DR KELLY FERGUSON (Orcid ID : 0000-0001-8467-3250)

Article type : Original article

Associations between repeated ultrasound measures of fetal growth and biomarkers of maternal oxidative stress and inflammation in pregnancy

Short version of title: Inflammation and oxidative stress in fetal growth

Kelly K. Ferguson[1], Elizabeth M. Kamai[2], David E. Cantonwine[3], Bhramar Mukherjee[4], John D. Meeker[5], Thomas F. McElrath[3]

[1]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA
[2]Department of Epidemiology, University of North Carolina Gillings School of Global Public Health
[3]Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School
[4]Departments of Biostatistics and Epidemiology, University of Michigan School of Public Health
[5]Department of Environmental Health Sciences, University of Michigan School of Public Health

Correspondence: Kelly K. Ferguson, Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA. Email: kelly.ferguson2@nih.gov

Acknowledgements: We thank Drs. Allen Wilcox and Quaker Harmon for providing comments on an earlier version of this manuscript. We also thank Elizabeth Hurst and colleagues for

analysis of urinary oxidative stress biomarkers and Joel Whitfield for analysis of plasma inflammation biomarkers. This research was funded by the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, grant numbers: R01ES018872, P42ES017198, and P30ES017885, and by the Intramural Research Program of NIEHS (ZIA103313). Funding for EMK was provided by an NIEHS institutional training grant (T32ES007018). The original cohort was supported by an unrestricted grant from Abbott Diagnostics Division (9MZ-04-06N03). The authors have no conflicts of interest to declare.

Abstract

Problem: Perturbations in normal fetal growth during pregnancy are associated with poor child and adult health outcomes. Inflammation and oxidative stress are recognized as important mechanisms in preeclampsia and preterm birth but have been examined less in relation to fetal growth. We hypothesized that maternal inflammation and oxidative stress in pregnancy would be associated with reduced fetal growth and sought to identify windows of vulnerability.

Method of study: In a secondary analysis of 482 women from the LIFECODES birth cohort study, we measured inflammation (C-Reactive Protein [CRP] and the cytokines IL-1β, IL-6, IL-10, and TNF-α) and oxidative stress (8-isoprostane and 8-hydroxydeoxyguanosine [8-OHdG]) biomarkers in plasma and urine, respectively, at four time points during pregnancy. We examined associations between repeated measures of each marker and ultrasound (head and abdominal circumference, femur length, and a summary measure of estimated fetal weight) as well as delivery (birth weight) metrics of growth.

Results: In adjusted repeated measures models, an interquartile range (IQR) increase in CRP was associated with a 0.12 standard deviation decrease in fetal weight z-score (95% confidence interval, CI, -0.21, -0.02), which corresponds to approximately 50 grams at 40 weeks gestation. The association was greatest in magnitude (i.e., most negative) with CRP measured later in pregnancy. Oxidative stress markers were not associated with fetal weight, although both were inversely associated with head circumference and femur length.

Conclusions: Inflammation and oxidative stress markers measured later in pregnancy were associated with reduced fetal growth as measured by repeated ultrasound scans.

Keywords: biomarkers, circulation, cytokines, intrauterine growth restriction, birth weight, inflammation, isoprostane.

1 INTRODUCTION

Intrauterine growth restriction is a serious complication of pregnancy that is a major predictor of neonatal mortality and morbidities.[1] Decreased weight for gestational age at birth, which comprises normal as well as pathologic variation, is associated with consequences that last into childhood and even adult life.[2,3] Known contributors to pathologic fetal growth restriction include congenital anomalies and extreme maternal dietary restriction. However, numerous other factors can alter implantation and development of the placenta, hormone transfer to the fetus, and supply and demand of nutrients that can adversely affect growth.

