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Kripa Venkatakrishnan Date

The Effect of Pre-Pregnancy Obesity on Stillbirth: Mediation by Systemic and Placental Inflammatory Markers

By

Kripa Venkatakrishnan

Master of Public Health

Department of Epidemiology

Carolyn Drews-Botsch, PhD, MPH

Committee Chair

The Effect of Pre-Pregnancy Obesity on Stillbirth: Mediation by Systemic and Placental Inflammatory Markers

By

Kripa Venkatakrishnan

B.S.

University of Pittsburgh

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Faculty Thesis Advisor: Dr. Carolyn Drews-Botsch, PhD, MPH

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Abstract

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By Kripa Venkatakrishnan

BACKGROUND: Maternal obesity is a known risk factor for stillbirth, and both obesity and pregnancy are known to induce chronic, low-grade inflammation in the body. Obesity in pregnancy has been associated with a systemic increase in plasma proinflammatory cytokines which then accumulate in the placenta and cause a proinflammatory intrauterine environment. Placental inflammatory conditions such as chorioamnionitis have been shown to be more common in stillbirths compared to live births. Therefore, it is plausible that markers of both systemic and placental inflammation might mediate the relationship between pre-pregnancy obesity and stillbirth.

METHODS: Using data from the Stillbirth Collaborative Research Network, univariate and multivariate logistic regression was used to evaluate the relationship between pre-pregnancy obesity and stillbirth. The relationship between obesity and inflammation as well as inflammation and stillbirth were also evaluated. Systemic mediators of interest included maternal serum C-reactive protein, ferritin, and white blood cell count. Placental markers of inflammation included histologic chorioamnionitis of the chorionic plate and placental free membranes. Mediation analyses were preformed using Valeri and VanderWeele's SAS macro for mediation.

RESULTS: In crude and adjusted models, obesity was significantly associated with stillbirth (OR_{crude} = 1.67, 95% CI: 1.28, 2.18, $OR_{adjusted}$ = 1.68, 95% CI: 1.27, 2.22). With the exception of maternal elevated serum C-reactive protein, strong associations were found between maternal inflammatory markers and stillbirth with ORs ranging from 2.53 (95% CI: 1.98, 3.25) to 4.02 (95% CI: 2.99, 5.42). Little to no association was found between maternal obesity and inflammatory markers. Similarly, adjusted ORs for the effect of obesity that operates through systemic and placental inflammatory markers (indirect effects) were weak, ranging from 0.97 to 1.08.

CONCLUSION: Markers of both systemic or placental inflammation do not appear to mediate the relationship between pre-pregnancy obesity and stillbirth. However, stillbirth does appear to be associated with inflammatory markers. More research is required to further explore the role of inflammation as an intermediate.

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INTRODUCTION

Stillbirth is an unexpected adverse birth outcome with negative physical and psychological health effects for parents and clinicians. In the United States, approximately 1 in 160 pregnancies end in a stillbirth, and approximately 24,000 stillbirths occur each year (1). Since the 1990s and early 2000s, the rate of stillbirth has steadily declined in the US due to improvements in maternal and perinatal care (3, 4). However, as of 2014, this decline has stagnated, suggesting that the rate can be reduced even further (2, 3). Although several risk factors have been identified in recent years, stillbirth remains to be poorly understood, and the causes of many stillbirths are still largely unknown despite investigation. Therefore, in order to reduce burden, it is necessary to better understand the etiology of stillbirth and to elucidate the specific mechanisms that give rise to this devasting birth outcome.

Adult obesity in the United States has been increasing at an alarming rate in the last few decades, and approximately 42.4% of all adults are now obese (7). The impacts of the obesity epidemic have also extended to women of reproductive age as the prevalence of normal pre-pregnancy weight has been decreasing, and nearly half of all women giving birth are either overweight or obese (5, 6). Increased pre-pregnancy weight has negative consequences for both maternal and fetal health. Maternal obesity is a known risk factor for a number of pregnancy complications, including gestational diabetes, hypertension, preeclampsia, and placental abruption (9-11). Management of maternal obesity in addition to some of these associated antenatal comorbidities poses a challenge for obstetric care and greatly increases the risk of poor fetal outcomes such as macrosomia, low birthweight, small or large for gestational age, preterm birth, and most

notably stillbirth (11, 12). Therefore, it is clear that the increasing prevalence of pre-pregnancy obesity increases the likelihood of adverse birth outcomes and is a significant public health concern in the US.

Stillbirth is associated with maternal obesity. Epidemiologic studies have demonstrated between a 2 to 5-fold increase in the risk of stillbirth amongst obese women compared to non-obese women, and this risk appears to increase in a dose-dependent fashion as BMI or BMI class increases (13-16). Despite the breadth, magnitude, and consistency of reports supporting this association, the underlying pathophysiologic mechanisms that link obesity to stillbirth still remain unclear. However, recent studies have suggested that systemic and placental inflammation might play a key role.

