort as well as SGA and LGA groups.

RMSE	**Model**$_{\text{(Overall: BW\_ZS vs. UA)}}$	**Model**$_{\text{(SGA: BW\_ZS vs. UA, k=4.1)}}$	**Model**$_{\text{(LGA: BW\_ZS vs. UA)}}$
10- or 5-fold cross-validation:			
RMSE1	1.01	0.30	0.67
RMSE2	1.16	0.34	0.56
RMSE3	0.97	0.48	0.56
RMSE4	0.95	0.29	0.49
RMSE5	0.91	0.33	0.37
RMSE6	0.93	na	na
RMSE7	0.95	na	na
RMSE8	0.91	na	na
RMSE9	0.96	na	na
RMSE10	0.94	na	na
Average $RMSE_{\text{(cross-validation)}}$	0.97	0.35	0.53
$RMSE_{\text{(full sample)}}$	0.96	0.34	0.51
ΔRMSE	0.01	0.01	0.02
% Change in RMSE	1.04	2.86	3.92

Due to the non-significance of uric acid associated with birth weight Z-score in the AGA group, 10-fold cross-validation was conducted only for the final models in overall subcohort and SGA/LGA groups and the results indicated that the over-fitting percentage was less than 4% (1.04% for overall subcohort, 2.86% for SGA group, and 3.92% for LGA group) (Table 4.5).

Excluding the pregnant women who had been diagnosed with GH and PE did not significantly change the direction and effect size of parameters in all models (Table 4.6, Figure 4.5), suggesting that the fitted final models were relatively robust regardless of the inclusion of the women with GH and PE.

Table 4.6 Sensitivity analysis for birth weight Z-score vs. maternal serum UA in overall subcohort as well as AGA, SGA, and LGA groups after removing the women who had the diagnosed GH and PE.

		Linearized		Bootstrap	
	Coef.	SE	p	SE	p
Overall:					
Intercept	-0.26	0.20	0.21	0.21	0.22
Uric acid	0.12	0.06	0.06	0.06	0.06
Maternal race	-0.48	0.07	<0.01	0.07	<0.01
Maternal age	0.31	0.09	<0.01	0.09	<0.01
Underweight before pregnant	-0.34	0.19	0.07	0.19	0.07
Parity	0.21	0.08	0.01	0.08	0.01
Smoke during pregnancy	-0.53	0.10	<0.01	0.09	<0.01
Alcohol during pregnancy	0.14	0.09	0.14	0.09	0.11
AGA:					
Intercept	-0.13	0.14	0.35	0.14	0.33
Uric acid	0.08	0.43	0.07	0.04	0.06
Maternal race	-0.26	0.05	<0.01	0.06	<0.01
Maternal age	0.19	0.07	0.01	0.06	<0.01
Underweight before pregnant	-0.31	0.14	0.03	0.13	0.01
Smoke during pregnancy	-0.23	0.07	<0.01	0.07	<0.01
Alcohol during pregnancy	0.12	0.07	0.10	0.07	0.11
SGA:					
Intercept	2.05	0.22	<0.01	0.22	<0.01
UA<4.10 mg/dL	-0.17	0.07	0.02	0.07	0.01
UA>4.10 mg/dL	1.13	0.25	<0.01	0.23	<0.01
Medicaid	0.22	0.07	<0.01	0.07	<0.01
Alcohol during pregnancy	-0.19	0.05	<0.01	0.05	<0.01
LGA:					
Intercept	1.06	0.29	<0.01	0.27	<0.01
Uric acid (mg/dL)	0.19	0.09	0.04	0.09	0.03
Age	0.29	0.12	0.01	0.11	0.01
Obesity	0.33	0.13	0.01	0.12	0.01
SMK	-0.39	0.10	<0.01	0.10	<0.01

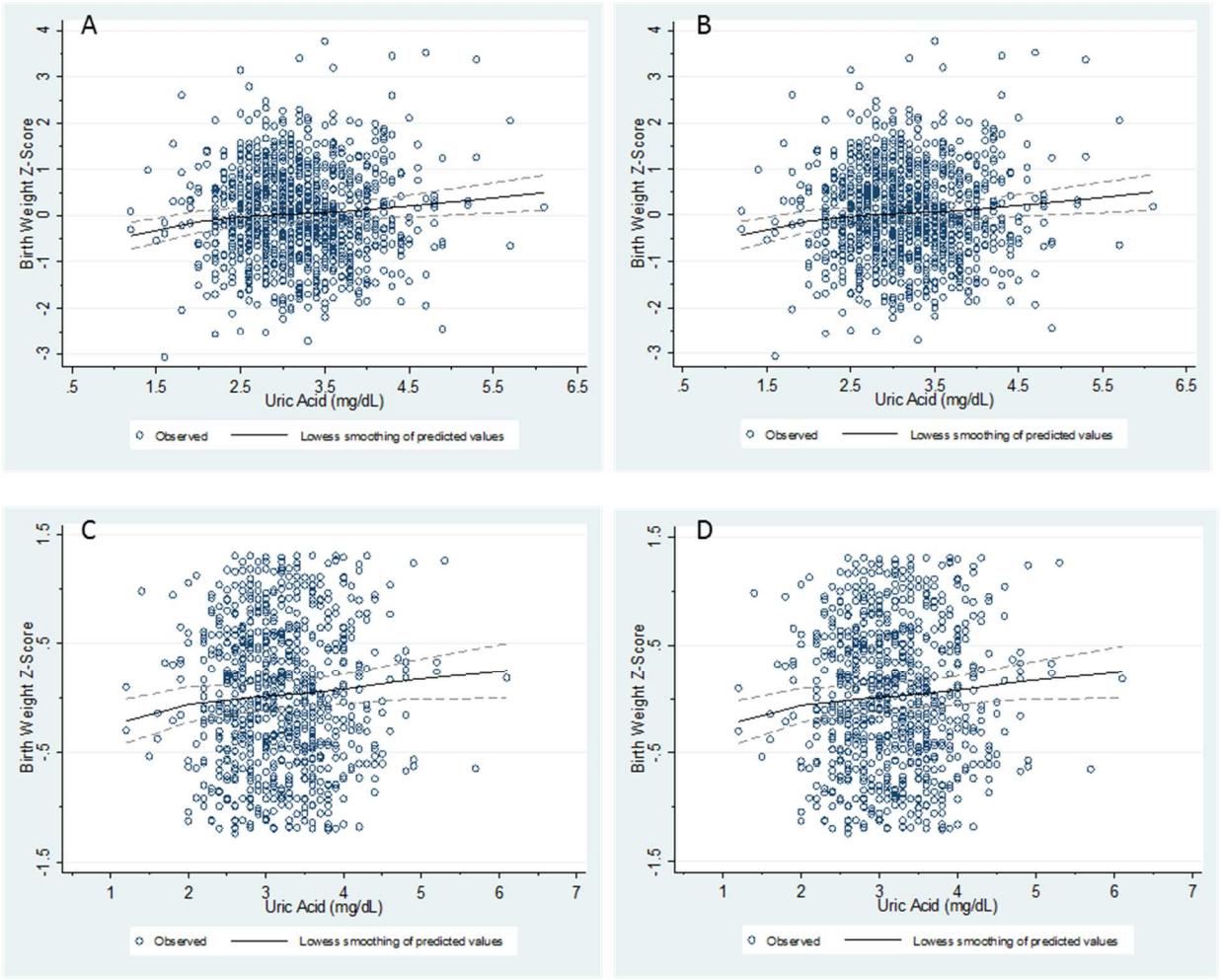
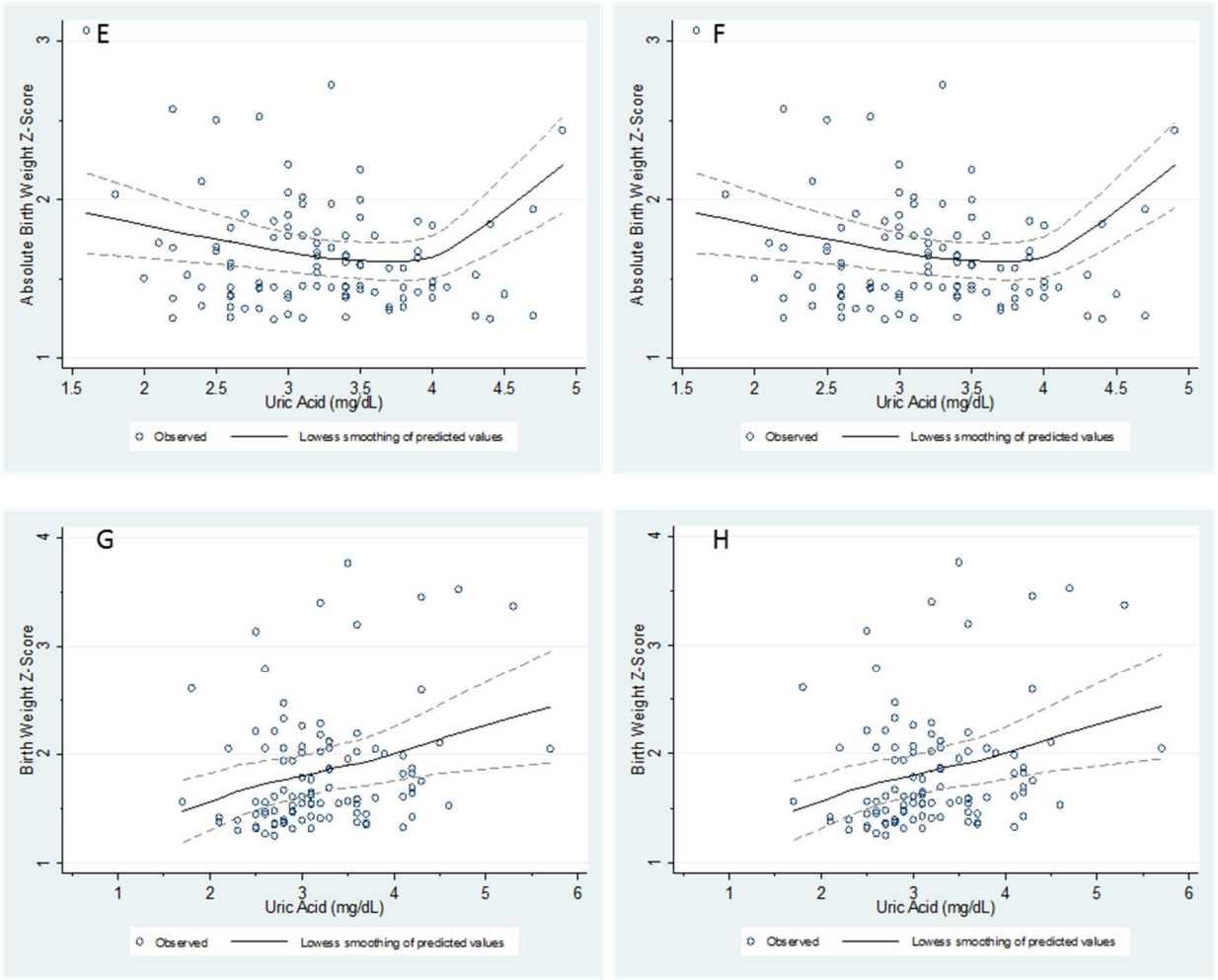