Maternal infection with diseases like malaria, which is characterized by activation of inflammation and oxidative stress pathways, is strongly associated with fetal growth restriction.[4] However, the impact of elevated but subclinical levels of inflammation and oxidative stress is less well known. Data from animal and cellular models suggest that inflammation and oxidative stress early in pregnancy can interfere with normal placentation, namely by inducing apoptosis of the syncytiotrophoblast and impairing invasion of the spiral arterioles.[5] Studies in humans, however, are limited by the availability of biomarker measurements from single time points during gestation or the use of birth weight alone as a proxy for growth.[6-14]

In this study we sought to address whether maternal inflammation and oxidative stress biomarker concentrations measured longitudinally across pregnancy were associated with repeated ultrasound as well as delivery measures of fetal growth. Additionally, we examined whether associations between biomarkers and growth differed depending on when they were measured during pregnancy, what parameter was used to assess growth (e.g., weight or head circumference), and sex of the fetus.

2 MATERIALS AND METHODS

2.1 Study Population

LIFECODES is an ongoing prospective birth cohort conducted at Brigham and Women's Hospital (BWH) in Boston, MA. Women are recruited early in gestation (prior to 15 weeks) and provide repeated biological specimens at up to four study visits. Recruitment has been ongoing since 2006. For the present analysis, we included women who were part of a nested case-control study of preterm birth that was originally designed to assess the relationship between phthalate exposure and prematurity in pregnancy, and to investigate longitudinal biomarkers of oxidative stress and inflammation that were hypothesized to mediate that assocation.[15,16] This study comprised all cases of preterm birth (defined as delivery prior to 37 weeks gestation; n=130) as well as 3:1 randomly selected controls (n=352) who delivered between 2006 and 2008.[17] The present secondary analysis leveraged this existing data, which, to our knowledge, are not available in any other epidemiologic study, to investigate the relationship between inflammation and oxidative stress biomarkers in pregnancy and fetal growth. Unadjusted analysis within the case-control population would bias effect estimates, since biomarkers are elevated in cases of preterm birth and babies born preterm are smaller and in many cases growth-restricted. Thus, we applied inverse probability weights to all analyses to account for the case-control study design.[18] This approach effectively downweights associations observed between biomarkers and growth parameters in cases of preterm to proportion at which they would be observed in the base LIFECODES population (i.e., 12%) and ensures that the results from this analysis are generalizable.

2.2 Inflammation and Oxidative Stress Biomarkers

Urine and plasma samples were collected at enrollment (median 10 weeks), and at three subsequent visits (median 18, 26, and 35 weeks). In urine, we measured two biomarkers of oxidative stress in each sample: total 8-isoprostane, an indicator of lipid peroxidation; and 8-hydroxydeoxyguanosine (8-OHdG), an indicator of oxidative DNA damage. For 8-isoprostane, samples underwent an affinity purification step. Concentrations of both analytes were measured using enzyme immunoassay. All analyses were performed at Cayman Chemical (Ann Arbor, MI) using methods described in detail elsewhere.[15] To adjust for urine dilution, specific gravity was measured by a handheld refractometer (Atago Co., Ltd., Tokyo, Japan).

To assess inflammation, we measured C-reactive protein (CRP) using enzyme immunoassay, and a panel of cytokines (ILβ, IL-6, IL-10, and TNF-α) using a Milliplex MAP High Sensitivity Human Cytokine Magnetic Bead Panel (EMD Millipore Corporation, St. Charles, MO). All inflammation markers were measured in plasma by the Cancer Center Immunology Core (University of Michigan, Ann Arbor, MI), with methods described elsewhere as well.[16]

Oxidative stress and inflammation markers measured in this study population showed good reliability over the course of pregnancy (intraclass correlation coefficients ranging from 0.60 to 0.81 for inflammation markers and 0.32 and 0.60 for 8-OHdG and 8-isoprostane, respectively).[15,16] Thus, we utilized a last observation carried forward approach to impute biomarker measurements missing from each time point as follows. Across all four collection times, 250 (13%) of 8-OHdG or 8-isoprostane measures were missing, while 343 (17.8%) of inflammation biomarkers were missing because samples were not provided by participants at those respective visits. Most missing measures, 220 (88%) for oxidative stress and 245 (71%) for inflammation, were imputable by levels measured at the previous visit. The remaining 30 missing oxidative stress measures and 88 missing inflammation measures were imputed using measures from 2 or more visits prior to the index visit. This resulted in 250 oxidative stress biomarker imputations: 61 (13%) at visit 2; 73 (15%) at visit 3; and 108 (22%) at visit 4. Likewise, 333 inflammation measurements were imputed: 72 (15%) at visit 2; 93 (19%) at visit 3; and 103 (21%) at visit 4. Additionally, since no biomarker measurements were examined at delivery, we used the latest biomarker measure available (visit 3 or 4 for 95% of participants) to represent levels at that time point for analysis.

