ASSOCIATIONS OF TRIMETHYLAMINE N-OXIDE AND ITS PRECURSORS WITH GESTATIONAL DIABETES AND PRE-ECLAMPSIA AMONG WOMEN DELIVERING AT AN URBAN SAFETY NET HOSPITAL

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Abstract

Introduction: Trimethylamine N-oxide (TMAO) and its precursors choline, betaine, and carnitine have been associated with cardiometabolic disease in non-pregnant adults. However, studies examining TMAO and its precursors in relation to cardiometabolic conditions during pregnancy are lacking.

Methods: We used data from the Boston Birth Cohort between 1998 and 2013. We examined associations of TMAO, choline, betaine, and carnitine measured in both cord blood and maternal blood with gestational diabetes mellitus (GDM) and pre-eclampsia (PE). We used logistic regression to estimate odds ratios for each outcome according to tertiles and a standard deviation (SD) increment of TMAO, choline, betaine, and carnitine. Final models were adjusted for potential confounders.

Results: Among our analytic sample of 1496 women, 115 developed GDM and 158 PE. Inter-metabolite correlations of TMAO and its precursors were stronger within cord blood (r=0.38-0.87) than within maternal blood (r=0.08-0.62). Among the maternal blood metabolites analyzed, TMAO was associated with higher odds of GDM (3^{rd} vs. 1^{st} tertile OR=1.75; 95% CI 1.04, 2.94), while TMAO precursor metabolites were not associated with GDM. Concentrations of TMAO and choline in maternal blood were not associated with PE, while maternal blood carnitine was associated with higher odds of PE (OR=1.92; 95% CI 1.21, 3.04) and betaine was associated with lower odds of PE (OR=0.37; 95% CI 0.23, 0.59). In cord blood, TMAO was not associated with GDM or PE, but choline, betaine, and carnitine measured in cord blood were associated with higher odds of PE (OR=3.11; 95% CI 1.62, 5.96; OR=2.65; 95% CI 1.42, 4.93); OR=2.56; 95% CI 1.39, 4.69; respectively). In addition, cord choline was associated

with lower odds of GDM (OR=0.52; 95% CI 0.27, 0.99), while other cord metabolites were not associated with GDM.

Conclusion: Our study adds to the evidence that TMAO and its precursors play an etiologic role in development of GDM and PE.

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Introduction

Gestational diabetes (GDM) and pre-eclampsia (PE) are leading contributors to maternal and infant morbidity and mortality, which continue to rise in the United States and globally.[1-5] In addition to contributing to perinatal morbidity and mortality, these conditions are risk factors for cardiometabolic disease later in life.[6-9] Given their important contribution to health of the mother and the fetus, there is critical need to identify factors that can be targeted for the prevention of these disorders.

Trimethylamine N-oxide (TMAO) is a metabolite produced by an interaction between dietary precursors (i.e. choline, betaine, and carnitine) and gut microbiota.[10-14] TMAO and its precursors have been associated with type 2 diabetes, hypertension, stroke, major adverse cardiac events, and all-cause mortality in adults.[11,15-19] A few case-control studies have also shown an association between circulating maternal TMAO and GDM and PE.[20-24] All were conducted in China and measured TMAO in maternal blood (plasma or serum). These studies were relatively small, especially those examining PE (n=60-264).[20,23,24] Further, results from the studies of GDM were mixed, finding either a positive or an inverse association between TMAO and GDM.[21,22] One study examining maternal concentrations of the TMAO precursor carnitine found that carnitine was elevated among women with PE compared to normotensive pregnant controls.[25] Two studies found that carnitine in routine neonatal dried blood spots[26] and carnitine and choline in cord blood[27] were elevated among infants born to mothers with PE.

To date, there have been no studies of these metabolites with pregnancy outcomes in a U.S. population of pregnant women, let alone one that is racially and ethnically diverse. Furthermore, no studies have examined TMAO and its precursors measured in

both maternal blood and cord blood, which may be important because of cross-placental transfer of these metabolites.[25,28-31] Choline is an essential nutrient and plays a key role in fetal growth and neural development; active transport of choline across the placenta ensures adequate fetal choline nutrition.[28-31] The transporter for carnitine, which is involved in fatty acid metabolism and the removal of toxic metabolites, has also been identified.[25,31] Further, choline and betaine are important methyl donors, playing a role in fetal gene expression and epigenetic regulation.[32] Given the aforementioned gaps in the literature, the purpose of the current study is to evaluate the associations of TMAO and its precursor metabolites with GDM and PE in a U.S. prebirth cohort of urban women of predominantly Black and Hispanic race and ethnicity.

Methods

Study participants

The Boston Birth Cohort (BBC) began in 1998 with the original aim of determining the environmental and genetic determinants of preterm birth and low birthweight among women delivering at the Boston Medical Center (Boston, Massachusetts, USA).[33] The Boston Medical Center sees a large proportion of low-income, city-dwelling patients, as well as a large proportion of patients from racial and ethnic backgrounds that have been historically underrepresented in research. The study population has been described previously.[33-35] Briefly, women delivering singleton, live births were invited to participate. Pregnancies resulting in multiple gestations or major birth defects, as well as those conceived via *in vitro* fertilization, were excluded, as were women delivering pre-term due to non-obstetric factors (e.g., trauma).

The BBC continues to enroll women and their infants at birth and has followed up participants for up to 20 years. It has also expanded its objectives to examine a variety of additional exposures (e.g. metabolites) and outcomes. We used data from women in the BBC who enrolled between 1998-2016, had sufficient cord and/or maternal blood samples for metabolite analysis, and who had complete information on exposure variables, outcome variables, and key covariates (i.e., those that were included in the fully-adjusted models).

Exposure variables

Our exposures of interest were TMAO and its precursor metabolites choline, betaine, and carnitine. Metabolites were measured in umbilical cord blood (collected at birth) and maternal blood (collected within 1-3 days after delivery) red blood cells. Red blood cells have an average lifespan of 115 days, and so metabolites measured in red blood cells after delivery may reflect the internal environment earlier in pregnancy, roughly the third trimester.[36] Metabolites were analyzed by the Broad Institute of MIT and Harvard (Cambridge, MA, USA) using liquid chromatography mass spectrometry and metabolite concentrations were inversely normalized. Inverse normalization preserves the rank order of the metabolite concentrations while approximately normally distributing the values and centering them around zero and reducing the influence of outliers. Pooled samples were implemented as quality control; the coefficient of variation of these metabolites was between 1-3%. Women with missing values on both maternal and cord metabolites were excluded from the analytic sample.

Outcome variables

The outcomes of interest in our study included physician-diagnosed GDM and PE. GDM and PE were diagnosed during routine prenatal care visits and were recorded in electronic health records. Outcome data were extracted from medical records after study enrollment and diagnoses were reviewed for consistency with American College of Obstetrics and Gynecology (ACOG) definitions. GDM was diagnosed between weeks 24-28 on the basis of an oral glucose tolerance test (OGTT); if information on OGTT was missing, blood glucose after 14 weeks gestation was used to ascertain GDM status. PE was defined by new-onset hypertension (systolic blood pressure of 140 mmHg or higher or diastolic blood pressure of 90 mmHg or higher) plus proteinuria (excretion of 300 mg or more in a 24-hour urine collection) measured after 20 weeks gestation; in the absence of proteinuria, PE may be diagnosed if headache, blurred vision, abdominal pain, or abnormal lab results occur.[3,37,38]

Covariates

Potential confounders were selected *a priori* based on a causal diagram of the hypothesized relationships between these variables (Figure 1).[39] Confounders are variables that are associated with both exposure and outcome, and are not on the causal path; adjustment for confounders reduces bias and bring us closer to estimation of the causal effect of exposure on outcome. Maternal age, parity, and pre-pregnancy hypertension and diabetes were extracted from medical records. Other demographic characteristics including self-reported race and ethnicity, educational attainment, marital status, and pre-pregnancy weight and height were ascertained via a questionnaire

administered within 1-3 days after delivery. Pre-pregnancy body mass index (BMI) was calculated as weight (kg) divided by height (meters) squared.

Statistical analyses

We performed exploratory data analysis to describe participant characteristics. Percentages were used to describe categorical variables and median (IQR) were used to describe continuous variables. Inversely normalized metabolites were modeled both in tertiles and as continuous variables. We computed Spearman's rank correlation coefficients to examine the correlations between metabolites in cord and maternal blood. We used complete case analysis for each exposure-outcome pairing such that crude and adjusted models for each pairing utilize the same number of observations. For example, if a mother had provided maternal blood, but not cord blood, she would be included in analyses of maternal metabolites. If a mother had missing information on GDM diagnosis, she could still be included in the analyses for PE. Mothers with missing covariate data were excluded from all analyses.

Maternal age, race/ethnicity, education, marital status, pre-pregnancy BMI, parity, and chronic pre-pregnancy hypertension, were included as potential confounders in the models for GDM. The same variables, plus pre-pregnancy diabetes, were used in the analyses of GDM. Women with pre-pregnancy diabetes were excluded from the GDM analyses because they were not at risk of developing new-onset, gestational diabetes.

We used logistic regression to estimate the relative odds (i.e., odds ratio) and 95% confidence interval of GDM and PE according to tertiles and a standard deviation (SD) increment of TMAO, choline, betaine, and carnitine.

Finally, we ran all analyses stratified by race to examine consistency of results across groups, and then tested race-by-exposure interactions to examine the possibility of effect measure modification by race.

Sensitivity analyses

To reduce the possibility that our associations were due to reverse causation, we performed sensitivity analyses restricting only to those without pre-pregnancy hypertension (for both GDM and PE), without pre-pregnancy diabetes (for PE only), and without either (for PE only). To try to disentangle the independent associations of each metabolite, we also performed sensitivity analyses including all four metabolites (as continuous variables) in a single regression model. To address potential for selection bias, we first compared the population that was included in our analytic sample to the larger BBC, and then we calculated inverse probability weights for selection into our metabolite sub-study and applied these weights in our logistic regression models. To estimate the weights we modeled the probability of selection into the study based on all variables in our final model plus gestational age at delivery (weeks), delivery type (cesarian vs. vaginal), first sign of labor (contractions, membrane rupture, both, medically induced, or unknown), low birthweight, baby's sex, year, and maternal smoking (ever vs. never). Analyses were performed in Stata (Version 15, College Station, TX). Significance levels were set at 0.05 for all main effects and interactions.