Obesity and pregnancy, independent of each other, are both known to cause chronic, low-grade inflammation throughout the body. Thus, having both conditions simultaneously enhances and heightens the body's natural inflammatory response and can lead to placental inflammation (17-20). The body's systemic, immune response to inflammation involves the increase in plasma pro-inflammatory macrophages that express pro-inflammatory cytokines such as interleukin 6 (IL-6), C-reactive protein, and leptin (17-20). In the presence of maternal obesity, proinflammatory macrophages infiltrate and accumulate in the placenta, causing inflammatory lesions to form on the placental villous tree, free membranes, chorionic plate, and basal plate (21-26). Increased presence of inflammatory lesions creates a proinflammatory intrauterine environment for the fetus and has implications for both placental function and fetal development (24).

Optimal placental function is essential for the transfer of oxygen and nutrients to the fetus. Therefore, a sub-optimal placental environment caused by increased

inflammatory lesions can hinder these important processes and threaten healthy fetal growth and development. Pathologic placental findings such as chorioamnionitis of the chorionic plate or free membranes are more common in stillbirths than in livebirths, which suggest that chronic placental inflammation is associated with fetal death (33-36). The biologic pathway between placental inflammation and stillbirth is not clear-cut as few studies have examined the association between placental inflammation and stillbirth specifically. However, stillbirths are closely related to placental dysfunction, and as many as 65% of all stillbirths are thought to be caused by placental abnormalities (32). Since placental inflammation is associated with impaired placental function, it is plausible that it plays a key role in the biologic pathway that leads to stillbirth.

Maternal obesity is associated with systemic and placental inflammation (17-26). Placental inflammation is associated with placental dysfunction, which is associated with stillbirth. Despite being related to one another, there are few studies in the literature that examine maternal obesity, inflammation, and stillbirth together or that explore the role of inflammation as an intermediate. To address this gap in content, we hypothesize that markers of systemic or placental inflammatory markers mediate the relationship between maternal obesity and stillbirth. Markers of systemic inflammation that are of interest include maternal serum C-reactive protein (CRP), ferritin, and white blood cell (WBC) count. Markers of placental inflammation include histologic chorioamnionitis of the chorionic plate and the placental free membranes.

METHODS

STUDY DESIGN AND DATA SOURCE:

Data for this analysis come from the Stillbirth Collaborative Research Network (SCRN) which was a multi-site, population-based, case-control study to investigate causes, risk factors, and geographic incidence of stillbirth. Recruitment of cases and controls occurred between March 2006 and September 2008 and took place at 59 hospitals associated with five clinical sites in the United States. Hospitals were selected to ensure access to at least 90% of all stillbirths and livebirths within geographic catchment areas located in counties in Massachusetts, Georgia, Texas, Utah and all of Rhode Island.

CASE AND CONTROL ASCERTAINMENT:

Enrollment for both cases and controls was prospective, and women were approached and consented into the study during their in-patient hospital stay for delivery. SCRN coordinators at site hospitals monitored all births to identify, screen, and register all women who had experienced a fetal death at greater than or equal to 20 weeks of gestation. Prior to enrollment, delivery records of stillbirths were reviewed to ensure that APGAR scores weren't greater than 0 at 1 and 5 minutes after delivery. This further distinguished stillbirths from live births.

Since stillbirths were more likely to be very preterm than term, all live births delivered between 20 to 23 weeks of gestation were registered and approached for enrollment, and live births delivered between 24 to 31 weeks of gestation were oversampled to match specified selection probabilities for each week of gestational age.

Women who had live births at greater than or equal to 32 weeks of gestation were approached for enrollment based on randomly specified hospital dates and times.

The existing study sampling design led to a 2:1 ratio of live births (greater than 32 weeks of gestation) to stillbirths for the entire study population and for the non-Hispanic white and Hispanic racial groups. For the non-Hispanic black racial group, this ratio was closer to 1:1. Therefore, non-Hispanic black women were oversampled to achieve closer to a 2:1 ratio and to also account for the increased burden of stillbirth in African American women. Out of 953 eligible stillbirths and 3088 eligible live births, the recruitment process resulted in a total of 663 (69.6%) stillbirths and 1932 (62.6%) selected live births enrolled into the study.

DATA COLLECTION:

Data on enrolled stillbirths and live births were collected by a two-part post-partum maternal interview after delivery and by chart abstraction from delivery and prenatal medical records. Information on social and demographic characteristics, reproductive history, and pregnancy complications were obtained from the first portion of the maternal interview which took place while women were still hospitalized for delivery. Information on psychosocial data and medical history were collected as part of the second portion of the maternal interview which mostly occurred in-hospital but also took place via phone call within 2-4 weeks after delivery. Chart abstraction from delivery and prenatal records supplemented social, demographic, and reproductive information gained from maternal interview but also provided more data on prenatal exposures and medications, genetic history, laboratory results, stillbirth assessments, and placental cultures.