Figure 4.5 Visualization of sensitivity analysis for birth weight Z-score vs. maternal serum UA after removing the women who had diagnosed GH and PE. Note: panels A, C, E, G: linearized estimates of variance; panels B, D, F, H: Bootstrapped estimates of variance. Panel A & B: for overall subcohort; panel C & D: for AGA subgroup; panel E & F: for SGA subgroup; panel G & H: for LGA subgroup. The solid lines represent point estimates and the upper and lower dashed lines indicate 95% confidence bands by nonparametric smoothing with locally weighted regression (lowess).

Figure 4.5 (cont'd)



4.4 Discussion

We found that there is heterogeneity in the relationship between birth weight Z-score and maternal serum uric acid concentration across AGA, SGA, and LGA subgroups in our study population. A J-shaped relationship existed between absolute birth weight Z-score and maternal serum uric acid in mid-pregnancy with a breakpoint at the 4.10 mg/dL in the SGA group, after adjusting for maternal Medicaid insurance status and maternal alcohol drinking during pregnancy as confounders. A significantly positive linear trend was found for the relationship between maternal serum uric acid and birth weight Z-score in the LGA group with an adjustment of the confounders including maternal age, maternal pre-pregnancy obesity, and maternal

smoking during pregnancy. In contrast, birth weight Z-score was not associated with maternal serum uric acid in the AGA group.

Studies have shown that maternal serum oxidative stress was associated with both LBW (Nakatsukasa et al., 2013; Weber et al., 2014) and macrosomia (Grissa et al., 2007; Yessoufou and Moutairou, 2011). On the other hand, as discussed in chapter 3, both low and high UA may increase maternal serum oxidative stress. Thus, taken together, both low and high maternal serum UA may be associated with extremes in birth weight Z-score in SGA infants by uric acid-related oxidative stress.

The mechanism underlying such a J-shaped birth weight Z score-maternal uric acid relationship for SGA infants is unclear. Both low and high maternal serum uric acid may accompany maternal vascular lesions in placenta, which consequently increase the risk of SGA infants. Evidence has also shown that nutrient (including amino acids, glucose, fatty acids, and cholesterol) transport from mother to fetus in the placenta was reduced during intrauterine growth restriction (IUGR) (Brown et al., 2011; Lager and Powell, 2012; Brett et al., 2014). Thus, it could be that both low and high maternal serum uric acid decreased the birth weight Z-score in SGA infants via the interruption of nutrient transport in placenta caused by uric acid-related oxidative stress and/or oxidative stress-related maternal vascular lesions in placenta. Another mechanism could be related to the inhibition of mTOR (a nutrient sensor) signaling by uric acid-induced oxidative stress and consequently resulting in the decrease of amino acid transport from mother to fetus based on a link between oxidative stress markers and mTOR signaling (Chen et al., 2010; Martino et al., 2016).

With respect to LGA infants, uric acid-oxidative stress might increase birth weight in LGA infants by decreasing maternal and fetal ghrelin (a hormone with a major influence on

energy balance). Several lines of evidence support such a hypothesis: 1) there were strong positive correlations between maternal and cord plasma biomarkers of oxidative stress and negative correlations between these biomarkers and maternal or cord plasma ghrelin concentrations (Luo et al., 2015); and 2) women with GDM (a major risk factor for macrosomia) had lower umbilical ghrelin (Gomez-Diaz et al., 2016; Karakulak et al., 2016) that was inversely associated with birth weight (Karakulak et al., 2016).

High/low UA-related maternal placental vascular lesions might be a contributing factor to reducing the transfer of nutrients from mother to the SGA fetus. However, for the LGA fetus, high UA-related oxidative stress might mainly function as a signal to trigger or inactivate the relevant signaling pathways and thus contribute to fetal overgrowth while low UA-related oxidative stress may not play a significant role. Few pregnant women with an LGA infant had blood uric acid concentrations below 2.1 mg/dL. More studies with a large cohort of LGA infants are needed to further clarify the phenomenon that we observed in the current study.

Interestingly, we found that SGA infants had a J-shaped birth weight Z score-maternal UA pattern with a higher breakpoint value (4.10 mg/dL of UA). It could be that SGA infants prefer a higher maternal serum uric acid, suggesting that the normal range of maternal serum uric acid concentration in women carrying an SGA fetus should be studied further. Our results also indicated that the analysis of an association between birth weight Z-score and maternal serum uric acid using the overall subcohort is less informative and the stratification of the overall subcohort into AGA, SGA, and LGA groups is essential. This heterogeneity may be useful to consider in analyses of other biomarkers that are potentially associated with birth weight in a non-linear way.