Results

Study sample

Among all 8553 women enrolled in the BBC up to 2016, there were 1605 women that contributed either maternal and/or cord blood. We excluded 108 women who were missing information on covariates. We further excluded 1 woman who was missing information on both GDM and PE diagnosis, leaving us with an analytic sample of 1496 women. Of these, 922 contributed cord blood and 1324 contributed maternal blood (750 contributed both). In total, 876 women contributed to the analyses of these metabolites in cord blood with GDM and 921 with PE; 1262 contributed to the analyses of the maternal blood metabolites with GDM and 1324 with PE (Figure 2).

Participant characteristics of the women contributing cord blood overall and stratified by cord TMAO tertile are shown in Table 1. Women included in the analytic sample were enrolled between 1998-2013. The median age was 27.9 years. The majority of women were Black (57.9%) or Hispanic (23.8%). Most had a high school education or equivalent (38.5%) or greater (33.5%) and approximately one third were married (34.5%). The median pre-pregnancy BMI was 25.3 kg/m^2. Most women (58.7%) had at least one previous live birth. Approximately 6% reported pre-existing chronic hypertension and 4.9% pre-pregnancy diabetes (4.9%).

Women with higher cord blood TMAO were more likely to be older, have a history of diabetes, and have lower educational attainment. Women with higher cord TMAO were also more likely to be multiparous vs. nulliparous (61.3% vs. 38.7%). Race/ethnicity, marital status, pre-pregnancy BMI, and the prevalence of pre-pregnancy chronic hypertension were similar across cord TMAO tertiles. Details of the study sample

stratified by other metabolites (both in cord blood and maternal blood) are shown in Tables 2-8.

Inter-metabolite correlations

Spearman's rank correlations among metabolites were stronger within cord blood (r=0.38-0.87) than within maternal blood (r=0.08-0.62; see Figure 3). Correlations between the same metabolite measured in cord vs. maternal blood was low for choline (r=0.09) and betaine (r=0.08) and moderate for TMAO (r=0.29) and carnitine (r=0.23). There was low correlation between other metabolites across maternal and cord blood (r=-0.03-0.09).

Gestational diabetes and pre-eclampsia

In our analytic sample, 115 women developed GDM and 158 developed PE (Table 9). Among the maternal metabolites analyzed, TMAO was associated with higher odds of GDM (3^{rd} vs. 1^{st} tertile multivariable-adjusted OR=1.75; 95% CI 1.04, 2.94), while TMAO precursors (choline, betaine, and carnitine) were not associated with GDM (OR=0.78; 95% CI 0.47, 1.30; OR=0.76; 95% CI 0.45, 1.28; OR=0.63; 95% CI 0.37, 1.07, respectively; see Figure 4 and Table 10). Concentrations of TMAO and choline in maternal blood were not associated with PE (OR=0.95; 95% CI 0.59, 1.51; OR=0.84; 95% CI 0.53, 1.34), while maternal blood carnitine was associated with higher odds of PE (OR=1.92; 95% CI 1.21, 3.04) and betaine was associated with lower odds of PE (OR=0.37; 95% CI 0.23, 0.59). In cord blood, TMAO was not associated with GDM or PE (OR=0.88; 95% CI 0.47, 1.63 for GDM; OR=1.21; 95% CI 0.66, 2.23 for PE), but TMAO precursors choline, betaine, and carnitine were associated with higher odds of PE (OR=3.11; 95% CI 1.62, 5.96; OR=2.65; 95% CI 1.42, 4.93); OR=2.56; 95% CI 1.39,

4.69, respectively). Cord blood choline was associated with lower odds of GDM (OR=0.52; 95% CI 0.27, 0.99); other cord blood metabolites were not associated with GDM (OR=0.88; 95% CI 0.47, 1.63; OR=0.68; 95% CI 0.37, 1.24; OR=0.55; 95% CI 0.30, 1.02, for TMAO, betaine, and carnitine, respectively). Results modeling these metabolites as continuous variables were consistent with the results according to tertiles (Table 11). Results were consistent across strata of self-reported race (Black vs. all other race/ethnicities) and we found no statistical evidence of exposure-by-race interactions (all P>0.05; see Table 12).

Sensitivity analyses

Among women entering pregnancy without hypertension, diabetes, or either, odds of PE were still increased among those in the highest vs. lowest tertile of cord choline, betaine, and carnitine (Table 13). Sensitivity analyses for maternal blood betaine and carnitine with PE, and maternal blood TMAO with GDM were also consistent with the main analyses (Table 13).

The association of maternal TMAO with GDM was robust to adjustment for precursor metabolites (multivariable-adjusted OR per SD-increment = 1.23; 95% CI 1.00, 1.52; Table 14). The inverse association of cord choline with GDM was also robust to adjustment for other cord metabolites (OR=0.62; 95% CI 0.35, 1.07). The associations of maternal betaine and carnitine with PE were both preserved when adjusting for all other exposures (OR=0.46; 95% CI 0.36, 0.59 and OR=1.86; 95% CI 1.49, 2.31, respectively). Finally, the associations of cord blood betaine and carnitine with PE disappear when adjusting for other metabolites, but the association of choline with PE remains (OR=1.69; 95% CI 1.02, 2.79).

In terms of potential selection bias, we found some differences in participant characteristics between women in the entire cohort vs. women selected for the metabolite sub-study (Tables 15-16), including differences in race, education, pre-pregnancy BMI, pre-pregnancy chronic hypertension, and pre-pregnancy diabetes. Those who had metabolites analyzed were also more likely to be Black and less likely to be white or Hispanic compared to those who did not have metabolites analyzed. They were also more likely to have a high school education or equivalent. They had higher BMI and were more likely to enter into pregnancy with type 1 or type 2 diabetes. Those who contributed cord blood were more likely to enter into pregnancy with chronic hypertension than those who did not contribute cord blood. To address this potential selection bias we conducted inverse probability weighting for selection into the sample, and we found that the estimates were similar to our main model (Table 17).

Discussion

In our urban, racially and ethnically diverse, and predominantly low-income population, we found that TMAO concentration in maternal blood was associated with higher odds of GDM, while cord blood choline was associated with lower odds. TMAO in cord or maternal blood was not associated with risk of PE, however, higher concentrations of cord blood choline, betaine, and carnitine and maternal carnitine were associated with greater odds of PE, while higher maternal blood betaine was associated with lower odds of PE.

Our finding of a positive dose-response-like association of maternal TMAO with GDM adds to a mixed literature base on this association. Two studies, both case-control

studies conducted in China using maternal blood, have reported on TMAO and GDM. Li and colleagues (2018) conducted both a nested (433 cases and 433 controls) and a non-nested (276 cases and 552 controls) matched case-control study.[22] In the non-nested case-control study, women in the highest quartile of maternal TMAO (measured at the same time as GDM diagnosis, between 24-32 weeks gestation) had 1.94 times (95% CI 1.28, 2.93) the odds of GDM compared to those in the lowest quartile, after controlling for potential confounders. The nested study, which collected maternal blood at the first prenatal care visit (up to 16 weeks gestation), found similar results (OR for highest vs. lowest quartile = 2.06; 95% CI 1.28, 3.31; p for trend <0.01).[22] Huo and colleagues (2019) conducted an age-matched, nested case-control study (243 cases and 243 controls), with maternal blood collected at approximately 10 weeks gestation.[21] Contrary our findings, and those of Li and colleagues, Huo et al. found an inverse association of maternal TMAO and GDM, which tended toward U-shaped.[21,22] Additionally, they found an inverse association of maternal betaine and l-carnitine with GDM, and a U-shaped association for choline chloride.[21] Our study found similar patterns for betaine and carnitine, though neither reached statistical significance. It may be possible that the TMAO precursors are harmless or even protective against GDM, but it is their conversion to TMAO, dependent on the composition of the gut microbiome, that is associated with GDM. Future studies might aim to disentangle whether these associations are modified by gut microbiome composition.

Contrary to existing studies, we did not find an association of maternal TMAO with PE. Other studies, again case-control studies conducted in China using maternal blood, have found positive associations between maternal TMAO and PE. Wen and colleagues

(2019) found that third trimester TMAO was higher among women with PE, and tended to be higher in a dose-dependent manner with severity of PE.[24] Wang and colleagues (2019) found that TMAO was higher among women with PE compared to their gestational age-matched controls.[23] Huang and colleagues (2020) found that TMAO measured during the second trimester was not associated with PE but TMAO measured at delivery was strongly associated with PE, early-onset PE, and severe PE.[20] This discrepancy in findings may be partially explained by differences in the timing of blood collection, assay methods, or underlying confounding structure between the study populations.

In addition to TMAO, our finding that cord carnitine was associated with higher odds of PE in both maternal and cord blood is in line with the results of existing studies.[25-27] Jääskelainen and colleagues (2018) and Ryckman and colleagues (2013) found higher carnitine in cord blood and in neonatal dried blood spots, respectively, of infants born to mothers with PE.[26,27] Our finding that maternal carnitine was associated with higher odds of PE replicates the findings of Thiele and colleagues (2004).[25] Finally, our finding that cord choline is associated with higher odds of PE aligns with Jääskelainen and colleagues' finding of the higher cord blood choline among infants born to women with PE.[27] Our finding that, after adjustment for other cord blood metabolites, only choline remained significantly associated with PE suggests that of choline, betaine, and carnitine, choline may be the most important for PE pathology—a finding that deserves further investigation.