Maternal blood was collected after enrollment while women were still hospitalized for their delivery. Both maternal blood and fetal cord blood samples were frozen and stored after collection until they were assayed to assess for various biomarkers 2 to 5 years later. Other biologic specimens such as umbilical cord and placental tissue sections were collected after delivery and stored for later analysis in both stillbirths and livebirths. Postmortem blood, fetal tissue, and meconium samples were also collected and stored but for stillbirths only.

Assessments of placental specimens were conducted by SCRN anatomic pathologists who met on several occasions to ensure standard examination procedures, definitions, and diagnoses. Women had the option to consent to placental examination, and for those who consented, examinations were further classified as adequate or inadequate. Among the 663 enrolled women who had stillbirths, 654 (98.6%) consented to placental examination, and 632 (95.3%) of these were considered to be adequate. For live births, 1804 (93.4%) out of 1932 enrolled women with live births consented, and 1347 (69.7%) had examinations that were considered to be adequate. Inadequate placental examinations were most often caused by failure to collect the placenta at delivery. Pathologic examinations of the placenta and other tissue samples occurred within two days of collection and delivery to site specified SCRN pathologists. Data and results from these examinations were recorded on designated SCRN study forms.

STUDY POPULATION AND MISSING DATA:

Analyses on systemic and placental inflammatory markers were conducted separately using two different study samples. Both study samples were restricted to singleton births, and excluded stillbirths with genetic or structural abnormalities as the

possible cause of fetal death for those stillbirths seemed more likely to be related to the genetic or structural abnormalities rather than the mechanism of interest in this analysis. Subjects were also excluded if data on BMI was missing (n=78, n=54 for systemic and placental samples, respectively). For analysis on systemic inflammatory mediators, any subjects with missing data on CRP, ferritin, or WBC count were excluded (n=592). Similarly, any subjects with missing data on chorioamnionitis of the chorionic place or placental free membranes were excluded for analyses of placental inflammatory mediators (n=807). Subjects were also excluded from both analyses if there were missing data for any covariates (n=43, n=62 for systemic and placental samples, respectively).

After all restrictions and exclusions, the analytic sample for systemic inflammatory markers resulted in 381 stillbirths and 1284 livebirths. Approximately 19.0% of all enrolled stillbirths and 30.4% of all enrolled live births were excluded due to missing information on the exposure, systemic inflammatory mediators, and covariates. For analysis on placental inflammatory markers, the analytic sample was restricted to 373 stillbirths and 1098 livebirths. Approximately 20.7% of all enrolled stillbirths and 40.7% of all enrolled live births were excluded due to lack of placental examination or missing information on exposure, placental inflammatory mediators, and covariates.

DATA WEIGHTS:

Data weights for the different sampling probabilities were constructed separately for live births delivered at less than 32 weeks of gestation, live births delivered at greater than 32 weeks of gestation, and all stillbirths. Base weights adjusted for staggered enrollment start dates and differential participation rates across site hospitals. Base weights also took into account any variables that were significant predictors of whether a

woman was approached or consented. Final analytic weights were constructed for the entire cohort by taking the product of all the weighting factors and adjustments. To account for differential losses in placental specimens, a placental weighting factor was also created. Placental analysis weights were constructed by taking the product of the placental weighting factor and the final analytic weight.

OBESITY:

The primary exposure in this analysis was pre-pregnancy obesity defined as having a body mass index (BMI) of greater than or equal to 30. Data on pre-pregnancy BMI was primarily collected via chart abstraction but was also collected from maternal interview if it could not be found in the medical record.

MEDIATORS:

Systemic inflammatory markers of interest included maternal serum CRP, ferritin, and WBC count. Serum CRP and ferritin levels were assayed from the maternal blood specimens collected at the time of enrollment, and WBC count was collected via chart abstraction of clinical lab results from prenatal and delivery records. If available, WBC counts were taken from the date of delivery. However, if not, WBC counts were taken from the next closest date within three days prior to delivery.

Elevated levels for all three systemic inflammatory markers were determined by looking at the interquartile range of the entire sample. Serum levels were considered elevated if they were at or above the 75^{th} percentile value for live births. Elevated CRP was defined as greater than or equal to 7.2 mg/dL, elevated serum ferritin was defined as

greater than or equal to 42 ng/mL, and elevated WBC count was defined as greater than or equal to 12.4 uL × 10^3.

Maternal placental inflammatory markers consisted of histologic chorioamnionitis of the chorionic plate and of the placental free membranes. Presence of chorioamnionitis at either of these sites was confirmed and recorded on study forms by SCRN pathologists during pathologic placental examinations.