With respect to the non-significant relationship between maternal serum uric acid and birth weight Z-score in AGA infants, it may be related to that mothers who had an AGA fetus may have a better health status and thus a better anti-oxidant capacity for buffering the effects of both low and high maternal serum uric acid in pregnancy. This interpretation is supported by a study, in which AGA infants and their mothers had higher plasma total antioxidant activity (TAC) and vitamin C and E concentrations, compared to their SGA and LGA counterparts (Saker et al., 2008).

To the best of our knowledge, this is the first report regarding a non-linear relationship between maternal serum uric acid and birth weight Z-score in SGA infants and a positive linear relationship between them in LGA infants. Previous studies showed that elevated maternal serum uric acid increased the risk of SGA infant in pregnant women with gestational hypertension (Bellomo et al., 2011) or was negatively associated with infant birth weight in normotensive pregnant women (Akahori et al., 2012). However, these studies had small sample sizes and used simple linear or logistic regressions based on assumptions of normal distribution of the residuals, equal variance (i.e., homoscedasticity), and a linear relationship between infant birth weight or the risk of SGA infant and maternal uric acid; these assumptions may not hold up. On the other hand, to date, no other studies have been found that address the relationship between maternal serum uric acid and birth weight Z-score in LGA infants.

In addition to the strengths that have been discussed in chapters 3, the major strength in the present study was that birth weights were standardized as Z-scores specifically for the corrected gestational age and sex, and thus, minimized the measurement error of outcome and increased the statistical power.

Similar to the discussion in chapter 3, our present study has some limitations such as inability to verify time order between exposure and outcomes, possible common underlying pathology in pregnancy associated with both UA and outcomes, unmeasured confounding variable, e.g., diet, one time measurement of maternal serum uric acid concentration, possible residual confounding from the dichotomization of maternal age, and relatively small proportion of the women who had SGA or LGA infants.

In conclusion, there was heterogeneity in the relationship between birth weight Z-score and maternal serum uric acid concentration across AGA, SGA, and LGA groups in our study. Specifically, the association between maternal serum uric acid concentration and birth weight Z-score was J-shaped in SGA infants, positively linear in LGA infants, and non-significant in AGA infants. Our findings may provide clues: 1) to guide the study of biological mechanisms underlying the non-linear and the positive linear relationships between maternal serum uric acid and birth weight Z-score in SGA and LGA infants, respectively; 2) to motivate a re-evaluation of the normal range of maternal serum uric acid concentration in mid-pregnancy for women who are carrying a SGA fetus; 3) to consider maternal serum uric acid in pregnancy as a marker along with ultrasound and/or other measurements to predict the progression of fetal growth during pregnancy; and 4) to explore maternal UA as a target for early intervention for SGA and LGA fetuses.

CHAPTER 5: A J-SHAPED RELATIONSHIP BETWEEN MATERNAL SERUM URIC ACID IN MID-PREGNANCY AND MATERNAL VASCULAR LESIONS IN PLACENTA

5.1 Introduction

In chapter 3 and 4, we found that maternal serum uric acid was associated with maternal blood pressure and with birth weight Z-score in SGA and LGA infants. Because placental pathology has been shown to be related to both maternal blood pressure (Jain et al., 2007; Furuya et al., 2008; Krielessi et al., 2012; Salmani et al., 2014; Nahar et al., 2015) and fetal growth (Krishna and Bhalerao, 2011; Vedmedovska et al., 2011; Ouyang et al., 2013; Mifsud and Sebire, 2014), we hypothesized that maternal serum uric acid might also be associated with placental pathology.

Placenta is formed from a fertilized egg (Benirschke et al., 2012). It is an interface between mother and fetus and plays a critical role in regulating nutrient supply to the fetus and producing hormones that control both the fetal and the maternal metabolisms (Illsley, 2011; McNamara & Kay, 2011). Thus, placenta can not only create a protective and stable environment to the fetus under the normal condition, but also present an adverse intrauterine environment to the fetus through mediating harmful environmental exposures and/or various maternal health conditions.

To date, no studies on the relationship between maternal serum uric acid and placental pathology in humans have been found. An in vitro trophoblast cell model study revealed that uric acid-induced a concentration-dependent reduction of trophoblast invasion and integration into a uterine microvascular endothelial cell monolayer (Bainbridge et al., 2009). In the same study, pooled serum from women with PE decreased the ability of trophoblast cells' integration into the endothelial cell monolayers compared with pooled serum from healthy controls, and this

response was partially rescued when endogenous uric acid was previously removed with uricase, suggesting that elevated serum uric acid in women with PE contributed to the pathogenesis of PE, at least, via decreasing normal trophoblast invasion and spiral artery vascular remodeling (Bainbridge et al., 2009). Other indirect evidence was from an in vivo study in which antenatal serum uric acid was significantly higher in women with placentas that had 8OHdG (an oxidative stress biomarker) positive immunostaining from pregnancy-induced hypertension (PIH) (Fukushima et al., 2011). This in vivo study implicates connections among maternal serum uric acid, placental oxidative stress, and gestational hypertension.

Thus, in the present study, based on our findings in the chapters 3 and 4, we aimed to test whether placental vascular lesions were associated with maternal serum uric acid concentration in a non-linear way using data from the POUCH cohort study.

5.2 Materials and Methods

5.2.1 Study population and sampling

As described in chapter 3, we used the POUCH cohort as our study population that was composed of 3,019 pregnant women enrolled in the 16th-27th week of pregnancy in Michigan (Holzman et al., 2013). The women who had missing placental measurements (n=198), birth weight Z-score (n=2), and maternal serum uric acid concentration during pregnancy (n=48), or had diagnosed chronic hypertension (n=38) and renal diseases (n=14) were excluded from a subcohort of 1371 participants and finally about 1071 pregnant women were included for the final analysis (Figure 5.1). The re-calculation of the sampling weights was based on such specific inclusions. The IRB approval was obtained from the Michigan State University for the POUCH

Cohort Study and all data used in the current studies were de-identified according to the HIPAA Security Guidelines.

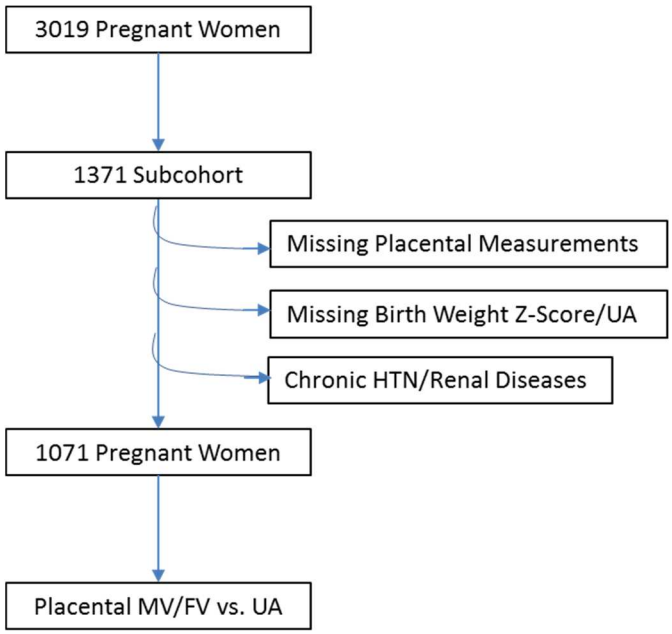


Figure 5.1 A flowchart to indicate the selection of the participants in the POUCH cohort for the current study in Chapter 5. Note: UA=uric acid, MV=maternal vascular constructs, FV=fetal vascular constructs.