Our finding that several metabolites trended in opposite directions, depending on whether they were measured in cord blood vs. maternal blood, was an unexpected

observation that merits further attention. This was most evident for betaine, which was positively associated odds of PE when measured in cord blood, but inversely associated with PE odds when measured in maternal blood. Metabolites may be differentially associated with these outcomes depending on whether they were measured in cord or maternal blood due to cross-placental transfer of these metabolites. For example, folate, an essential nutrient for both mother and fetus, is preferentially allocated to the fetus, even when maternal supply is low, perhaps due to its involvement in neural tube development among other essential functions.[40-42] Both choline and betaine are important methyl donors, but the use of betaine as a methyl donor may be increased during pregnancy.[43] As such, it is possible that other essential nutrients are allocated similarly, and that an increased demand for betaine, along with other TMAO precursors, during pregnancy contributes to lower circulating levels among those with low supply. Further, choline, carnitine, and betaine were highly correlated in cord blood, while betaine in cord blood was not strongly correlated with betaine in maternal blood; in a model including all cord blood metabolites, only the association of choline with PE remained significant, suggesting that cord betaine appeared associated with PE due to its correlation with cord blood choline, rather than because of its own contribution to PE risk. Maternal blood betaine, on the other hand, remained inversely associated with PE when including all metabolites in the regression model, suggesting maternal betaine status may play a more important role in the development of PE than does cord blood betaine. It should be noted that it is also possible that regression adjustment for other metabolites is not the most etiologically accurate way to model this association, and that sums or ratios of these metabolites may better reflect PE risk; however, such analyses

were beyond the scope of this paper. Finally, it is possible that our finding occurred by chance, and therefore needs to be replicated in other studies.

Interestingly, we found stronger correlation between these metabolites measured in cord blood as compared to maternal blood. Because we measured these metabolites in red blood cells, which have a lifespan of nearly 4 months, we expect that our measurements reflect metabolite concentrations during the third trimester; however, since cord blood was collected at birth but maternal blood was collected 1-3 days later, it is possible that measurements in maternal blood might be affected by one's diet in the hospital, which may be different than their usual diet. The stronger correlation between metabolites measured in cord blood compared to those measured in maternal blood could be a product of this differential timing of sample collection, and suggests that cord blood may be a better matrix to measure TMAO and its precursors for maternal pregnancy complications.

This study is not without limitations. First, blood was taken at or shortly after delivery rather than prior to or simultaneously with diagnosis of GDM or PE. Among non-pregnant adults, choline and betaine have been shown to be quite stable over one year (r=0.81 and r=0.61, respectively), but TMAO was more variable (r=0.29).[44] While we attempted to mitigate the possibility for reverse causation by using red blood cells, it is possible that changes to the diet during pregnancy as a result of diagnosis of these conditions, or even changes to the diet in the hospital, are reflected in the measured values. Mothers with GDM or PE may have been hospitalized longer prior to delivery than mothers with uncomplicated pregnancies, potentially inducing an association between the metabolites and these conditions. Second, even with the red blood cells

ideally reflecting metabolite levels during the third trimester, our data are cross-sectional and so we cannot rule out the possibility that differences in metabolite concentrations are the result of the cardiometabolic conditions, rather than vice-versa. To address this limitation, future studies should aim to measure these metabolites early in pregnancy and to describe not only differences in cord and maternal metabolite concentrations between-person, but within-person differences over the course of pregnancy. Third, since TMAO, choline, betaine, and carnitine are in the same metabolic pathway, future studies may aim to use more advanced methods to examine the effects of summed exposures on cardiometabolic disease risk, rather than regression adjustment to look at each metabolite in isolation. Methods for assessing exposure mixtures, such as quantile g-computation or Bayesian kernel machine regression, should be used to assess the effects of summed exposures and their components.[45-49]

Despite these limitations, this study has a number of strengths. As mentioned, this is the first study to our knowledge to consider the group of precursors in addition to TMAO as potential risk factors for GDM and PE and, further, the first to our knowledge to conduct a targeted analysis of any of these metabolites in cord blood. Second, we have objective measures of these metabolites which, while they are largely influenced by diet, unlike diet they are not subject to recall or social desirability biases. Third, our sample size is larger than many existing studies of TMAO and these outcomes. This allowed us to adjust for a number of potential confounders and to conduct race-specific analyses and to test for effect measure modification by race/ethnicity. Fourth, and relatedly, our study population contains a large proportion of Black and Hispanic mothers, low-income mothers, and mothers with urban residence. These populations have been historically

under-represented in research, and our population is distinct from the populations of existing studies on TMAO and these outcomes. It is important to examine these associations in diverse populations because sources of TMAO may differ between populations, and sources of TMAO may matter for disease risk. For example, TMAO is contained in fish and other seafoods, though fish has paradoxically been associated with lower cardiometabolic risk.[50-53] Further, it is important that we examined these associations in a cohort of predominantly Black mothers since Black mothers experience pregnancy complications and maternal and perinatal morbidity and mortality at disproportionate rates in the U.S.

Conclusion

In our urban, racially and ethnically diverse cohort, we found that TMAO in maternal blood and related metabolites in cord blood are associated with development of GDM and PE. These findings add to the evidence that TMAO and related metabolites play a role in cardiometabolic disease pathology during pregnancy. Future studies using longitudinal data are warranted to establish temporality of these associations, as well as studies examining these exposures in relation to the components of GDM and PE (e.g., blood pressure, proteinuria, blood glucose). As understanding of dietary and microbiome contributors to TMAO and these related metabolites advances, we may hope to prevent cardiometabolic disease in pregnancy and beyond through targeted interventions.

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Appendix A: Tables

Table 1: Characteristics of study participants by cord blood TMAO tertile

			Cord TMAO Tertile	
	Overall (n=1000)	**Lowest (n=334)**	**Middle (n=333)**	**Highest (n=333)**
Cord TMAO units	0 (-3.29, 3.29)	-0.97 (-3.29, -0.43)	0.00 (-0.43, 0.43)	0.97 (0.43, 3.29)
Maternal age (years)	27.9 (22.9-33.4)	26.6 (21.9-31.3)	28.1 (23.1-33.5)	29.7 (24.4-34.9)
Missing age	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Race/ethnicity				
Black	579 (57.9)	189 (56.6)	185 (55.6)	205 (61.6)
White	54 (5.4)	17 (5.1)	19 (5.7)	18 (5.4)
Hispanic	238 (23.8)	75 (22.5)	84 (25.2)	79 (23.7)
Other	127 (12.7)	52 (15.6)	44 (13.2)	31 (9.3)
Missing race/ethnicity	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Education				
< High school	272 (27.2)	106 (31.7)	92 (27.6)	74 (22.2)
High school or equivalent	385 (38.5)	114 (34.1)	125 (37.5)	146 (43.8)
> High school	335 (33.5)	111 (33.2)	112 (33.6)	112 (33.6)
Missing education	8 (0.8)	3 (0.9)	4 (1.2)	1 (0.3)
Marital status				
Unmarried	642 (64.2)	213 (63.8)	207 (62.2)	222 (66.7)
Married	345 (34.5)	118 (35.3)	121 (36.3)	106 (31.8)
Missing marital status	13 (1.3)	3 (0.9)	5 (1.5)	5 (1.5)
Pre-pregnancy BMI (kg/m2)	25.3 (22.3-29.8)	24.9 (22.0-29.3)	25.7 (22.2-30.2)	25.2 (22.5-29.3)
Missing BMI	57 (5.7)	14 (4.2)	23 (6.9)	20 (6.0)
Parity				
0	411 (41.1)	151 (45.2)	131 (39.3)	129 (38.7)
≥1	587 (58.7)	182 (54.5)	201 (60.4)	204 (61.3)
Missing parity	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Chronic hypertension	60 (6.0)	22 (6.6)	16 (4.8)	22 (6.7)
Missing hypertension	7 (0.7)	2 (0.6)	2 (0.6)	3 (0.3)
Diabetes (1 or 2)	49 (4.9)	13 (3.9)	16 (4.8)	20 (6.0)
Missing diabetes	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)

TMAO = Trimethylamine N-oxide; BMI = body mass index.

Median (IQR) is presented for continuous variables; N (%) is reported for categorical variables

Table 2: Characteristics of study participants by cord blood choline tertile

		Cord Choline Tertile		
	Overall (n=1000)	**Lowest (n=334)**	**Middle (n=333)**	**Highest (n=333)**
Cord Choline units, Median (range)	0.00 (-0.67, 0.67)	-0.97 (-1.38, -0.67)	0.00 (-0.21, 0.21)	0.97 (0.67, 1.38)
Maternal age (years)	27.9 (22.9-33.4)	27.7 (22.9-33.0)	28.0 (22.8-33.9)	28.1 (23.5-33.2)
Missing age	2 (0.2)	0 (0.0)	2 (0.6)	0 (0.0)
Race/ethnicity				
Black	579 (57.9)	195 (58.4)	199 (59.8)	185 (55.6)
White	54 (5.4)	12 (3.6)	19 (5.7)	23 (6.9)
Hispanic	238 (23.8)	64 (19.2)	80 (24.0)	94 (28.2)
Other	127 (12.7)	63 (18.9)	33 (9.9)	31 (9.3)
Missing race/ethnicity	2 (0.2)	0 (0.0)	2 (0.6)	0 (0.0)
Education				
< High school	272 (27.2)	83 (24.9)	93 (27.9)	96 (28.8)
High school or equivalent	385 (38.5)	136 (40.7)	125 (37.5)	124 (37.2)
> High school	335 (33.5)	113 (33.8)	110 (33.0)	112 (33.6)
Missing education	8 (0.8)	2 (0.6)	5 (1.5)	1 (0.3)
Marital status				
Unmarried	642 (64.2)	211 (63.2)	216 (64.9)	215 (64.6)
Married	345 (34.5)	122 (36.5)	110 (33.0)	113 (33.9)
Missing marital status	13 (1.3)	1 (0.3)	7 (2.1)	5 (1.5)
BMI (kg/m2)	25.3 (22.3-29.8)	24.9 (22.2-29.1)	25.4 (22.4-30.0)	25.7 (22.2-30.0)
Missing BMI	57 (5.7)	17 (5.1)	21 (6.3)	19 (5.7)
Parity				
0	411 (41.1)	119 (35.6)	146 (43.8)	146 (43.8)
≥1	587 (58.7)	215 (64.4)	185 (55.6)	187 (56.2)
Missing parity	2 (0.2)	0 (0.0)	2 (0.6)	0 (0.0)
Chronic hypertension	60 (6.0)	14 (4.2)	22 (6.6)	24 (7.2)
Missing hypertension	7 (0.7)	1 (0.3)	5 (1.5)	1 (0.3)
Diabetes (1 or 2)	49 (4.9)	11 (3.3)	18 (5.4)	20 (6.0)
Missing diabetes	2 (0.2)	0 (0.0)	2 (0.6)	0 (0.0)