CONFOUNDERS:

Maternal age, race/ethnicity, education, insurance status, parity, and smoking status were identified by literature review as potential confounders of the relationship between obesity and stillbirth (Figure 1). Gestational diabetes and gestational hypertension were identified as confounders of the association between placental inflammation and stillbirth. However, they were not controlled for because they may exist on the causal pathway between maternal obesity and stillbirth.

ANALYTIC PLAN:

All analyses were conducted using SAS Version 9.4 (SAS Institute INC, Cary, North Carolina), and statistical significance was assessed at an alpha level of 0.05. Analyses on systemic inflammatory markers utilized the final analytic data weights since there was no dependence on placental examination. However, placental analysis weights were used for analyses on placental inflammatory markers.

Univariate and multivariable logistic regression was used to examine the association between obesity and stillbirth. The association between inflammatory markers and stillbirth and the association between inflammatory markers and obesity was also

examined, but analyses on the latter only took place amongst livebirths. Collinearity between covariates was assessed by examining condition indices and variance decomposition proportions (VDPs). Presence of collinearity was defined as having condition indices greater than 30 and two or more VDPs greater than 0.5. No collinearity was found in any analyses.

Mediation analysis was performed to examine whether the relationship between obesity and stillbirth is mediated by systemic and/or placental inflammation. All mediation analyses were performed utilizing Valeri and VanderWeele's SAS macro for mediation (47). This macro was modified to allow for data weighting and analysis on complex survey data. Logistic models were used for both the mediator and the outcome and all models were adjusted for potential exposure-outcome, exposure-mediator, and mediator-outcome confounding. Additionally, the percentage of the total effect that was due to mediation was also estimated.

RESULTS

Table 1 provides a summary of descriptive characteristics for both the systemic and placental inflammatory marker samples. Women who delivered live born infants were less likely to be nulliparous (35.2%) and to have smoked within 3 months of pregnancy (12.5%) compared to women who delivered stillbirths (45.0% and 21.4%, respectively). After oversampling, a higher proportion of women who delivered stillbirths were non-Hispanic black (20.1%) compared to women who delivered live births (9.1%). Women who delivered live births had a higher proportion of non-Hispanic white women (44.6%) compared to women who delivered stillbirths (34.6%). Descriptive characteristics for the restricted sample used in placental inflammatory marker analyses followed similar patterns for parity, smoking status, and race/ethnicity.

Obesity was more prevalent in women who had stillbirths than in women who had live births in both systemic (33.3% vs. 23.0%) and placental inflammatory samples (29.8% vs. 23.3%) (Table 2). In crude and adjusted models, obesity was significantly associated with stillbirth in both study samples. However, the observed associations were stronger in the sample used for systemic inflammatory markers compared to those of the restricted placental sample. In unadjusted analyses for systemic inflammatory markers, obese women had 1.67 times the odds of stillbirth compared to non-obese women (95% CI: 1.28, 2.18), and this association was unchanged adjusting for maternal age, race/ethnicity, parity, education, insurance, and smoking status (OR:1.68, 95% CI: 1.27, 2.22). These associations were similar yet slightly weaker when limited to women for whom placentas were available for analysis (OR_{crude} = 1.40, 95% CI: 1.07, 1.83, $OR_{adjusted}$ = 1.51, 95% CI: 1.13, 2.03).

Markers of systemic or placental inflammation were generally more common in pregnancies ending in stillbirth compared to pregnancies ending in live birth (Table 2). With the exception of elevated CRP levels, pregnancies ending in a stillbirth had a higher proportion of women with elevated serum levels of both ferritin and WBC count (47.5%, 45.7%) compared to pregnancies ending in a live birth (25.1%, 25.1%). For placental inflammatory markers, chorioamnionitis of the chorionic plate or the placental free membranes was also more common in pregnancies ending in a stillbirth (24.6%, 33.6%) compared to live birth (11.2%, 11.3%). Women who had markers of systemic and placental inflammation were more likely to experience stillbirth (Table 2). The odds of stillbirth amongst women with elevated serum ferritin and serum WBC count were more than two times that of women without elevated serum ferritin (OR: 2.69, 95% CI: 2.10, 3.45) or elevated serum WBC count (OR: 2.53, 95% CI: 1.98, 3.25). This effect was similar in women who had chorioamnionitis of the chorionic plate (OR: 2.60, 95% CI: 1.90, 3.56). However, women with chorioamnionitis of the placental free membranes had approximately 4 times the odds of stillbirth compared to women without chorioamnionitis of the placental membranes (OR: 4.02, 95% CI: 2.99, 5.42).