5.2.2 Measurement of placental vascular lesions

The POUCH placental measurements were described by Kelly et al (2009). Briefly, placentas were formalin-fixed. The central tissues with or without grossly abnormal findings were sampled followed by paraffin-embedded, sectioned, and stained with hematoxylin and eosin for microscopic assessment by a pathologist who was blinded to gestational age at delivery, all clinical data, and gross examination findings. Placental pathological findings from a computer-based data collection instrument were categorized into 5 constructs: 1) maternal vascular–obstructive (MV-O) (evidence of major placental disc infarcts and decidual vessel atherosis); 2) maternal vascular–disturbance of integrity (MV-I) (evidence of retroplacental hemorrhage and bleeding in the decidua; 3) maternal vascular–developmental (MV-D) (evidence

of abnormal or incomplete trophoblast remodeling of maternal spiral arteries); 4) fetal vascular–obstructive (FV-O) (evidence of large and small fetal vessel obstruction); and 5) fetal vascular–disturbance of integrity (FV-I) (evidence of abnormalities of fetal villous blood flow such as fetal-to-maternal hemorrhage). The findings used to reliably capture these constructs were determined by 1) literature review; 2) factor analysis of our own data; and 3) elimination of findings that showed poor reliability, i.e., kappa<0.40, in an inter-rater reliability study with 10% of placental samples. Each of the remaining vascular findings within a construct was scored by evaluating the frequency with which it appeared within a placenta. An overall distribution of frequencies of positive samples per placenta across women was dichotomized as above (high) and below (not high) the top quintile. All of these dichotomized vascular findings within each of the 5 constructs were summed for each placenta. A construct-specific score was further dichotomized based on a uniform cut-point for "high" at the top 12^{th}-21^{st} percentiles (except for the construct FV-I, which was cut at the top 36^{th} percentile). The typical frequency distributions of such construct scores were determined by using data from women with term delivery and normal maternal serum α-fetoprotein levels. The reliabilities of the 5 constructs were evaluated using a 10% of random samples and all vascular constructs had a range of moderate to excellent concordances (κ=0.42 for MV-O, 0.62 for MV-I, 0.48 for MV-D, 0.66 for FV-O, 0.64 for FV-I). Because placentas often have more than one maternal or fetal vascular 'construct' and POUCH Study women who had more than one construct in their placentas were at greater risk of adverse outcomes (Kelly et al., 2009), we generated two new multinomial variables with three levels – maternal and fetal vascular lesions (MV and FV) by recoding 5 constructs as follows: 1) MV=0 if none of three maternal constructs (MV-O, MV-I, and MV-D) was "high", MV=1 if one of them was "high", and MV=2 if at least two of them were "high"; 2) FV=0 if none of two fetal

constructs (FV-O and FV-I) was “high”, FV=1 if one of them was “high”, and FV=2 if two of them were “high”.

5.2.3 Measurements of independent variables – maternal serum uric acid concentration and potential confounding covariates

As described in chapter 3, at the time of the enrollment, maternal serum uric acid (mg/dL) was measured using the method developed by Fossati et al (1980) and covariates including maternal age at the enrollment, maternal race/education/Medicaid/obesity before pregnancy, parity, and maternal smoking and alcohol drinking during pregnancy were self-reported. The LMP or ultrasound method was used to estimate gestational age at the enrollment.

5.2.4 Data management and statistical analysis

Two placental variables including MV and FV were presented as weighted percentage. Maternal serum uric acid concentration was expressed as mean and standard error. All covariates were categorized as binary and presented as weighted percentages (Table 5.1). A t-test (for continuous variable) or chi-square test (for discrete variable) was used to examine whether an outcome or exposure was associated with individual potential confounding variable.

The relationship between the observed risk of each placental variable (MV or FV) and maternal serum uric acid concentration that had been divided into three tertiles was plotted as a histogram to show whether the relationship is linear or non-linear. For the linear trend, a classical logistic regression model was used; and for the non-linear one, a restricted cubic spline (RCS) with a logistic regression model was applied. Further analyses were terminated if the results from both linear and non-linear analyses were not significant.

A process of model selection was applied for generating a final model followed by a 10-fold cross-validation to evaluate the overfitting problem. To obtain a robust variance estimation

for the parameters in the final model, a bootstrap procedure with sampling weights and 1000 bootstrap replications for complex survey data was applied. A sensitivity analysis was carried out with the final model by excluding women who had diagnosed GE or PE. The statistical significance level, α, was set as 0.05 for a two-sided test. SAS v9.4 (SAS Institute, Cary, North Carolina) was used for data management and STATA v13.0 (StataCorp LP, College Station, Texas) for all statistical analyses with the POUCH sampling weights.

5.3 Results

5.3.1 Study population and maternal characteristics

Figure 5.1 showed that our study population was a cohort of 3019 pregnant women, which was composed of 24.6% pregnant women who were African-American, 28.5% with age beyond 30 years, 17.8% with education less than 11^{th} grade, 25.0% with pre-pregnancy obesity, 48.6% with Medicaid insurance, 58.1% with 1 or more parities, 16.9% and 17.4% with smoking alcohol drinking during pregnancy, respectively, and 14.1% with gestational age at enrollment less than 20 weeks (Table 5.1). Table 5.1 also indicated that of all included covariates, only maternal alcohol drinking during pregnancy had a marginally significant relationship with both MV and FV and only maternal pre-pregnancy obesity and Medicaid significantly with maternal uric acid.

Table 5.1 Maternal characteristics and distributions of MV/FV and maternal serum UA in the subcohort (N=1071, weighted).

		MV				FV				Uric Acid	
Variable	**N (%)**	**0**	**1**	**2**	**p**	**0**	**1**	**2**	**p**	**Mean (SE)**	**p**
Maternal Race:											
Non-Black	659 (75.4)	425 (66.6)	176 (25.8)	58 (7.6)	0.01	273 (43.8)	313 (46.2)	73 (10.0)	<0.01	3.22 (0.03)	0.90
Black	412 (24.6)	239 (14.0)	138 (8.4)	35 (2.3)		228 (55.8)	155 (37.2)	29 (7.0)		3.21 (0.03)	
Maternal Age:											
<30	783 (71.5)	478 (63.9)	233 (27.4)	72 (8.7)	0.55	381 (49.2)	341 (41.9)	71 (9.0)	0.12	3.24 (0.03)	0.20
≥30	288 (28.5)	186 (64.9)	81 (28.7)	21 (6.4)		120 (40.5)	137 (49.4)	31 (10.1)		3.17 (0.04)	
Maternal Education:											
12-17	844 (82.2)	540 (65.6)	234 (26.9)	70 (7.5)	0.18	385 (45.0)	376 (45.5)	83 (9.5)	0.10	3.22 (0.03)	0.85
7-11	227 (17.8)	124 (57.7)	80 (32.0)	23 (10.3)		116 (54.7)	92 (37.2)	19 (8.1)		3.21 (0.06)	
Maternal Pre-Pregnancy Obesity:											
no	779 (75.0)	493 (65.4)	220 (26.2)	66 (8.4)	0.23	373 (47.9)	335 (43.5)	71 (8.7)	0.45	3.11 (0.03)	0.00
yes	292 (25.0)	171 (60.6)	94 (32.5)	27 (6.9)		128 (43.3)	133 (45.5)	31 (11.2)		3.53 (0.05)	
Maternal Medicaid Insurance Status:											
no	487 (51.4)	312 (64.9)	133 (28.1)	42 (7.0)	0.54	217 (43.9)	214 (44.8)	56 (11.2)	0.10	3.14 (0.03)	0.00
yes	583 (48.6)	352 (63.5)	180 (27.3)	51 (9.1)		284 (49.7)	253 (43.1)	46 (7.2)		3.30 (0.04)	
Parity:											
0	451 (41.9)	275 (64.7)	140 (28.1)	36 (7.3)	0.81	214 (46.4)	196 (43.1)	41 (10.5)	0.65	3.17 (0.04)	0.14
≥1	619 (58.1)	389 (63.9)	173 (27.5)	57 (8.5)		287 (47.0)	271 (44.6)	61 (8.4)		3.25 (0.03)	

Table 5.1 (cont'd)