Median (IQR) for continuous variables; N (%) for categorical variables

Table 3: Characteristics of study participants by cord blood betaine tertile

		Cord Betaine Tertile		
	Overall (n=1000)	**Lowest (n=334)**	**Middle (n=333)**	**Highest (n=333)**
Cord Betaine units, Median (range)	0.00 (-0.67, 0.67)	-0.97 (-1.38, -0.67)	0.00 (-0.21, 0.21)	0.97 (0.67, 1.38)
Maternal age (years)	27.9 (22.9-33.4)	27.6 (22.5-33.0)	28.2 (23.0-33.6)	28.0 (23.3-33.3)
Missing age	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Race/ethnicity				
Black	579 (57.9)	203 (60.8)	198 (59.5)	178 (53.5)
White	54 (5.4)	11 (3.3)	20 (6.0)	23 (6.9)
Hispanic	238 (23.8)	58 (17.4)	82 (24.6)	98 (29.4)
Other	127 (12.7)	61 (18.3)	32 (9.6)	34 (10.2)
Missing race/ethnicity	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Education				
< High school	272 (27.2)	88 (26.3)	95 (28.5)	89 (26.7)
High school or equivalent	385 (38.5)	134 (40.1)	116 (34.8)	135 (40.5)
> High school	335 (33.5)	108 (32.3)	120 (36.0)	107 (32.1)
Missing education	8 (0.8)	4 (1.2)	2 (0.6)	2 (0.6)
Marital status				
Unmarried	642 (64.2)	225 (67.4)	198 (59.5)	219 (65.8)
Married	345 (34.5)	107 (32.0)	126 (37.8)	112 (33.6)
Missing marital status	13 (1.3)	2 (0.6)	9 (2.7)	2 (0.6)
BMI (kg/m2)	25.3 (22.3-29.8)	24.9 (22.2-29.1)	25.0 (22.3-30.0)	26.1 (22.3-30.0)
Missing BMI	57 (5.7)	18 (5.4)	23 (6.9)	16 (4.8)
Parity				
0	411 (41.1)	115 (34.4)	137 (41.1)	159 (47.7)
≥1	587 (58.7)	218 (65.3)	195 (58.6)	174 (52.3)
Missing parity	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Chronic hypertension	60 (6.0)	14 (4.2)	19 (5.7)	27 (8.1)
Missing hypertension	7 (0.7)	4 (1.2)	3 (0.9)	0 (0.0)
Diabetes (1 or 2)	49 (4.9)	6 (1.8)	24 (7.2)	19 (5.7)
Missing diabetes	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)

Median (IQR) for continuous variables; N (%) for categorical variables

Table 4: Characteristics of study participants by cord blood carnitine tertile

		Cord Carnitine Tertile		
	Overall (n=1000)	**Lowest (n=334)**	**Middle (n=333)**	**Highest (n=333)**
Cord Carnitine units, Median (range)	0.00 (-0.67, 0.67)	-0.97 (-1.38, -0.67)	0.00 (-0.21, 0.21)	0.97 (0.67, 1.38)
Maternal age (years)	27.9 (22.9-33.4)	28.0 (23.0-33.4)	27.7 (22.8-33.5)	27.8 (23.1-33.2)
Missing age	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Race/ethnicity				
Black	579 (57.9)	189 (56.6)	180 (54.1)	210 (63.1)
White	54 (5.4)	14 (4.2)	23 (6.9)	17 (5.1)
Hispanic	238 (23.8)	73 (21.9)	85 (25.5)	80 (24.0)
Other	127 (12.7)	57 (17.1)	44 (13.2)	26 (7.8)
Missing race/ethnicity	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Education				
< High school	272 (27.2)	91 (27.2)	100 (30.0)	81 (24.3)
High school or equivalent	385 (38.5)	133 (39.8)	113 (33.9)	139 (41.7)
> High school	335 (33.5)	106 (31.7)	119 (35.7)	110 (33.0)
Missing education	8 (0.8)	4 (1.2)	1 (0.3)	3 (0.9)
Marital status				
Unmarried	642 (64.2)	215 (64.4)	215 (64.6)	212 (63.7)
Married	345 (34.5)	117 (35.0)	111 (33.3)	117 (35.1)
Missing marital status	13 (1.3)	2 (0.6)	7 (2.1)	4 (1.2)
BMI (kg/m2)	25.3 (22.3-29.8)	24.9 (22.4-29.0)	25.0 (22.2-30.0)	25.8 (22.3-29.8)
Missing BMI	57 (5.7)	17 (5.1)	20 (6.0)	20 (6.0)
Parity				
0	411 (41.1)	118 (35.3)	135 (40.5)	158 (47.4)
≥1	587 (58.7)	215 (64.4)	197 (59.2)	175 (52.6)
Missing parity	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)
Chronic hypertension	60 (6.0)	12 (3.6)	22 (6.6)	26 (7.8)
Missing hypertension	7 (0.7)	3 (0.9)	3 (0.9)	1 (0.3)
Diabetes (1 or 2)	49 (4.9)	13 (3.9)	18 (5.4)	18 (5.4)
Missing diabetes	2 (0.2)	1 (0.3)	1 (0.3)	0 (0.0)

Median (IQR) for continuous variables; N (%) for categorical variables

Table 5: Characteristics of study participants by maternal blood TMAO tertile

		Maternal TMAO Tertile		
	Overall (n=1412)	**Lowest (n=471)**	**Middle (n=471)**	**Highest (n=470)**
Maternal TMAO units, Median (range)	0.00 (-0.67, 0.67)	-0.97 (-1.38, -0.67)	0.00 (-0.21, 0.21)	0.97 (0.67, 1.38)
Maternal age (years)	28.2 (22.9-33.5)	26.8 (21.9-32.6)	27.9 (22.8-33.3)	29.6 (24.7-34.6)
Missing age	1 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)
Race/ethnicity				
Black	909 (64.4)	311 (66.0)	307 (65.2)	291 (61.9)
White	56 (4.0)	13 (2.8)	24 (5.1)	19 (4.0)
Hispanic	292 (20.7)	88 (18.7)	93 (19.7)	111 (23.6)
Other	154 (10.9)	58 (12.3)	47 (10.0)	49 (10.4)
Missing race/ethnicity	1 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)
Education				
< High school	387 (27.4)	129 (27.4)	131 (27.8)	127 (27.0)
High school or equivalent	535 (37.9)	182 (38.6)	169 (35.9)	184 (39.1)
> High school	483 (34.2)	156 (33.1)	170 (36.1)	157 (33.4)
Missing education	7 (0.5)	4 (0.8)	1 (0.2)	2 (0.4)
Marital status				
Unmarried	934 (66.1)	314 (66.7)	317 (67.3)	303 (64.5)
Married	464 (32.9)	149 (31.6)	149 (31.6)	166 (35.3)
Missing marital status	14 (1.0)	8 (1.7)	5 (1.1)	1 (0.2)
BMI (kg/m2)	25.5 (22.1-30.0)	24.9 (21.6-29.1)	25.8 (22.4-30.1)	25.7 (22.4-30.0)
Missing BMI	63 (4.5)	22 (4.7)	14 (3.0)	27 (5.7)
Parity				
0	619 (43.8)	233 (49.5)	204 (43.3)	182 (38.7)
≥1	792 (56.1)	237 (50.3)	267 (56.7)	288 (61.3)
Missing parity	1 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)
Chronic hypertension	99 (7.0)	27 (5.7)	30 (6.4)	42 (8.9)
Missing hypertension	9 (0.6)	5 (1.1)	1 (0.2)	3 (0.6)
Diabetes (1 or 2)	68 (4.8)	15 (3.2)	23 (4.9)	30 (6.4)
Missing diabetes	1 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)

Median (IQR) for continuous variables; N (%) for categorical variables

Table 6: Characteristics of study participants by maternal blood choline tertile

		Maternal Choline Tertile		
	Overall (n=1412)	**Lowest (n=471)**	**Middle (n=471)**	**Highest (n=470)**
Maternal Choline units, Median (range)	0.00 (-0.67, 0.67)	-0.97 (-1.38, -0.67)	0.00 (-0.21, 0.21)	0.97 (0.67, 1.38)
Maternal age (years)	28.2 (22.9-33.5)	27.6 (22.5-33.2)	28.8 (23.1-34.2)	28.1 (23.0-33.3)
Missing age	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)
Race/ethnicity				
Black	909 (64.4)	314 (66.7)	305 (64.8)	290 (61.7)
White	56 (4.0)	15 (3.2)	19 (4.0)	22 (4.7)
Hispanic	292 (20.7)	87 (18.5)	92 (19.5)	113 (24.0)
Other	154 (10.9)	55 (11.7)	55 (11.7)	44 (9.4)
Missing race/ethnicity	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)
Education				
< High school	387 (27.4)	115 (24.4)	135 (28.7)	137 (29.1)
High school or equivalent	535 (37.9)	186 (39.5)	177 (37.6)	172 (36.6)
> High school	483 (34.2)	168 (35.7)	156 (33.1)	159 (33.8)
Missing education	7 (0.5)	2 (0.4)	3 (0.6)	2 (0.4)
Marital status				
Unmarried	934 (66.1)	323 (68.6)	312 (66.2)	299 (63.6)
Married	464 (32.9)	142 (30.1)	154 (32.7)	168 (35.7)
Missing marital status	14 (1.0)	6 (1.3)	5 (1.1)	3 (0.6)
BMI (kg/m2)	25.5 (22.1-30.0)	24.9 (21.7-29.1)	26.4 (22.4-30.9)	25.6 (22.3-29.8)
Missing BMI	63 (4.5)	16 (3.4)	19 (4.0)	28 (6.0)
Parity				
0	619 (43.8)	207 (43.9)	201 (42.7)	211 (44.9)
≥1	792 (56.1)	264 (56.1)	270 (57.3)	258 (54.9)
Missing parity	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)
Chronic hypertension	99 (7.0)	36 (7.6)	30 (6.4)	33 (7.0)
Missing hypertension	9 (0.6)	2 (0.4)	4 (0.8)	3 (0.6)
Diabetes (1 or 2)	68 (4.8)	26 (5.5)	18 (3.8)	24 (5.1)
Missing diabetes	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)