After restricting to only live births, the association between obesity and inflammation was weak as markers of systemic or placental inflammation were only slightly more common in obese women compared to non-obese women (Table 3). Obese women were 1.30 and 1.16 times more likely to have elevated serum CRP (95% CI: 0.93, 1.81) or elevated serum WBC count (95% CI: 0.83, 1.63). However, confidence intervals for ORs were wide and contained the null value. This effect was similar for markers of placental inflammation as obese women were 1.15 and 1.33 times more likely to have

chorioamnionitis of the chorionic plate (95% CI: 0.79, 1.86) or the placental free membranes (95% CI: 0.83, 2.12). Elevated serum ferritin was the only marker of inflammation that was more common in non-obese women. Obese women were 21% less likely to have elevated serum ferritin (OR: 0.79, 95% CI: 0.55, 1.13).

Very little of the effect of obesity on stillbirth appears to operate though systemic inflammatory markers as ORs for adjusted indirect effects ranged from 0.97 to 1.06 and proportions mediated ranged from -6.3% to 11.0% (Table 4). For elevated serum CRP and serum ferritin, the adjusted natural direct effects of obesity on stillbirth, independent of elevated serum markers, resulted in ORs that were as strong or even stronger than the total effect (OR: 1.70, 95% CI: 1.28, 2.26 and OR: 1.68, 95%CI: 1.26, 2.23, respectively), which suggests that part of the effect of obesity on stillbirth might be masked by the protective indirect effects of elevated serum CRP and ferritin.

For analysis on placental inflammatory markers, the indirect effects of chorioamnionitis of the chorionic plate were relatively weak in comparison to that of the placental free membranes (Table 4). In adjusted models, the odds ratio for the association between obesity and stillbirth operating through chorioamnionitis of the chorionic plate was 1.02 (95% CI: 0.95, 1.10) and only approximately 4.9% of the total effect was accounted for by mediation. For chorioamnionitis of the placental free membranes, the adjusted odds ratio for the indirect effect was slightly higher with an OR of 1.08 (95% CI: 0.95, 1.22), and approximately 18.3% of the total effect was due to mediation. The natural direct effects of obesity on stillbirth not accounted for by chorioamnionitis of the chorionic plate or the free membranes were similar but reduced compared to the overall total effect of obesity on stillbirth.

DISCUSSION

PRINCIPAL FINDINGS:

The purpose of this analysis was to examine whether systemic or placental inflammatory markers mediate the relationship between pre-pregnancy obesity and stillbirth. Consistent with previous research, we found that obese women have a consistently higher odds of stillbirth compared to non-obese women in both crude and adjusted analyses. However, markers of systemic inflammation do not appear to mediate this relationship. For maternal serum CRP and serum ferritin, the effect of obesity on stillbirth appears to be slightly stronger when inflammatory markers are accounted for, which suggests that elevated serum CRP and serum ferritin actually mask part of the effect of obesity on stillbirth. This also suggests that the probability of having systemic inflammatory markers are lower in obese women, which was actually observed for maternal serum ferritin in our analyses on obesity and inflammatory markers. Markers of placental inflammation also do not appear to mediate the relationship between obesity and stillbirth. Although indirect effects were found for chorioamnionitis of the chorionic plate and placental free membranes, both effects were weak and statistically imprecise.

Overall, we found a strong association between stillbirth and markers of systemic or placental inflammation. Higher proportions of inflammatory markers were found in stillbirths compared to live births, and the odds of stillbirth were doubled in women who had elevated serum ferritin, elevated WBC count, and chorioamnionitis of the chorionic plate. This likelihood was quadrupled in women who had chorioamnionitis of the placental free membranes. Elevated serum CRP was actually found to be more common in live births compared to stillbirths. Data on the association between stillbirth and

maternal serum CRP specifically are sparse in the literature. Thus, future studies are required to determine the true nature of this association.

Previous studies have examined and established the pathophysiologic pathways that link obesity, inflammation, and inflammatory markers. CRP is an acute phase protein produced by the liver and is part of the body's innate immune response to inflammation (37, 38). Therefore, plasma CRP levels are expected to be elevated in the presence of obesity, which is a chronic, systemic inflammatory state (39). WBCs are produced from proinflammatory cytokines such as interleukin 6 (IL-6) and interleukin 8 (IL-8) (43). Since chronic inflammation due to obesity has been characterized by the increase of plasma pro-inflammatory cytokines, WBC count has also been shown to be elevated in obese individuals (44). Elevated serum ferritin levels are also associated with systemic inflammation because proinflammatory cytokines produce reactive oxygen species that release free iron from ferritin and then upregulate ferritin synthesis (40, 41). As such, serum ferritin has also been documented to be elevated in obese individuals (42). For markers of placental inflammation, chorioamnionitis of the chorionic plate and placental free membranes are characterized by the presence of inflammatory lesions and accumulation of proinflammatory cytokines (28). Thus, presence of both of these placental findings have also been associated with maternal obesity (27).