		MV				FV				Uric Acid	
Variable	**N (%)**	**0**	**1**	**2**	**p**	**0**	**1**	**2**	**p**	**Mean (SE)**	**p**
Maternal Smoke During Pregnancy:											
no	883 (83.1)	543 (64.3)	261 (27.2)	79 (8.5)	0.40	406 (46.3)	389 (43.9)	88 (9.8)	0.43	3.21 (0.03)	0.55
yes	188 (16.9)	121 (63.6)	53 (30.6)	14 (5.8)		95 (48.9)	79 (44.5)	14 (6.6)		3.25 (0.06)	
Maternal Alcohol Drinking During Pregnancy											
no	878 (82.6)	532 (62.8)	264 (28.4)	82 (8.8)	0.06	414 (46.6)	374 (43.3)	90 (10.1)	0.05	3.22 (0.03)	0.55
yes	183 (17.4)	127 (71.5)	45 (23.9)	11 (4.6)		83 (48.4)	89 (46.9)	11 (4.6)		3.18 (0.07)	
Gestational Age at Enrollment:											
≥20wk	909 (85.9)	564 (64.6)	271 (28.1)	74 (7.4)	0.36	429 (47.4)	396 (43.2)	84 (9.3)	0.57	3.23 (0.03)	0.08
<20wk	162 (14.1)	100 (61.9)	43 (26.1)	19 (12.0)		72 (42.3)	72 (48.6)	18 (9.1)		3.12 (0.06)	

5.3.2 Co-occurrence of two or more types of maternal vascular lesions in placenta had a J-shaped relationship with maternal serum uric acid concentration

The histograms plotted with the observed risks (unweighted) of placental vascular lesions (MV and FV) against maternal serum uric acid in Figure 5.2 demonstrated that a non-linear relationship might exist between maternal serum uric acid and the observed risks of MV=1, MV=2, and FV=2. Thus, a non-linear logistic regression such as restricted cubic spline with a logistic regression was applied.

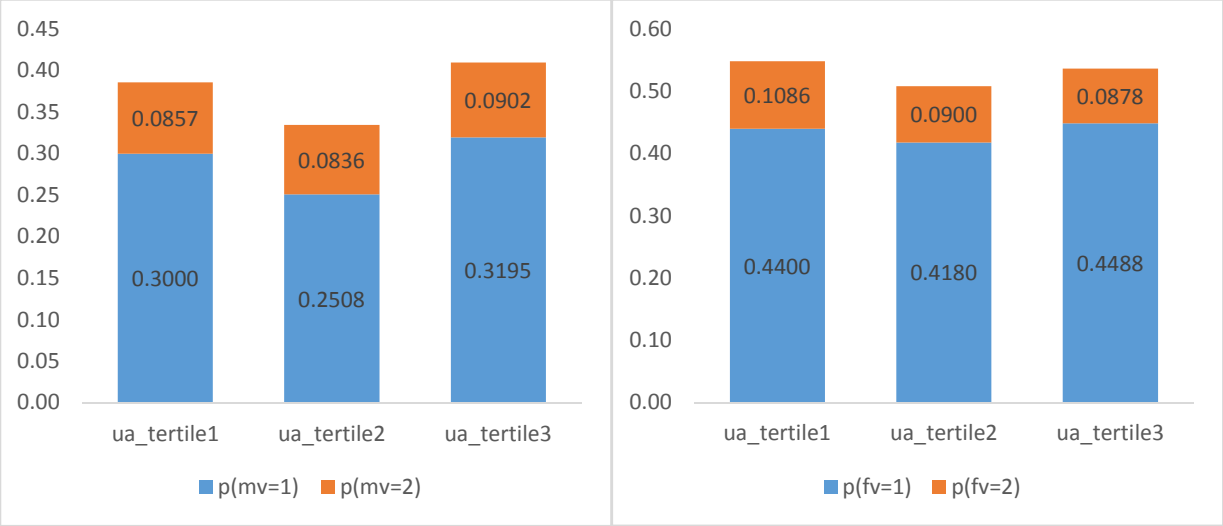


Figure 5.2 Distributions of the observed risks of MV or FV=1 and MV or FV=2 within the tertiles of uric acid concentration. Note: left panel: MV, right panel: FV.

Table 5.2 summarized the processes of model selections for MV and FV vs. maternal serum uric acid. For unadjusted model for MV vs. UA, the first spline covariate (rc1) for MV=2 vs. MV=0 was marginally significant and negative (β=-0.75, SE=0.47, p=0.11) and the second spline covariate (rc2) was significant and positive (β=1.17, SE=0.57, p=0.04) (Table 5.2). However, the relationship between the risk of MV=1 and maternal serum uric acid was not significant (β=-0.06, SE=0.30, p=0.84 for rc1; β=0.15, SE=0.38, p=0.69 for rc2) (Table 5.2).

Inclusion of all covariates did not significantly change the associations of exposure with the risk of MV at two levels (Table 5.2) and only the contribution of alcohol drinking in pregnancy to the final model for MV=2 vs. MV=0 was marginally significant (β=-0.80, SE=0.40, p=0.06) (Table 5.2, Table 5.3).

Table 5.2 Model selections for MV/FV vs. maternal serum UA using RCS with the number of knots=3 followed by a multinomial logistic regression (weighted).

	Unadjusted Model		Full Model		Final Model	
Parameter	**Coef. (SE)**	**p**	**Coef. (SE)**	**p**	**Coef. (SE)**	**p**
$MV_{1\ vs\ 0}$:						
Intercept	-0.69(0.85)	0.42	-0.62(0.87)	0.48	-0.54 (0.85)	0.52
rc1	-0.06 (0.30)	0.84	-0.11 (0.30)	0.71	-0.10 (0.30)	0.74
rc2	0.15 (0.38)	0.69	0.23(0.38)	0.55	0.20 (0.38)	0.61
Race (Black vs. Non-Black)	na	na	0.51 (0.17)	<0.01	na	na
Age (≥30 vs. <30)	na	na	0.14 (0.21)	0.50	na	na
Education (7-11th vs. ≥12th)	na	na	0.26(0.22)	0.24	na	na
Pre-pregnancy underweight (Yes vs. No)	na	na	-0.51(0.41)	0.22	na	na
Medicaid (Yes vs. No)	na	na	-0.24 (0.21)	0.25	na	na
Parity (≥1 vs. 0)	na	na	-0.01 (0.17)	0.96	na	na
Smoke during pregnancy (Yes vs. No)	na	na	0.17 (0.24)	0.48	na	na
Alcohol during pregnancy (Yes vs. No)	na	na	-0.32 (0.23)	0.16	-0.31 (0.23)	0.19
GA_Visit (<20 vs. >20)	na	na	-0.07 (0.25)	0.78	na	na
$MV_{2\ vs\ 0}$:						
Intercept	-0.16 (1.30)	0.91	-0.34 (1.32)	0.80	0.03 (1.29)	0.98
rc1	-0.75 (0.47)	0.11	-0.73 (0.46)	0.12	-0.77 (0.46)	0.10
rc2	1.17 (0.57)	0.04	1.17 (0.58)	0.04	1.20 (0.57)	0.04
Race (Black vs. Non-Black)	na	na	0.14 (0.30)	0.65		
Age (≥30 vs. <30)	na	na	-0.15 (0.37)	0.68	na	na
Education (7-11th vs. ≥12th)	na	na	0.41 (0.32)	0.20	na	na
Underweight before pregnancy (Yes vs. No)	na	na	-0.05 (0.73)	0.95	na	na
Medicaid (Yes vs. No)	na	na	0.07 (0.35)	0.84	na	na
Parity (≥1 vs. 0)	na	na	0.20 (0.29)	0.49	na	na
Smoke during pregnancy (Yes vs. No)	na	na	-0.49 (0.37)	0.18	na	na
Alcohol during pregnancy (Yes vs. No)	na	na	-0.68 (0.43)	0.12	-0.80 (0.42)	0.06
GA_Visit (<20 vs. >20)	na	na	0.49 (0.33)	0.14	na	na

Table 5.2 (cont'd)