Median (IQR) for continuous variables; N (%) for categorical variables

Table 7: Characteristics of study participants by maternal blood betaine tertile

		Maternal Betaine Tertile		
	Overall (n=1412)	**Lowest (n=471)**	**Middle (n=471)**	**Highest (n=470)**
Maternal Betaine units, Median (range)	0.00 (-0.67, 0.67)	-0.97 (-1.38, -0.67)	0.00 (-0.21, 0.21)	0.97 (0.67, 1.38)
Maternal age (years)	28.2 (22.9-33.5)	28.6 (22.6-33.5)	28.2 (23.3-33.7)	27.8 (22.8-33.3)
Missing age	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)
Race/ethnicity				
Black	909 (64.4)	312 (66.2)	292 (62.0)	305 (64.9)
White	56 (4.0)	12 (2.5)	20 (4.2)	24 (5.1)
Hispanic	292 (20.7)	99 (21.0)	104 (22.1)	89 (18.9)
Other	154 (10.9)	48 (10.2)	55 (11.7)	51 (10.9)
Missing race/ethnicity	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)
Education				
< High school	387 (27.4)	133 (28.2)	133 (28.2)	121 (25.7)
High school or equivalent	535 (37.9)	179 (38.0)	183 (38.9)	173 (36.8)
> High school	483 (34.2)	156 (33.1)	153 (32.5)	174 (37.0)
Missing education				
Marital status				
Unmarried	934 (66.1)	311 (66.0)	312 (66.2)	311 (66.2)
Married	464 (32.9)	152 (32.3)	156 (33.1)	156 (33.2)
Missing marital status	14 (1.0)	8 (1.7)	3 (0.6)	3 (0.6)
BMI (kg/m2)	25.5 (22.1-30.0)	25.3 (22.1-29.3)	25.6 (22.3-30.0)	25.6 (22.0-30.2)
Missing BMI	63 (4.5)	22 (4.7)	15 (3.18)	26 (5.5)
Parity				
0	619 (43.8)	198 (42.0)	208 (44.2)	213 (45.3)
≥1	792 (56.1)	273 (58.0)	263 (55.8)	256 (54.5)
Missing parity	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)
Chronic hypertension	99 (7.0)	41 (8.7)	34 (7.2)	24 (5.1)
Missing hypertension	9 (0.6)	3 (0.6)	2 (0.4)	4 (0.9)
Diabetes (1 or 2)	68 (4.8)	25 (5.3)	18 (3.8)	25 (5.3)
Missing diabetes	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.2)

Median (IQR) for continuous variables; N (%) for categorical variables

Table 8: Characteristics of study participants by maternal blood carnitine tertile

		Maternal Carnitine Tertile		
	Overall (n=1412)	**Lowest (n=471)**	**Middle (n=471)**	**Highest (n=470)**
Maternal Carnitine units, Median (range)	0.00 (-0.67, 0.67)	-0.97 (-1.38, -0.67)	0.00 (-0.21, 0.21)	0.97 (0.67, 1.38)
Maternal age (years)	28.2 (22.9-33.5)	27.9 (22.6-33.4)	28.4 (23.2-33.7)	28.1 (22.9-33.6)
Missing age	1 (0.1)	0 (0.0)	1 (0.2)	0 (0.0)
Race/ethnicity				
Black	909 (64.4)	322 (68.4)	281 (59.7)	306 (65.1)
White	56 (4.0)	18 (3.8)	21 (4.5)	17 (3.6)
Hispanic	292 (20.7)	93 (19.7)	105 (22.3)	94 (20.0)
Other	154 (10.9)	38 (8.1)	63 (13.4)	53 (11.3)
Missing race/ethnicity	1 (0.1)	0 (0.0)	1 (0.2)	0 (0.0)
Education				
< High school	387 (27.4)	124 (26.3)	129 (27.4)	134 (28.5)
High school or equivalent	535 (37.9)	171 (36.3)	177 (37.6)	187 (39.8)
> High school	483 (34.2)	173 (36.7)	161 (34.2)	149 (31.7)
Missing education	7 (0.5)	3 (0.6)	4 (0.8)	0 (0.0)
Marital status				
Unmarried	934 (66.1)	311 (66.0)	315 (66.9)	308 (65.5)
Married	464 (32.9)	154 (32.7)	155 (32.9)	155 (33.0)
Missing marital status	14 (1.0)	6 (1.3)	1 (0.2)	7 (1.5)
BMI (kg/m2)	25.5 (22.1-30.0)	25.2 (22.0-29.8)	25.7 (22.3-30.2)	25.3 (22.1-30.0)
Missing BMI	63 (4.5)	16 (3.4)	24 (5.1)	23 (4.9)
Parity				
0	619 (43.8)	200 (42.5)	195 (41.4)	224 (47.7)
≥1	792 (56.1)	271 (57.5)	275 (58.4)	246 (52.3)
Missing parity	1 (0.1)	0 (0.0)	1 (0.2)	0 (0.0)
Chronic hypertension	99 (7.0)	37 (7.9)	27 (5.7)	35 (7.4)
Missing hypertension	9 (0.6)	3 (0.6)	4 (0.8)	2 (0.4)
Diabetes (1 or 2)	68 (4.8)	25 (5.3)	18 (3.8)	25 (5.3)
Missing diabetes	1 (0.1)	0 (0.0)	1 (0.2)	0 (0.0)

Median (IQR) for continuous variables; N (%) for categorical variables

Table 9: Sample sizes and number of cases for each exposure-outcome pairing

		Analytic sample overall	Gestational diabetes				Pre-eclampsia			
				Metabolite tertile				Metabolite tertile		
			Overall	Lowest	Middle	Highest	Overall	Lowest	Middle	Highest
Cord blood	**Cord TMAO**	n = 1496 115 cases GDM 158 cases PE	n=876 76 cases				n=921 85 cases			
	n			304	288	284		315	302	304
	Cases			26	27	23		23	31	31
	Cord choline									
	n			303	285	288		312	302	307
	Cases			30	28	18		14	26	45
	Cord betaine									
	n			304	277	295		309	299	313
	Cases			31	20	25		16	21	48
	Cord carnitine									
	n			299	289	288		312	304	305
	Cases			31	24	21		17	21	47
Maternal blood	**Maternal TMAO**		n=1262 103 cases				n=1324 142 cases			
	n			422	429	411		436	450	438
	Cases			26	31	46		44	49	49
	Maternal choline									
	n			420	425	417		445	441	438
	Cases			37	33	33		49	52	41
	Maternal betaine									
	n			413	432	417		436	450	438
	Cases			36	37	30		70	43	29
	Maternal carnitine									
	n			419	426	417		443	443	438
	Cases			38	37	28		37	41	64

TMAO = trimethylamine N-oxide; GDM = gestational diabetes mellitus; PE = pre=eclampsia

Table 10: [a] Multivariable-adjusted models adjusted for maternal age (years, continuous), race/ethnicity (Black, white, Hispanic, other), educational attainment (less than high school, high school or equivalent, greater than high school), marital status (unmarried, married), pre-pregnancy BMI (kg/m2, continuous), parity (0, ≥1), chronic pre-pregnancy hypertension (no/yes), and pre-pregnancy diabetes (no/yes, for pre-eclampsia models only)

Table 10: Associations of metabolites with gestational diabetes and pre-eclampsia in reference to the lowest tertile

	Unadjusted			Multivariable adjusted[a]		
Gestational diabetes	**Lowest**	**Middle**	**Highest**	**Lowest**	**Middle**	**Highest**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Cord TMAO	REF	1.11 (0.63, 1.94)	0.94 (0.52, 1.69)	REF	0.92 (0.51, 1.68)	0.88 (0.47, 1.63)
Cord choline	REF	0.99 (0.54, 1.62)	0.57 (0.31, 1.06)	REF	0.92 (0.52, 1.62)	**0.52 (0.27, 0.99)**
Cord betaine	REF	0.69 (0.38, 1.23)	0.82 (0.47, 1.42)	REF	0.55 (0.30, 1.03)	0.68 (0.37, 1.24)
Cord carnitine	REF	0.78 (0.45, 1.37)	0.68 (0.38, 1.21)	REF	0.68 (0.38, 1.23)	0.55 (0.30, 1.02)
Maternal TMAO	REF	1.19 (0.69, 2.03)	**1.92 (1.16, 3.17)**	REF	1.12 (0.64, 1.94)	**1.75 (1.04, 2.94)**
Maternal choline	REF	0.87 (0.53, 1.42)	0.89 (0.54, 1.45)	REF	0.74 (0.45, 1.22)	0.78 (0.47, 1.30)
Maternal betaine	REF	0.98 (0.61, 1.59)	0.81 (0.49, 1.34)	REF	0.88 (0.53, 1.43)	0.76 (0.45, 1.28)
Maternal carnitine	REF	0.95 (0.59, 1.53)	0.72 (0.43, 1.20)	REF	0.83 (0.51, 1.35)	0.63 (0.37, 1.07)