Despite having evidence and explanations of the biologic mechanisms underlying obesity and inflammation, little to no association between systemic or placental inflammatory markers and obesity was found in our analysis. Although elevated serum CRP, elevated WBC count, and chorioamnionitis at either site were more common in obese women than non-obese women, the difference between the two groups was

marginal and the ORs for obesity were imprecise. Elevated serum ferritin was actually more common in non-obese women, which was contrary to what was expected. Serum ferritin is also a marker of maternal iron status in addition to inflammation, and a few recent studies in the literature have suggested that maternity obesity might be associated with iron deficiency (45, 46). This could be a possible reason as to why we observed this result. However, more studies are required to determine the true relationship between maternal obesity and serum ferritin specifically.

LIMITATIONS:

There are several limitations to consider when interpreting the results of this study. The potential for selection bias is a significant concern as all analyses were complete case analyses, and a significant number of stillbirths and live births were excluded due to missing information on exposure, mediators, or covariates. Lack of adequate placental examination affected a larger proportion of live births compared to stillbirths and was also a big source of potential selection bias as approximately 40% of all enrolled livebirths were excluded for analyses on placental inflammatory markers. This was further supported by the differences in overall OR for the association of obesity and stillbirth between systemic and placental samples. However, since these associations were relatively consistent in strength and direction, we are more confidence that selection bias did not create or mask the observed association. Although placental analysis weights were constructed to account for inadequate placental examinations, this was only limited to women who had placental examinations in the first place. The most common reason for an inadequate placental examination was failure to collect the placenta at delivery.

Another limitation of this study is the use of self-reported pre-pregnancy weight from maternal interview. Women and individuals of higher weight are more likely to underreport their weight, which could lead to misclassification of the exposure. However, the primary method of obtaining pre-pregnancy weight was through chart abstraction, and self-reported pre-pregnancy weight was used for only 5 women in placental inflammatory marker analyses only. No self-reported pre-pregnancy weight data was used for any analyses on systemic inflammatory markers.

The use of BMI as a measure for maternal obesity is another potential limitation as definitions of maternal obesity should ideally reflect maternal body fat content. BMI is calculated using weight in kilograms, and weight does not differentiate muscle from fat. Therefore, individuals with lean muscle mass could have higher BMI's despite not necessarily having unhealthy levels of body fat. Dichotomization of obesity was another limitation as it rid of any subtle differences in effect that may have existed between specific BMI classes. Ideally, we would have liked to analyze BMI categorically. However, nominal independent variables were not supported in Valeri and VanderWeele's SAS macro for mediation.

CONCLUSION

This study provides important insights into the relationship between maternal obesity and inflammation and the relationship between inflammation and stillbirth. The main finding of this analysis is that systemic and placental inflammatory markers do not appear to mediate the relationship between stillbirth and obesity. This is likely due to the weak association found between maternal obesity and inflammatory markers. Stillbirth, on the other hand, was found to be highly associated with markers of systemic and placental inflammation with the exception of elevated maternal serum CRP.

Our analysis aimed to fill the gap in current research regarding the role of inflammation as an intermediate between maternal obesity and stillbirth. As our knowledge of risk factors associated with stillbirth continues to grow, it is important that time and effort are spent towards improving our understanding of the underlying mechanisms that cause this adverse birth outcome. Despite our limitations and null findings, this was one of the first few studies to explore the relationship between specific markers of inflammation, obesity, and stillbirth altogether. Further research is necessary to determine whether a pathway exists between maternal obesity, inflammation, and stillbirth.

TABLES AND FIGURES

Table 1. Descriptive characteristics of the study population used in systemic and placental inflammatory mediator analyses

	Systemic Inflammatory Sample		Placental Inflammatory Sample	
	Stillbirths	Live births	Stillbirths	Live births
Characteristic, weighted %	N = 381 N_W = 382	N = 1284 N_W = 965	N = 373 N_W = 377	N = 1098 N_W = 885
Maternal age at delivery (years)				
< 20	13.5	10.5	14.2	10.4
20 – 34	72.3	76.4	71.8	76.3
35 – 39	9.7	11.5	10.4	10.9
40+	4.5	1.6	3.5	2.5
Maternal race/ethnicity				
Non-Hispanic white	34.6	44.6	32.2	44.7
Non-Hispanic black	20.3	9.1	25.5	11.9
Hispanic	38.9	39.0	36.4	36.1
Other	6.1	7.3	5.9	7.3
Maternal education				
0 – 11 (none/primary/some secondary)	23.2	19.7	21.3	18.0
12 (completed secondary)	31.1	25.9	32.1	26.6
13+ (college)	45.7	54.4	46.6	55.4