Parameter	Unadjusted Model		Full Model		Final Model	
	Coef. (SE)	p	Coef. (SE)	p	Coef. (SE)	p
$FV_{1\ vs\ 0}$:						
Intercept	-0.46 (0.78)	0.55	-0.38 (0.82)	0.64	-0.46 (0.78)	0.56
rc1	0.13 (0.28)	0.65	0.11 (0.29)	0.71	0.12 (0.28)	0.66
rc2	-0.03 (0.36)	0.94	0.001 (0.37)	1.00	-0.02 (0.36)	0.96
Race (Black vs. Non-Black)	na	na	-0.39 (0.17)	0.02	na	na
Age (≥30 vs. <30)	na	na	0.27 (0.20)	0.18	na	na
Education (7-11th vs. ≥12th)	na	na	-0.25 (0.21)	0.23	na	na
Underweight before pregnancy (Yes vs. No)	na	na	-0.50 (0.41)	0.22	na	na
Medicaid (Yes vs. No)	na	na	0.09 (0.19)	0.64	na	na
Parity (≥1 vs. 0)	na	na	-0.06 (0.16)	0.73	na	na
Smoke during pregnancy (Yes vs. No)	na	na	0.06 (0.22)	0.77	na	na
Alcohol during pregnancy (Yes vs. No)	na	na	-0.04 (0.21)	0.86	0.05 (0.21)	0.82
GA_Visit (<20 vs. >20)	na	na	0.27 (0.22)	0.22	na	na
$FV_{2\ vs\ 0}$:						
Intercept	-1.95 (1.25)	0.12	-1.14 (1.24)	0.36	-1.66 (1.23)	0.18
rc1	0.14 (0.45)	0.75	0.03 (0.45)	0.96	0.07 (0.44)	0.87
rc2	-0.36 (0.60)	0.55	-0.16 (0.59)	0.79	-0.26 (0.59)	0.67
Race (Black vs. Non-Black)	na	na	-0.43 ()0.31	0.16	na	na
Age (≥30 vs. <30)	na	na	0.14(0.33)	0.67	na	na
Education (7-11th vs. ≥12th)	na	na	-0.04 (0.39)	0.91	na	na
Underweight before pregnancy (Yes vs. No)	na	na	-0.88 (0.98)	0.37	na	na
Medicaid (Yes vs. No)	na	na	-0.27 (0.32)	0.40	na	na
Parity (≥1 vs. 0)	na	na	-0.31 (0.27)	0.26	na	na
Smoke during pregnancy (Yes vs. No)	na	na	-0.15 (0.41)	0.72	na	na
Alcohol during pregnancy (Yes vs. No)	na	na	-0.90 (0.47)	0.05	-0.82 (0.45)	0.07
GA_Visit (<20 vs. >20)	na	na	0.13 (0.39)	0.73	na	na

In the final model, converting the coefficients for the level of MV=2 into odds ratio (OR), when uric acid concentration was below the breakpoint (i.e., an optimal uric acid value at around 3.1 mg/dL that corresponded to the lowest risk of MV=2 generated by RCS with 3 knots generated), the OR for MV=2 vs. MV=0 increased 2.17 units (i.e., the inverse of OR=0.46 for

the left trend) if UA decreased 1 unit; when UA was beyond the breakpoint, the OR for MV=2 vs. MV=0 increased 3.32 units if UA increased 1 unit (Table 5.3).

Table 5.3 Final models for MV vs. maternal serum UA using RCS (the number of knots=3) followed by multinomial logistic regression (weighted).

	Coef	OR	Linearized			Bootstrap		
			SE for Coef	95% CI for OR	p	SE for Coef	95% CI for OR	p
$MV_{1 \text{ vs } 0}$:								
Intercept	-0.54	-	0.85	-	0.52	0.82	-	0.51
rc1	-0.10	0.90	0.30	0.50, 1.63	0.74	0.29	0.51, 1.60	0.73
rc2	0.20	1.22	0.38	0.57, 2.59	0.61	0.37	0.61, 2.51	0.60
Alcohol	-0.31	0.73	0.23	0.46, 1.16	0.19	0.23	0.47, 1.15	0.17
$MV_{2 \text{ vs } 0}$:								
Intercept	0.03	-	1.29	-	0.98	1.20	-	0.98
rc1	-0.77	0.46	0.46	0.19, 1.15	0.10	0.43	0.20, 1.08	0.08
rc2	1.20	3.32	0.57	1.08, 10.28	0.04	0.54	1.15, 9.58	0.03
Alcohol	-0.80	0.45	0.42	0.20, 1.03	0.06	0.37	0.22, 0.92	0.03

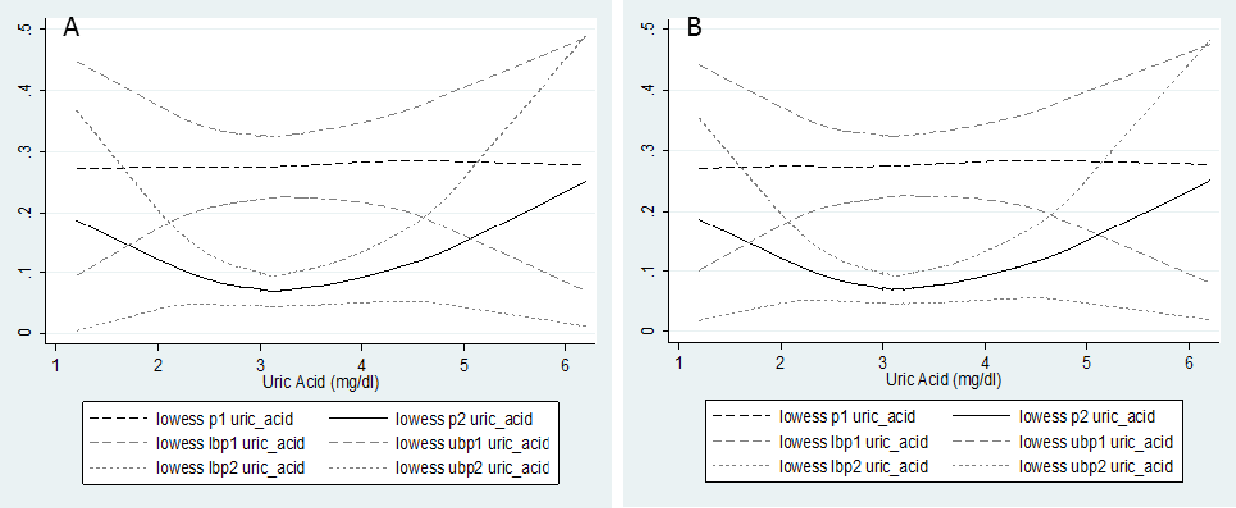


Figure 5.3 Visualization of the final model for MV vs. maternal serum UA. Note: panel A: linearized estimates of variance; panel B: Bootstrapped estimates of variance. The black solid line represents point estimate for MV=2 vs. MV=0 while the black long dashed line represents point estimate for MV=1 vs. MV=0. The upper and lower grey short or long dashed lines indicate 95% confidence bands by nonparametric smoothing with locally weighted regression (lowess) for MV=2 vs. MV=0 and MV=1 vs. MV=0, respectively.

Table 5.4 Sensitivity analysis for the final model MV vs. maternal serum UA after removing the women who had the diagnosed GH and PE using RCS (the number of knots=3) followed by multinomial logistic regression (weighted).