	Unadjusted			Multivariable adjusted[a]		
Pre-eclampsia	**Lowest**	**Middle**	**Highest**	**Lowest**	**Middle**	**Highest**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Cord TMAO	REF	1.45 (0.83, 2.55)	1.44 (0.82, 2.53)	REF	1.45 (0.80, 2.62)	1.21 (0.66, 2.23)
Cord choline	REF	**2.01 (1.03, 3.92)**	**3.66 (1.96, 6.81)**	REF	1.70 (0.85, 3.40)	**3.11 (1.62, 5.96)**
Cord betaine	REF	1.38 (0.71, 2.71)	**3.32 (1.84, 5.98)**	REF	1.24 (0.62, 2.48)	**2.65 (1.42, 4.93)**
Cord carnitine	REF	1.29 (0.67, 2.49)	**3.16 (1.77, 5.64)**	REF	1.17 (0.59, 2.31)	**2.56 (1.39, 4.69)**
Maternal TMAO	REF	1.09 (0.71, 1.67)	1.12 (0.73, 1.73)	REF	1.05 (0.67, 1.66)	0.95 (0.59, 1.51)
Maternal choline	REF	1.08 (0.71, 1.64)	0.83 (0.54, 1.29)	REF	1.11 (0.71, 1.73)	0.84 (0.53, 1.34)
Maternal betaine	REF	**0.55 (0.37, 0.83)**	**0.37 (0.24, 0.58)**	REF	**0.51 (0.33, 0.78)**	**0.37 (0.23, 0.59)**
Maternal carnitine	REF	1.12 (0.70, 1.78)	**1.88 (1.22, 2.88)**	REF	1.22 (0.74, 2.00)	**1.92 (1.21, 3.04)**

Table 11: Continuous associations of metabolites with gestational diabetes and pre-eclampsia

Gestational diabetes	**Unadjusted[a] OR (95% CI)**	**Multivariable adjusted[ab] OR (95% CI)**
Cord TMAO	1.01 (0.80, 1.27)	0.99 (0.76, 1.28)
Cord choline	0.81 (0.64, 1.02)	**0.74 (0.57, 0.95)**
Cord betaine	0.90 (0.69, 1.10)	0.82 (0.64, 1.05)
Cord carnitine	0.82 (0.65, 1.04)	0.73 (0.56, 0.94)
Maternal TMAO	**1.24 (1.01, 1.52)**	1.21 (0.98, 1.50)
Maternal choline	0.97 (0.79, 1.19)	0.91 (0.73, 1.13)
Maternal betaine	0.94 (0.77, 1.15)	0.90 (0.73, 1.11)
Maternal carnitine	0.90 (0.73, 1.10)	0.85 (0.68, 1.05)
Pre-eclampsia	**Unadjusted[a] OR (95% CI)**	**Multivariable adjusted[ab] OR (95% CI)**
Cord TMAO	1.22 (0.98, 1.53)	1.13 (0.89, 1.45)
Cord choline	**1.87 (1.48, 2.36)**	**1.79 (1.39, 2.29)**
Cord betaine	**1.82 (1.44, 2.29)**	**1.67 (1.30, 2.14)**
Cord carnitine	**1.75 (1.39, 2.21)**	**1.61 (1.25, 2.06)**
Maternal TMAO	1.04 (0.88, 1.25)	1.00 (0.83, 1.21)
Maternal choline	0.91 (0.71, 1.17)	0.94 (0.71, 1.23)
Maternal betaine	**0.67 (0.56, 0.80)**	**0.65 (0.53, 0.78)**

[a] Per SD-increment of exposure

[b] Multivariable-adjusted models adjusted for maternal age (years, continuous), race/ethnicity (Black, white, Hispanic, other), educational attainment (less than high school, high school or equivalent, greater than high school), marital status (unmarried, married), pre-pregnancy BMI (kg/m2, continuous), parity (0, ≥1), chronic pre-pregnancy hypertension (no/yes), and pre-pregnancy diabetes (no/yes, for pre-eclampsia models only)

Table 12: Analyses for effect measure modification by race/ethnicity

	Gestational diabetes[c] OR (95% CI)			Preeclampsia[c] OR (95% CI)		
	Black[a]	**All other race/ethnicities[a]**	**P-value for interaction[b]**	**Black[a]**	**All other race/ethnicities[a]**	**P-value for interaction[b]**
Cord metabolites						
Cord TMAO	1.29 (0.57, 2.92)	0.48 (0.17, 1.35)	0.27	1.10 (0.52, 2.32)	1.86 (0.62, 5.57)	0.40
Cord choline	0.55 (0.23, 1.32)	0.48 (0.18, 1.25)	0.59	4.24 (1.72, 10.40)	2.49 (0.94, 6.57)	0.91
Cord betaine	0.74 (0.32, 1.72)	0.63 (0.27, 1.47)	0.76	3.30 (1.45, 7.52)	2.21 (0.85, 5.76)	0.89
Cord carnitine	0.94 (0.41, 2.19)	0.27 (0.10, 0.74)	0.35	2.53 (1.13, 5.67)	2.60 (1.01, 6.68)	0.40
Maternal metabolites						
Maternal TMAO	2.14 (1.10, 4.18)	1.21 (0.52, 2.81)	0.25	0.80 (0.46, 1.41)	1.79 (0.73, 4.41)	0.06
Maternal choline	0.62 (0.32, 1.21)	1.02 (0.45, 2.30)	0.90	1.00 (0.57, 1.75)	0.56 (0.23, 1.33)	0.51
Maternal betaine	0.59 (0.30, 1.16)	1.21 (0.52, 2.82)	0.40	0.42 (0.24, 0.75)	0.25 (0.10, 0.64)	1.00
Maternal carnitine	0.59 (0.30, 1.15)	0.66 (0.28, 1.57)	0.79	1.97 (1.16, 3.36)	1.74 (0.69, 4.41)	0.95

[a] Odds ratios (95% CIs) are from stratified models estimating the relative odds of gestational diabetes or pre-eclampsia among those in the highest exposure tertile in reference to those in the lowest exposure tertile, separately for Black mothers vs. all other race/ethnicities.

[b] P-values for the interaction between binary race/ethnicity (Black vs. all other race/ethnicities) x continuous exposure.

[c] Multivariable-adjusted models adjusted for maternal age (years, continuous), race/ethnicity (Black, white, Hispanic, other), educational attainment (less than high school, high school or equivalent, greater than high school), marital status (unmarried, married), pre-pregnancy BMI (kg/m2, continuous), parity (0, ≥1), chronic pre-pregnancy hypertension (no/yes), and pre-pregnancy diabetes (no/yes, for pre-eclampsia models only)

Table 13: Sensitivity analyses excluding those with pre-pregnancy hypertension and diabetes

	Gestational diabetes[a] OR (95% CI)	Pre-eclampsia[a] OR (95% CI)		
	Excluding chronic hypertension	Excluding chronic hypertension	Excluding pre-pregnancy diabetes	Excluding anyone with either
Cord metabolites				
Cord TMAO	0.85 (0.44, 1.67)	1.26 (0.65, 2.48)	1.13 (0.60, 2.15)	1.07 (0.58, 1.96)
Cord choline	0.48 (0.24, 0.95)	2.65 (1.36, 5.16)	3.03 (1.57, 5.85)	3.14 (1.64, 5.98)
Cord betaine	0.65 (0.35, 1.23)	2.34 (1.20, 4.57)	2.74 (1.44, 5.21)	2.88 (1.54, 5.40)
Cord carnitine	0.48 (0.25, 0.94)	2.79 (1.42, 5.48)	2.63 (1.41, 4.92)	2.88 (1.57, 5.29)
Maternal metabolites				
Maternal TMAO	1.64 (0.96, 1.08)	0.85 (0.51, 1.41)	0.92 (0.57, 1.49)	0.94 (0.60, 1.48)
Maternal choline	0.83 (0.49, 1.41)	0.71 (0.42, 1.21)	0.81 (0.50, 1.32)	0.82 (0.52, 1.31)
Maternal betaine	0.79 (0.46, 1.37)	0.31 (0.18, 0.53)	0.33 (0.20, 0.55)	0.34 (0.21, 0.55)
Maternal carnitine	0.63 (0.36, 1.09)	2.11 (1.25, 3.54)	2.14 (1.32, 3.47)	1.86 (1.18, 2.92)

[a] Multivariable-adjusted models adjusted for maternal age (years, continuous), race/ethnicity (Black, white, Hispanic, other), educational attainment (less than high school, high school or equivalent, greater than high school), marital status (unmarried, married), pre-pregnancy BMI (kg/m2, continuous), parity (0, ≥1), chronic pre-pregnancy hypertension (no/yes), and pre-pregnancy diabetes (no/yes, for pre-eclampsia models only)

Table 14: Sensitivity analyses including all exposures simultaneously

Gestational diabetes	Multivariable-adjusted continuous[ab] OR (95% CI)
Cord TMAO	1.17 (0.87, 1.55)
Cord choline	0.62 (0.35, 1.07)
Cord betaine	1.51 (0.87, 2.63)
Cord carnitine	0.72 (0.47, 1.11)
Maternal TMAO	**1.23 (1.00, 1.52)**
Maternal choline	0.96 (0.72, 1.29)
Maternal betaine	0.95 (0.72, 1.26)
Maternal carnitine	0.87 (0.68, 1.10)

Pre-eclampsia	Multivariable-adjusted continuous[a] OR (95% CI)
Cord TMAO	0.87 (0.66, 1.14)
Cord choline	**1.69 (1.02, 2.79)**
Cord betaine	1.02 (0.61, 1.70)
Cord carnitine	1.14 (0.77, 1.68)
Maternal TMAO	1.02 (0.83, 1.24)
Maternal choline	1.19 (0.92, 1.55)
Maternal betaine	**0.46 (0.36, 0.59)**
Maternal carnitine	**1.86 (1.49, 2.31)**

[a] Continuous measures are per SD-increment of exposure, holding all others constant, adjusting for maternal age, race/ethnicity, educational attainment, marital status, pre-pregnancy BMI, parity, chronic pre-pregnancy hypertension, and (for pre-eclampsia models only) pre-pregnancy diabetes type 1 or type 2

[b] Multivariable-adjusted models adjusted for maternal age (years, continuous), race/ethnicity (Black, white, Hispanic, other), educational attainment (less than high school, high school or equivalent, greater than high school), marital status (unmarried, married), pre-pregnancy BMI (kg/m2, continuous), parity (0, ≥1), chronic pre-pregnancy hypertension (no/yes), and pre-pregnancy diabetes (no/yes, for pre-eclampsia models only)

Table 15: Participant characteristics by selection into cord blood metabolites sub-study