Insurance				
No insurance	4.4	3.4	5.6	4.2
Any public/private assistance	58.1	49.1	56.6	48.8
Veteran's Affairs/commercial health insurance/health maintenance organization	37.5	47.4	37.8	47.0
Parity				
Nulliparous	45.0	35.2	46.4	34.5
Multiparous	55.0	64.8	53.6	65.5
Maternal smoking status[1]				
Did not smoke	78.6	87.4	79.5	87.6
< 10 cigarettes/day	11.8	6.5	11.0	6.2
10+ cigarettes/day	9.6	6.0	9.5	6.2
Gestational age at delivery (weeks)				
18 – 19	2.7	0.0	2.5	0.0
20 – 23	31.8	0.4	31.3	0.3
24 – 27	13.6	0.5	14.7	0.4
28 – 31	13.3	1.0	13.0	0.7
32 – 36	19.8	8.1	18.6	6.8
37+	18.9	90.0	19.7	91.8

Abbreviations: N_W, weighted sample size.

[1] Self-reported smoking status three months prior to pregnancy.

Table 2. Summary of obesity and markers of systemic or placental inflammation stratified by birth status

	Stillbirths	Live births		
	$\%_w$	$\%_w$	Crude OR (95% CI)	Adjusted OR[1] (95% CI)
Systemic Inflammatory Analyses				
Obesity[1]	33.3	23.0	1.67 (1.29, 2.16)	1.68 (1.27, 2.22)
Elevated serum levels				
CRP	23.0	25.4	0.88 (0.66, 1.16)	---
Ferritin	47.5	25.1	2.69 (2.10, 3.45)	---
WBC count	45.7	25.1	2.53 (1.98, 3.25)	---
Continuous levels, median (IQR)				
CRP	3.3 (1.5, 6.7)	3.9 (1.7, 7.2)	---	---
Ferritin	40.0 (22.0, 68.0)	22.0 (13.0, 42.0)	---	---
WBC count	12.0 (9.3, 15.4)	10.3 (8.6, 12.4)	---	---
Placental Inflammatory Analyses				
Obesity[1]	29.8	23.3	1.40 (1.07, 1.83)	1.51 (1.13, 2.03)
Chorioamnionitis				
Chorionic plate	24.7	11.2	2.60 (1.90, 3.56)	---
Placental membranes	33.9	11.3	4.02 (2.99, 5.42)	---

Abbreviations: $\%_w$, weighted percent; CRP, C-reactive protein; WBC, white blood cell; OR, odds ratio; CI, confidence interval; IQR, interquartile range.

[1] Multivariate models were adjusted for maternal age, race/ethnicity, parity, education, insurance, and smoking status.

Table 3. Summary of systemic and placental inflammatory mediators stratified by obesity status amongst live births

	Obese	Non-Obese	
	$\%_w$	$\%_w$	Crude OR (95% CI)
Systemic Inflammatory Analyses			
Elevated serum levels			
CRP	29.3	24.2	1.30 (0.93, 1.81)
Ferritin	21.8	26.1	0.79 (0.55, 1.13)
WBC count	27.3	24.4	1.16 (0.83, 1.63)
Placental Inflammatory Analyses			
Chorioamnionitis			
Chorionic plate	12.3	10.8	1.15 (0.79, 1.86)
Placental membranes	13.6	10.6	1.33 (0.83, 2.12)

Abbreviations: $\%_w$, weighted percent; CRP, C-reactive protein; WBC, white blood cell; OR, odds ratio; CI, confidence interval.

Table 4. Results examining mediation of the relationship between pre-pregnancy obesity and stillbirth by markers of systemic or placental inflammation

	Total Effect OR (95% CI)	Natural Direct Effect OR (95% CI)	Natural Indirect Effect OR (95% CI)	% Mediated
Systemic Inflammatory Analyses				
Crude	1.67 (1.29, 2.16)			
CRP[2]	---	1.68 (1.28, 2.19)	0.99 (0.98, 1.01)	-1.4
Ferritin[2]	---	1.68 (1.28, 2.21)	0.95 (0.87, 1.03)	-10.3
WBC count[2]	---	1.61 (1.22, 2.11)	1.03 (0.96, 1.11)	6.0
Adjusted[1]	1.68 (1.27, 2.22)			
CRP[2]	---	1.70 (1.28, 2.26)	0.98 (0.95, 1.01)	-4.0
Ferritin[2]	---	1.68 (1.26, 2.23)	0.97 (0.89, 1.05)	-6.3
WBC count[2]	---	1.56 (1.17, 2.08)	1.06 (0.99, 1.14)	11.0
Placental Inflammatory Analyses				
Crude	1.40 (1.07, 1.83)			
Chorionic plate[3]	---	1.37 (1.03, 1.83)	1.02 (0.94, 1.11)	5.6
Placental membranes[3]	---	1.31 (0.97, 1.76)	1.07 (0.93, 1.23)	19.4
Adjusted[1]	1.51 (1.13, 2.03)			
Chorionic plate[3]	---	1.48 (1.10, 2.00)	1.02 (0.95, 1.10)	4.9
Placental membranes[3]	---	1.40 (1.03, 1.91)	1.08 (0.95, 1.22)	18.3