Distributions of all inflammation and oxidative stress markers were right-skewed and natural log transformed for statistical analyses.

2.3 Measures of Fetal Growth

Gestational age for the LIFECODES study is assessed by last menstrual period with verification by crown-rump length (median 10 weeks) ultrasound.[19] In addition to the gestational dating ultrasound, a second trimester (median 18 weeks) morphology ultrasound is performed on all patients at BWH to screen for congenital abnormalities. Information on head circumference, abdominal circumference, and femur length is abstracted from this scan. For many patients,

ultrasound scans are performed at additional time points later in pregnancy, either due to obstetrical indications as determined by the provider or at the request of the patient. For this study population we estimated growth using all ultrasound parameters measured after the 18 week morphology screening ultrasound, as that time point has been shown to have low variability in individual parameters in this and other study populations.[20,21]

Thus, for the present analysis we included anthropometric ultrasound measurements that were performed closest in time to study visits 3 and 4 (median 26 and 35 weeks gestation). Measurements included head circumference, abdominal circumference, femur length, and we calculated a summary measure of estimated fetal weight using the formula of Hadlock[22] for 326 participants. Two ultrasound measurements were available for 148 participants and the remaining had one measurement available. All ultrasound parameters were converted to gestational-age-specific z-scores based on mean and standard deviation values obtained from approximately 19,000 pregnancies at BWH between 2006-2012.[23] Estimated fetal weight z-scores were based on estimated fetal weight means and standard deviations from that study population as well. In addition to ultrasound parameters, we calculated birth weight z-scores based on birth weight means and standard deviations from the same reference population for all 482 study participants.

2.4 Model Selection and Statistical Analysis

All analyses conducted in SAS version 9.4 (Cary, NC). Demographic characteristics of the study population were tabulated with weighted percentages. Linear mixed models (LMMs) were used to assess associations between repeated measures of log-transformed oxidative stress and inflammation biomarkers and each z-scored measure of fetal size using SAS Proc Mixed. These powerful models allow incorporation of multiple measures of exposure (i.e., inflammation or oxidative stress biomarker) and outcome (i.e., growth measurement) available on the same participant. Models for head circumference, abdominal circumference, and femur length included z-scores from ultrasound measurements at visits 3 and 4. Models of weight combined the estimated fetal weight z-scores from visits 3 and 4 as well as birth weight z-score at delivery. As examples, 1) We examined CRP (measured at median 26 and 35 weeks gestation) in relation to head circumference z-scores (also measured at 26 and 35 weeks gestation); and 2) We examined CRP, measured at median 26 and 35 weeks gestation and imputed at delivery, in

relation to estimated fetal weight z-scores from 26 and 35 weeks gestation and birth weight z-score at delivery. All models included a random intercept for participant and random slope for gestational age at the time of measurement (i.e., at ultrasound scan or delivery).

Sex, gestational age at the time of size measurement, and maternal age and race/ethnicity were included in models *a priori*. Additional covariates examined included: physician-recorded maternal body mass index (BMI) at enrollment (examined both continuously and as a categorical variable), education level, health insurance provider, any tobacco or alcohol use during pregnancy, parity, use of assisted reproductive technology, and use of *in vitro* fertilization specifically. Covariates were included in final models if they improved model fit, as assessed by Akaike Information Criterion values and likelihood ratio tests. In addition to *a priori* covariates, all final models were adjusted for maternal BMI at enrollment (<25 kg/m^2, 25-30 kg/m^2, >30 kg/m^2) and education level (high school or less, technical/some college, college graduate, graduate school). Models of oxidative stress biomarkers were additionally adjusted for urinary specific gravity (time-varying).