	Overall (N=8553)	Not in cord metabolites sub-study (N=7553)	In cord metabolites sub-study (N=1000)
Maternal age (years)	27.8 (23.0-32.9)	27.8 (23.0-32.9)	27.9 (22.9-33.4)
Missing age			
Race/ethnicity			
Black	4,031 (47.1%)	3,452 (45.7%)	579 (57.9%)
White	1,006 (11.8%)	952 (12.6%)	54 (5.4%)
Hispanic	2,423 (28.3%)	2,185 (28.9%)	238 (23.8%)
Other	1,049 (12.3%)	922 (12.2%)	127 (12.7%)
Missing race/ethnicity	44 (0.5%)	42 (0.6%)	2 (0.2%)
Education			
< High school	2,630 (30.7%)	2,358 (31.2%)	272 (27.2%)
High school or equiv	2,793 (32.7%)	2,408 (31.9%)	385 (38.5%)
> High school	2,926 (34.2%)	2,591 (34.3%)	335 (33.5%)
Missing education	204 (2.4%)	196 (2.6%)	8 (0.8%)
Marital status			
Married	5,469 (63.9%)	4,827 (63.9%)	642 (64.2%)
Unmarried	2,871 (33.6%)	2,526 (33.4%)	345 (34.5%)
Missing marital status	213 (2.5%)	200 (2.6%)	13 (1.3%)
BMI (kg/m2)	24.7 (21.6-28.9)	24.6 (21.5-28.7)	25.3 (22.3-29.8)
Missing BMI			
Parity			
0	3,663 (42.8%)	3,252 (43.1%)	411 (41.1%)
≥1	4,846 (56.7%)	4,259 (56.4%)	587 (58.7%)
Missing parity	44 (0.5%)	42 (0.6%)	2 (0.2%)
Chronic hypertension	442 (5.2%)	382 (5.1%)	60 (6.0%)
Missing hypertension	103 (1.2%)	96 (1.3%)	7 (0.7%)
Diabetes (1 or 2)	307 (3.6%)	258 (3.4%)	49 (4.9%)
Missing diabetes	53 (0.6%)	51 (0.7%)	2 (0.2%)

Table 16: Participant characteristics by selection into maternal blood metabolites sub-study

	Overall (N=8553)	Not in maternal metabolites sub-study (N=7553)	In maternal metabolites sub-study (N=1000)
Maternal age (years)	27.8 (23.0-32.9)	27.7 (23.0-32.8)	28.2 (22.9-33.5)
Missing age			
Race/ethnicity			
Black	4,031 (47.1%)	3,122 (43.7%)	909 (64.4%)
White	1,006 (11.8%)	950 (13.3%)	56 (4.0%)
Hispanic	2,423 (28.3%)	2,131 (29.8%)	292 (20.7%)
Other	1,049 (12.3%)	895 (12.5%)	154 (10.9%)
Missing race/ethnicity	44 (0.5%)	43 (0.6%)	1 (0.1%)
Education			
< High school	2,630 (30.7%)	2,243 (31.4%)	387 (27.4%)
High school or equiv	2,793 (32.7%)	2,258 (31.6%)	535 (37.9%)
> High school	2,926 (34.2%)	2,443 (34.2%)	483 (34.2%)
Missing education	204 (2.4%)	197 (2.8%)	7 (0.5%)
Marital status			
Married	5,469 (63.9%)	4,535 (63.5%)	934 (66.1%)
Unmarried	2,871 (33.6%)	2,407 (33.7%)	464 (32.9%)
Missing marital status	213 (2.5%)	199 (2.8%)	14 (1.0%)
BMI (kg/m2)	24.7 (21.6-28.9)	24.5 (21.5-28.6)	25.5 (22.1-30.0)
Missing BMI			
Parity			
0	3,663 (42.8%)	3,044 (42.6%)	619 (43.8%)
≥1	4,846 (56.7%)	4,054 (56.8%)	792 (56.1%)
Missing parity	44 (0.5%)	43 (0.6%)	1 (0.1%)
Chronic hypertension	442 (5.2%)	343 (4.8%)	99 (7.0%)
Missing hypertension	103 (1.2%)	94 (1.3%)	9 (0.6%)
Diabetes (1 or 2)	307 (3.6%)	239 (3.3%)	68 (4.8%)
Missing diabetes	53 (0.6%)	52 (0.7%)	1 (0.1%)

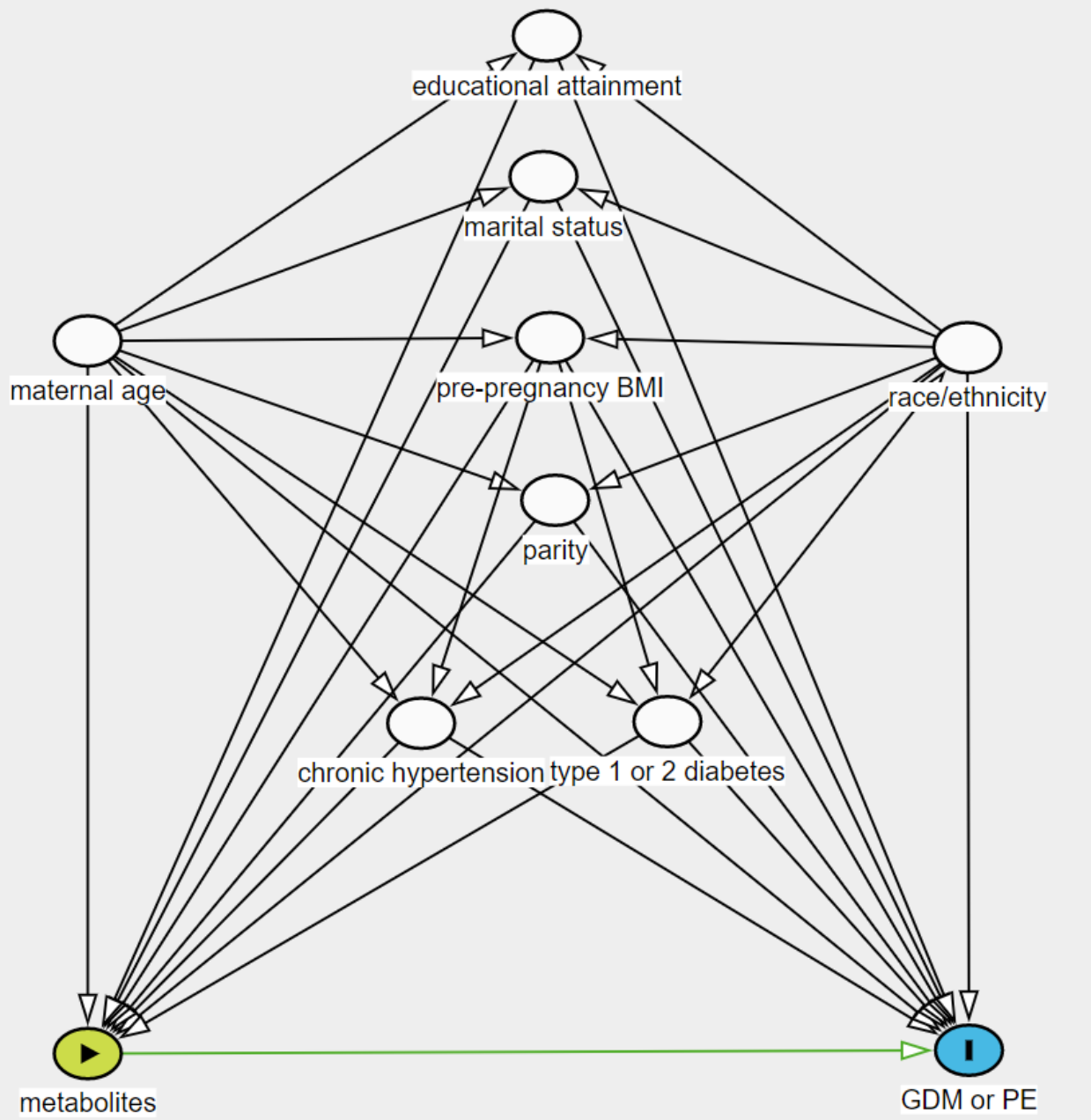
Table 17: Sensitivity analyses using inverse probability of selection weighted pseudopopulation

		Multivariable-adjusted[a]		
		Metabolite tertile		
Gestational diabetes	**Continuous[b] OR (95% CI)**	**Lowest OR (95% CI)**	**Middle OR (95% CI)**	**Highest OR (95% CI)**
Cord TMAO	0.99 (0.74, 1.33)	REF	0.91 (0.50, 1.67)	0.89 (0.47, 1.68)
Cord choline	0.74 (0.58, 0.94)	REF	0.90 (0.51, 1.58)	0.52 (0.27, 1.01)
Cord betaine	0.82 (0.63, 1.06)	REF	0.55 (0.30, 1.00)	0.69 (0.38, 1.25)
Cord carnitine	**0.73 (0.57, 0.94)**	REF	0.70 (0.39, 1.27)	0.56 (0.29, 1.05)
Maternal TMAO	1.23 (0.99, 1.53)	REF	1.15 (0.65, 2.02)	**1.81 (1.06, 3.08)**
Maternal choline	0.91 (0.75, 1.11)	REF	0.74 (0.45, 1.22)	0.77 (0.46, 1.28)
Maternal betaine	0.89 (0.73, 1.08)	REF	0.87 (0.53, 1.43)	0.73 (0.43, 1.24)
Maternal carnitine	0.84 (0.68, 1.04)	REF	0.83 (0.50, 1.37)	0.62 (0.37, 1.05)
Pre-eclampsia	**Continuous OR (95% CI)**	**Lowest OR (95% CI)**	**Middle OR (95% CI)**	**Highest OR (95% CI)**
Cord TMAO	1.12 (0.90, 1.40)	REF	1.42 (0.79, 2.55)	1.17 (0.62, 2.18)
Cord choline	**1.77 (1.37, 2.29)**	REF	1.69 (0.84, 3.42)	**3.08 (1.58, 5.99)**
Cord betaine	**1.65 (1.26, 2.17)**	REF	1.25 (0.63, 2.52)	**2.64 (1.40, 5.00)**
Cord carnitine	**1.58 (1.21, 2.08)**	REF	1.17 (0.60, 2.28)	**2.46 (1.36, 4.46)**
Maternal TMAO	0.98 (0.81, 1.19)	REF	1.03 (0.65, 1.63)	0.92 (0.58, 1.47)
Maternal choline	0.98 (0.80, 1.20)	REF	1.14 (0.73, 1.78)	0.87 (0.54, 1.40)
Maternal betaine	**0.65 (0.53, 0.81)**	REF	**0.51 (0.33, 0.80)**	**0.38 (0.23, 0.61)**
Maternal carnitine	**1.49 (1.22, 1.83)**	REF	1.21 (0.74, 1.99)	**1.90 (1.19, 3.03)**