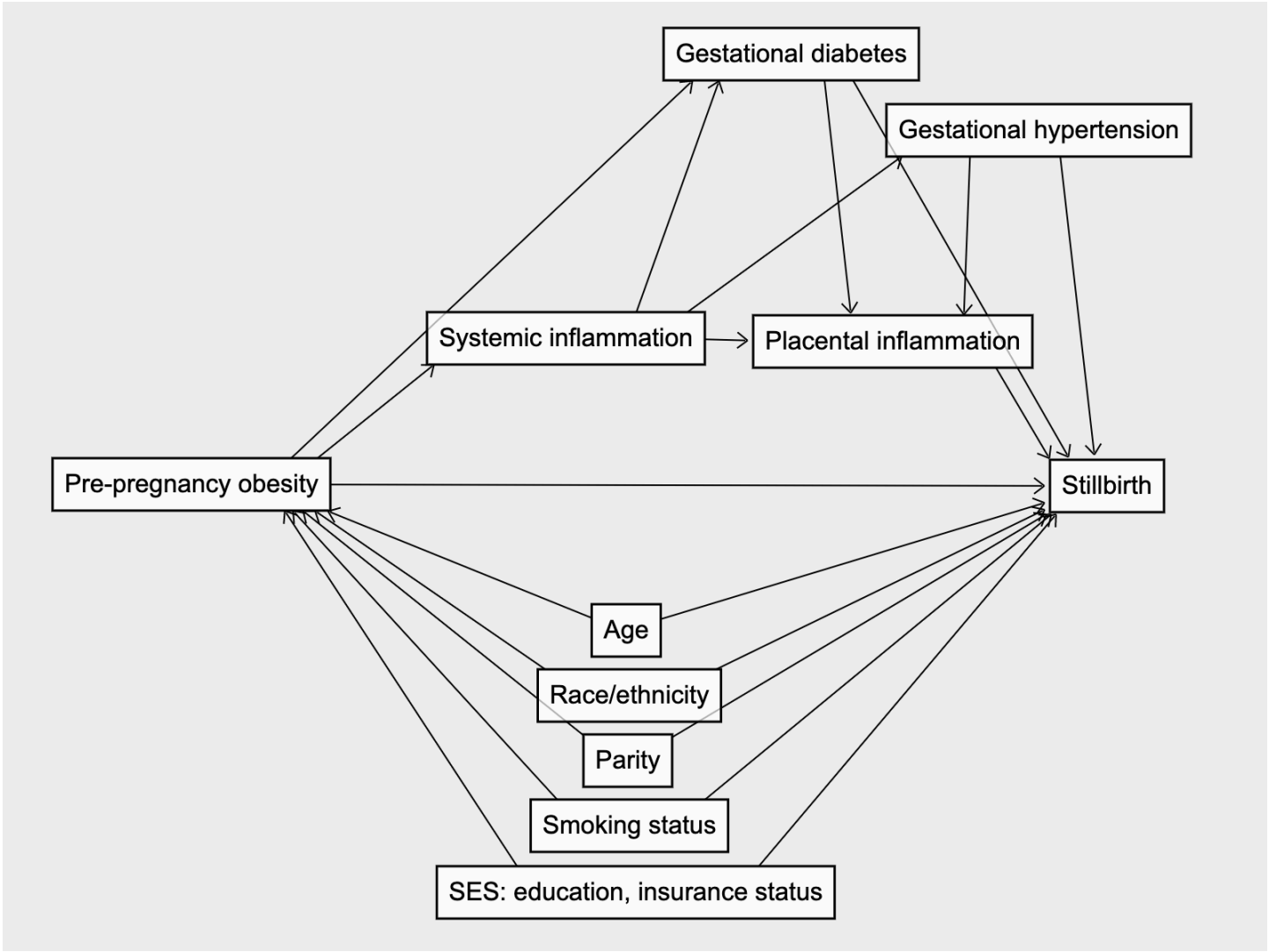
Abbreviations: CRP, C-reactive protein; WBC, white blood cell; OR, odds ratio; CI, confidence interval.

[1] Multivariate models were adjusted for maternal age, race/ethnicity, parity, education, insurance, and smoking status.

[2] Elevated serum levels of either c-reactive protein, ferritin, or white blood cell count.

[3] Histologic chorioamnionitis of either the chorionic plate or the placental free membranes.

Figure 1. Directed acyclic graph of the relationship between obesity and stillbirth



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