	Coef	OR	Linearized			Bootstrap		
			SE for Coef	95% CI for OR	p	SE for Coef	95% CI for OR	p
$MV_{1\ vs\ 0}$:								
Intercept	-0.73	-	0.89	-	0.42	0.87	-	0.41
rc1	-0.03	0.97	0.32	0.52, 1.80	0.92	0.31	0.53, 1.79	0.92
rc2	0.14	1.15	0.37	0.55, 2.39	0.71	0.36	0.57, 2.34	0.70
Alcohol	-0.42	0.66	0.25	0.40, 1.06	0.09	0.25	0.40, 1.07	0.09
$MV_{2\ vs\ 0}$:								
Intercept	0.16	-	1.40	-	0.91	1.38	-	0.90
rc1	-0.85	0.43	0.51	0.16, 1.17	0.10	0.47	0.17, 1.08	0.07
rc2	1.08	2.94	0.62	0.87, 9.97	0.08	0.57	0.97, 8.94	0.01
Alcohol	-0.61	0.54	0.43	0.23, 1.26	0.15	0.39	0.25, 1.17	0.12

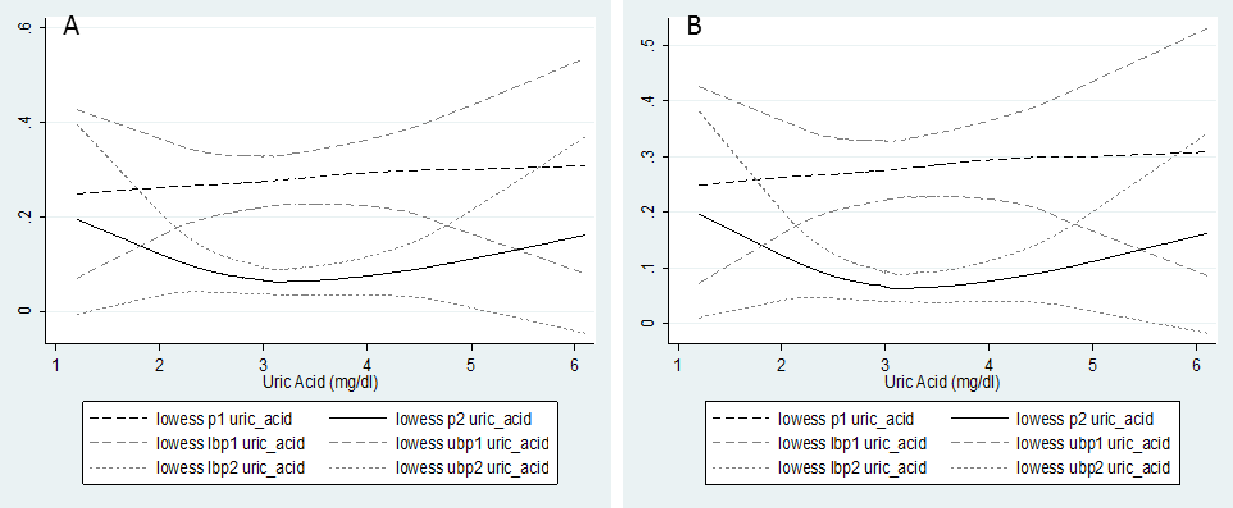


Figure 5.4 Visualization of the final model for MV vs. maternal serum UA after removing women with GH and PE. Note: panel A: linearized estimates of variance; panel B: Bootstrapped estimates of variance. The black solid line represents point estimate for MV=2 vs. MV=0 while the black long dashed line represents point estimate for MV=1 vs. MV=0. The upper and lower grey short or long dashed lines indicate 95% confidence bands by nonparametric smoothing with locally weighted regression (lowess) for MV=2 vs. MV=0 and MV=1 vs. MV=0, respectively.

Using bootstrap variance estimation increased the statistical power and narrowed the 95% confidence intervals for parameters. For example, for MV=2 vs. MV=0, both the first and second spline covariates (rc1 and rc2) had a smaller SE of β (0.43 and 0.54 in bootstrap vs. 0.46 and 0.57 in linearized for rc1 and rc2, respectively) and a narrower 95% CI for OR (0.20-1.08 and 1.15-9.58 in bootstrap vs. 0.19-1.15 and 1.08-10.28 in linearized for rc1 and rc2, respectively)

(Table 5.3, Figure 5.3). In contrast, non-significance of MV=1 vs. MV=0 was not influenced by the bootstrap estimation (Table 5.3, Figure 5.3). In addition, the relationship between FV and maternal uric acid was not significant regardless of inclusion of the covariates and thus terminated for further analysis (Table 5.2). Exclusion of women who had the diagnosed GH and PE did not significantly changed the estimates of parameters and corresponding variance in final model (Table 5.4, Figure 5.4). Ten-fold cross-validation indicated that there was about 9.72% overfitting for the final model of MV vs. UA (Table 5.5).

Table 5.5 Ten-fold cross-validation for the final model: MV vs. maternal serum UA.

RMSE	**Model$_{(MV\ vs.\ UA)}$**
10-fold cross-validation:	
RMSE1	0.64
RMSE2	0.75
RMSE3	0.66
RMSE4	0.55
RMSE5	0.63
RMSE6	0.67
RMSE7	0.66
RMSE8	0.70
RMSE9	0.64
RMSE10	0.62
Average RMSE (cross_validation)	0.72
$RMSE_{(full\ sample)}$	0.65
ΔRMSE	0.07
% Change in RMSE	9.72

Because we grouped all maternal vascular constructs (i.e., MV_O, MV_I, and MV_D) in one score (i.e., MV), separating the overall MV vs. UA into 3 component parts: MV_O vs. UA, MV_I vs. UA, and MV_D vs. UA simply by visualization using a RCS with logistic regression may provide clues about relative contributions of MV_O, MV_I, and MV_D to the overall MV vs. UA pattern. Figure 5.5 showed that when uric acid concentration was below the breakpoint, only MV_O and MV_I constructs contributed to the left-hand side of the breakpoint for MV vs. UA, whereas, when uric acid concentration was beyond the breakpoint, all three maternal

vascular constructs made contributions to the right-hand side of the breakpoint for MV vs. UA. It also indicated that the most contribution to the entire non-linear pattern of MV vs. UA appeared to be from MV_I construct, the second contribution was from MV_O, and the least from MV_D (Figure 5.5) although all relevant models were not statistically significant (data not shown) due to a relatively small sample size and/or large variance of data.

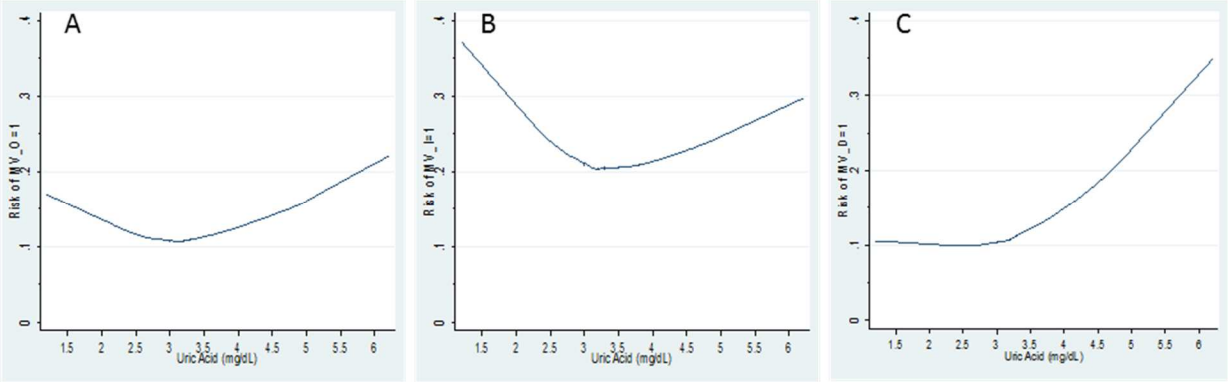


Figure 5.5 Visualization of the relationships between maternal serum UA concentration and single maternal vascular construct in placenta. Note: panel A: MV_O, panel B: MV_I, and panel C: MV_D) with a restricted cubic spline (RCS) regression followed by a logistic regression (weighted); the solid lines represent point estimates while the upper and lower dashed lines indicate 95% confidence bands by nonparametric smoothing with locally weighted regression (lowess).

5.4 Discussion

For the first time, we found that maternal serum uric acid concentration had a J-shaped relationship with the co-occurrence of two or more types of maternal vascular lesions in placenta, but was not associated with fetal vascular lesions in human population.

To date, no other human studies in this area in humans have been found to compare with our current study. However, our result regarding the association of high maternal serum uric acid with the increased risk of more severe maternal vascular lesions in placenta is, at least partially, consistent with an in vitro trophoblast cell model study, in which uric acid was documented to

induce a concentration-dependent reduction of trophoblast invasion and integration into a uterine microvascular endothelial cell monolayer (Bainbridge et al., 2009).