[a] Multivariable-adjusted models adjusted for maternal age (years, continuous), race/ethnicity (Black, white, Hispanic, other), educational attainment (less than high school, high school or equivalent, greater than high school), marital status (unmarried, married), pre-pregnancy BMI (kg/m2, continuous), parity (0, ≥1), chronic pre-pregnancy hypertension (no/yes), and pre-pregnancy diabetes (no/yes, for pre-eclampsia models only); [b] Per SD-increment of exposure

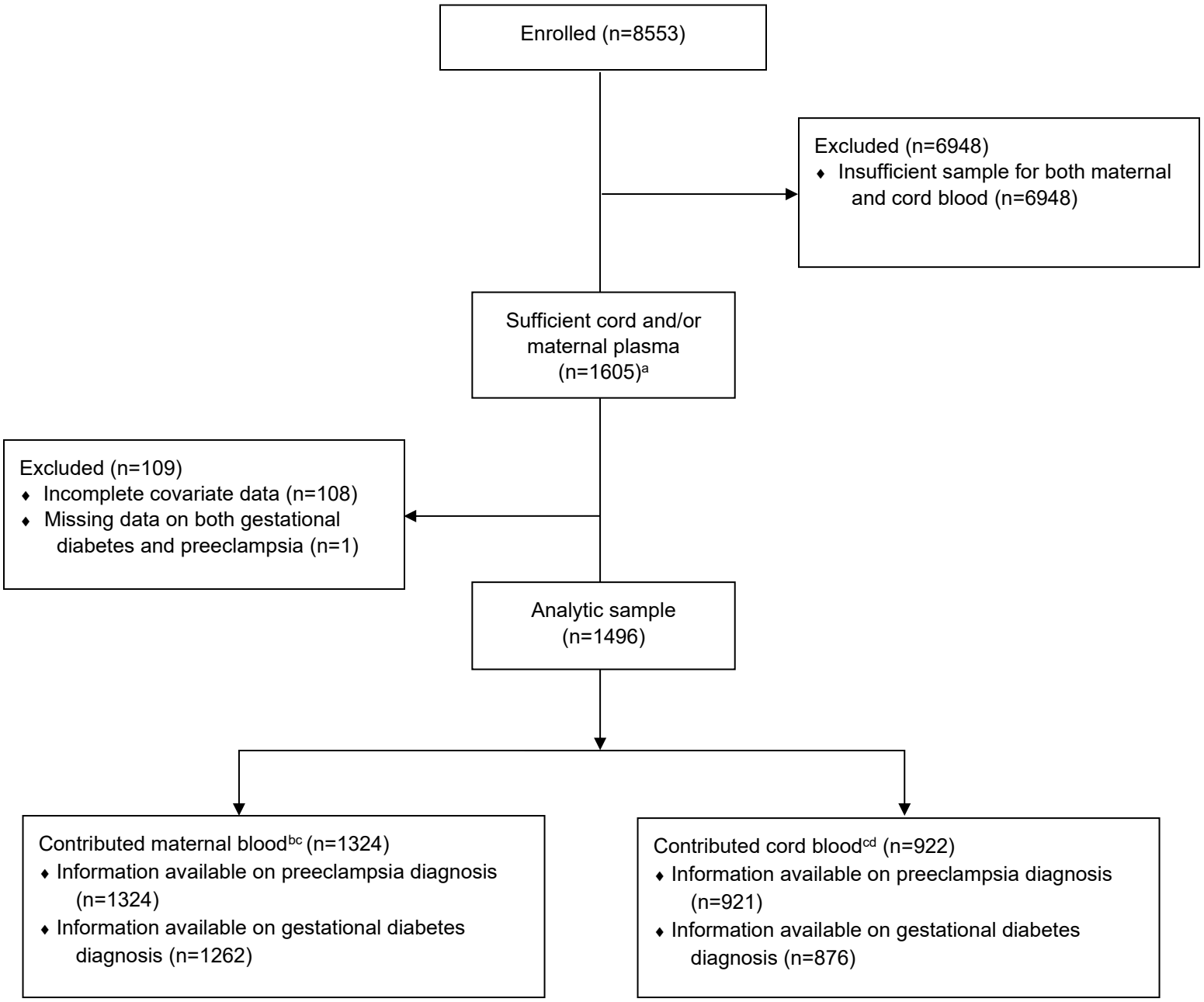
Appendix B: Figures

Figure 1: Directed acyclic graph (DAG)



Pre-pregnancy diabetes was included as a covariate only in models for pre-eclampsia; those with pre-pregnancy diabetes were not included in the analyses for gestational diabetes as they were not at risk for the outcome

Figure 2: Participant flow diagram



[a] Among 1605 women with sufficient cord and/or maternal plasma, 1000 contributed cord blood and 1412 contributed maternal blood (807 contributed both). These are the women whose characteristics are reported in Table 1 and Supplemental Tables 1-7.

[b] Among those included in the analytic sample, 1324 contributed maternal blood and 922 contributed cord blood (750 contributed both).

[c] Among those included in the analytic sample who contributed maternal blood, none were missing information on preeclampsia diagnosis and 62 were missing information on gestational diabetes diagnosis.

[d] Among those in the analytic sample who contributed cord blood, 1 was missing information on preeclampsia diagnosis and 46 were missing information on gestational diabetes diagnosis.

Figure 3: Spearman's rank correlation matrix for the included metabolites

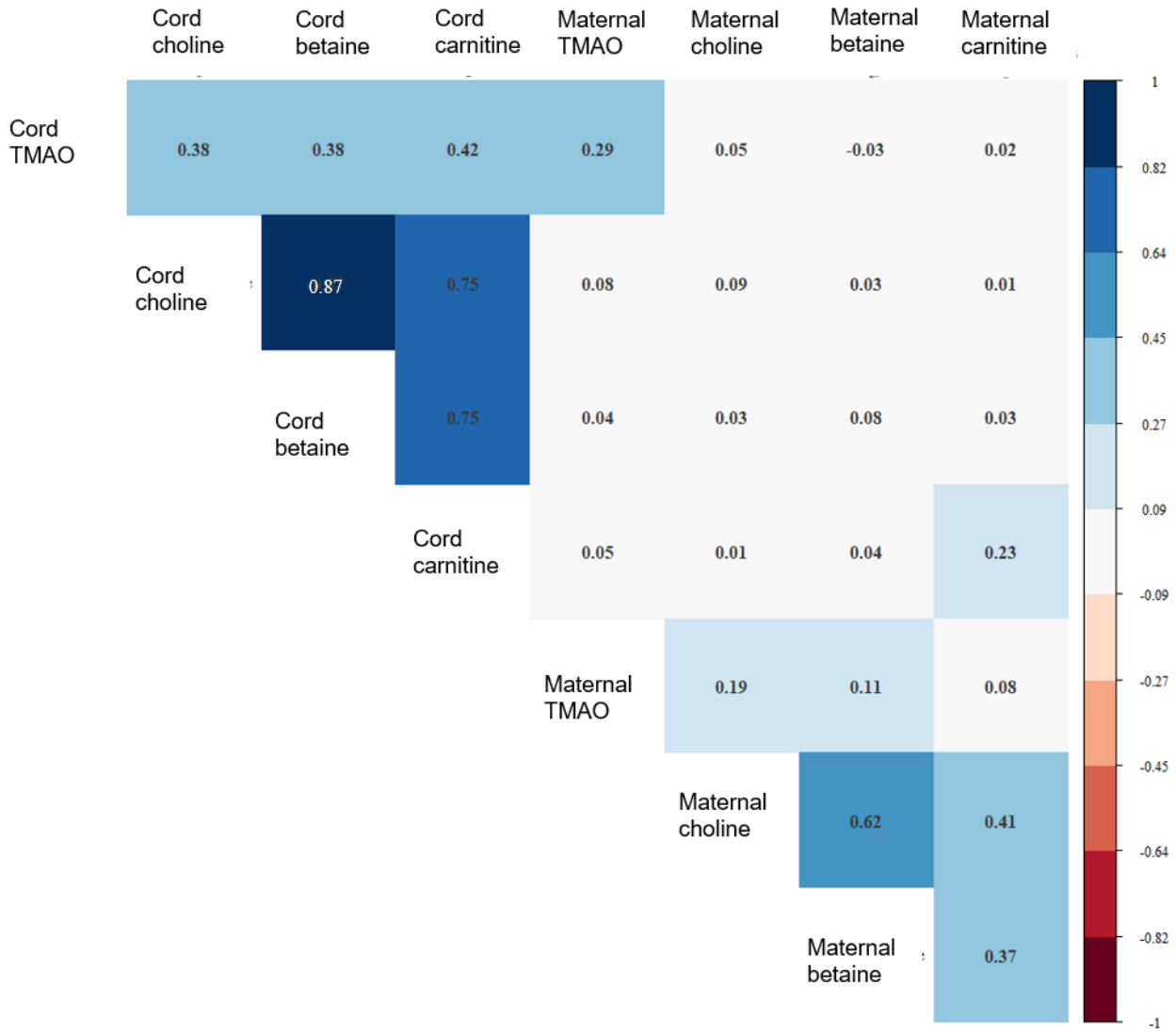


Figure 4: Odds ratios and 95% CIs for cord and maternal blood metabolites with gestational diabetes and pre-eclampsia