The underlying mechanism of our results is unknown. As discussed in chapter 3, both low and high blood UA may increase maternal serum oxidative stress. Evidence showed that placental oxidative stress is correlated to serum oxidative stress in women with PE (Das et al., 2012). Fukushima et al (2011) found that antenatal serum uric acid was significantly higher in placentas with 8OHdG (an oxidative stress biomarker) positive immunostaining from pregnancy-induced hypertensive women. A connection between placental oxidative stress and placental pathology has also been documented in a malaria infected mice model (Sharma et al., 2012). More recently, the possible complicated mechanisms underlying the relationship between oxidative stress and vasculopathy in placenta has been reviewed (Wu et al., 2015). Thus, the observed J-shaped relationship between maternal serum uric acid and maternal vascular lesions in placenta in our study could be related to low/high UA-induced placental oxidative stress.

Our finding regarding only co-occurrence of two or more types of maternal vascular lesions significantly contributed to such a non-linear relationship indicated that abnormal maternal serum uric acid might be a marker of severe maternal vascular lesions in placenta. Such an interpretation might be partially supported by the findings of the correlation of serum uric acid levels to the severity of primary pulmonary hypertension in patients (Nagaya et al., 1999).

In terms of the finding that fetal vascular lesions in placenta was not significantly associated with maternal serum uric acid, it reinforced that maternal and fetal placental pathologies are not entirely overlapping and biomarkers for each of them would also not necessarily be overlapping. Recently, acute atherosis (a lesion of the spiral arteries characterized by fibrinoid necrosis of the vessel wall) has been reported to be associated with placental lesions

that were consistent with maternal vascular underperfusion, and to a lesser extent, those consistent with fetal vascular thrombo-occlusive disease (Kim et al., 2015).

In chapter 4, we found that maternal serum uric acid had a different effect pattern with birth weight Z-score in SGA and LGA infants, whereas our study in this chapter indicated a non-linear relationship between maternal serum uric acid and placental pathology. Studies have consistently linked placental pathology and poor fetal growth (Althabe et al., 1985; Krishna and Bhalerao, 2011; Vedmedovska et al., 2011; Ouyang et al., 2013; Mifsud and Sebire, 2014). Thus, it seems that placental pathology might play a mediating role in the relationship between maternal serum uric acid and birth weight Z-score in SGA or LGA infants. However, our attempt to examine the relationship between maternal serum uric acid and MV in the SGA subgroup failed due to the sparse data issue (data not shown). It would be worthy of future investigation with a large cohort.

In addition to the strengths that have been discussed in chapters 3 and 4, the major strengths in the present study were that the 5 placental pathological constructs were developed by the pathologist who was blinded to all clinical information as well as the use of the distributions of placental vascular findings from term placentae as reference, and thus, minimizing the possible information bias.

Similarly, in addition to the limitations that were mentioned in chapters 3 and 4, the first major limitation in the present study was that the top quintile of each vascular finding was defined as “high” and the left quintiles as “not high” for each placenta; within these 2 categories there could be variation of risk not captured by dichotomization. The second major limitation was that in MV and FV composite variables, all involved placental constructs (MV-O, MV-I, and MV-D for MV; FV-O and FV-I for FV) were given the same weight; it is possible that one

subtype of vascular lesion has a stronger relation to maternal serum uric acid, which may be supported by our finding regarding different relative contributions to the entire non-linear pattern of MV vs. UA among MV_O, MV_I, and MV_D constructs.

In summary, there was a J-shaped relationship between maternal serum uric acid concentration during pregnancy and co-occurrence of two or more maternal vascular lesions in placenta. Our findings may provide clues to guide the study of biological mechanisms underlying the non-linear relationship between maternal serum uric acid and severe placental pathology; to allow researchers to consider maternal serum uric acid in pregnancy as a marker along with imaging measurements to predict the severity of placental pathology or as a target for early intervention against the progression of placental pathology.

CHAPTER 6: SUMMARY

Gestational hypertension in mother, atypical fetal growth, and placental pathology represent an interactive triad among mother, placenta, and fetus, which has been evidenced by numerous studies. We addressed two major research gaps: a lack of knowledge regarding the associations of low maternal serum uric acid during pregnancy with gestational hypertension in women and fetal growth as well as a lack of research examining the relationship between maternal serum uric acid and placental pathology in literatures.

Our studies aimed to test whether there is a J-shaped relationship between maternal serum uric acid in mid-pregnancy and maternal blood pressure, birth weight Z-score, and placental pathology, respectively. We considered maternal serum uric acid level as a continuous exposure variable and applied a linear spline with multiple linear regression models or a restricted cubic spline with multinomial logistic regression models. The robustness of our results was evaluated and assured by using bootstrap estimation of variance, sensitivity analysis, and 10- or 5-fold cross-validation.

We found a J-shaped relationship existing between maternal serum uric acid in mid-pregnancy and gestational DBP or MAP in pregnant women with a breakpoint of 2.6 mg/dL (for DBP) or 2.7 mg/dL (for MAP) of uric acid, respectively, after adjusting for potential confounding covariates. In contrast, the association of gestational SBP with maternal serum uric acid only followed a positive linear trend with an adjustment for confounders. We also found there is a heterogeneity in the relationship between birth weight Z-score and maternal serum uric acid concentration across AGA, SGA, and LGA subgroups. A J-shaped relationship existed between absolute birth weight Z-score and maternal serum uric acid in mid-pregnancy with a breakpoint at the 4.10 mg/dL of uric acid in the SGA group, after adjusting for confounders

while. In contrast, a positive linear relationship was found between maternal serum uric acid and birth weight Z-score in the LGA group. In addition, birth weight Z-score was not associated with maternal serum uric acid in the AGA group. Finally, we found that in placenta, there was a J-shaped relationship of maternal serum uric acid with maternal vascular lesions, but not with fetal vascular lesions. We proposed that a common mechanism underlying these findings may be related to low/high serum uric acid concentration-relevant oxidative stress.

Our findings may provide clues to guide the study of biological mechanisms underlying the non-linear relationship between maternal serum uric acid and maternal blood pressure, disproportionate fetal growth, and placental pathology; to allow researchers to consider maternal serum uric acid in pregnancy as a marker along with other measurements to predict the progression and/or severity of pregnancy-related health conditions or as a target for early intervention.

The strengths of our studies include: 1) the POUCH Study comprises a large, diverse cohort of pregnant women enrolled from multi-communities with detailed placental measures and many biomarker measurements; 2) the 5 placental pathological constructs were developed by the pathologist who was blinded to all clinical information as well as the use of the distributions of placental vascular findings from term placentae as reference, and thus, minimizing the possible information bias; 3) biological plausibility for the linear or non-linear relationships between outcome and exposure; 4) standardization of birth weights as Z-scores specifically for the corrected gestational age and sex, and thus, minimizing the measurement error of outcome and increased the statistical power; 5) improved internal validity due to the use of 10-fold cross-validation; 6) increased statistical power and reduced information bias due to the application of

non-linear regression models; and 7) enhanced robustness of the results due to the use of both bootstrap variance estimation and sensitivity analysis.

Our studies have some limitations including: 1) we abstracted DBP based on one single highest measurement, which may have measurement error and/or large variance; 2) the time order between exposure and outcomes cannot be determined and an inverse causal relationship is possible; 3) we cannot rule out common underlying pathology in pregnancy associated with both UA and outcomes; 4) one time measurement of maternal serum uric acid concentration; 5) possible residual confounding from the dichotomization of maternal age; 6) relatively small proportion of the participants who had low serum uric acid concentration; 7) diet may influence both maternal serum UA levels and pregnancy complications, thus, there might be unmeasured confounding in our studies; 8) the top quintile of each vascular finding was defined as "high" and the left quintiles as "not high" for each placenta, which could not capture the variation of risk; and 9) in MV and FV composite variables, all involved placental constructs (MV-O, MV-I, and MV-D for MV; FV-O and FV-I for FV) were given the same weight, and thus, it is possible that one subtype vascular lesion has a stronger relation to maternal serum uric acid.

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