In addition to these repeated measures analyses, we wanted to assess windows of vulnerability to oxidative stress and inflammation during gestation. To address this question we examined associations between biomarker concentrations at each individual visit in relation to repeated measures of weight z-scores. Finally, we also examined repeated measures of biomarkers in relation to fetal size measures stratified by sex in order to investigate any sex differences in the associations observed. To test for significance of interactions by sex, we extracted p-values from models that included interaction terms between sex and each covariate in the model.[24]

3 RESULTS

The overall study population (N=482) was primarily White and well-educated (Table 1). Slightly more than half of the babies were female (55%). Differences in birth weight z-scores by demographic characteristics in this study population have been previously reported.[23] As expected, birth weight z-scores were lower in mothers who self-identified as Black, had lower BMI, had public health insurance providers, and who were nulliparous compared to reference. Unexpectedly, birth weight z-scores were also slightly lower in male compared to female fetuses in this study population. Oxidative stress and inflammation biomarkers showed moderate to high

stability in repeated measures across pregnancy, and tended to be higher in mothers who were Black, had higher BMI, and who had lower socioeconomic status.[15,16]

Adjusted LMMs showed that each inflammation biomarker was inversely associated with fetal growth, as indicated by repeated z-scores of head circumference, abdominal circumference, femur length, and weight; however, few associations reached statistical significance (Table 2). The most consistent associations, and the effect estimates that were greatest in magnitude, were between CRP and growth measurements. For example, an interquartile range (IQR) increase in CRP was associated with a 0.12 standard deviation decrease in weight z-score (95% confidence interval, CI=-0.21, -0.02), which corresponds to a decrease in 50 grams at 40 weeks gestation (based on mean birth weight at week 40 in the BWH population).[23] Additionally, IL-1β was associated with a 0.08 standard deviation decrease in head circumference z-score (95% CI=-0.17, 0.00).

To identify windows of vulnerability during pregnancy, we next examined models of inflammation biomarkers by visit in relation to repeated measures of weight (i.e., estimated fetal weight z-scores at visits 3 and 4 and birth weight z-score at delivery). For CRP, we observed that associations between levels measured at visits 1 and 2 in pregnancy were not associated with weight; however, higher levels of CRP measured at visits 3 and particularly at 4 were associated with lower weight (Figure 1; effect estimates presented in Table S1). This suggests later pregnancy as a potentially vulnerable window when higher levels of inflammation could have a greater influence on fetal growth. Patterns were similar but less precise for IL-1β, and associations for other cytokines were null (Table S1).

We also investigated whether inflammation marker associations with fetal growth differed by sex of the fetus by creating stratified models. Associations between CRP and weight were similar in males and females (Figure 2; effect estimates presented in Table S2), but associations between IL-1β and weight were inverse for males and null for females (p for interaction=0.10). The latter suggests that inflammation as indicated by IL-1β may be have a stronger effect on fetal growth in male compared to female fetuses.

In regard to oxidative stress biomarkers, 8-OHdG and 8-isoprostane were both associated with lower fetal growth, as indicated by each anthropometric measurement; however, the effect estimates for associations with head circumference and femur length were greatest in magnitude (i.e., most negative; Table 3). An IQR increase in 8-OHdG concentration was associated with a

0.20 standard deviation decrease in head circumference z-score (95% CI=-0.37, -0.02). For 8-isoprostane, an IQR increase was associated with a 0.13 standard deviation decrease in head circumference z-score (95% CI=-0.24, -0.02) as well as a 0.13 standard deviation decrease in femur length z-score (95% CI=-0.24, -0.01).

When we examined associations by study visit to investigate windows of vulnerability, 8-isoprostane levels measured at visit 3 were inversely associated with weight and 8-OHdG levels at visit 1 were positively associated with weight (Figure 1; effect estimates in Table S2).

In models stratified by sex, associations were mostly null Figure 2; effect estimates presented in Table S2). However, we observed that the inverse association between 8-OHdG and weight was stronger in females compared to males (p for interaction=0.09).

4 DISCUSSION

Inflammation has long been suspected to play an important role in growth restriction and preeclampsia, although support is more consistent for the latter.[25] Animal evidence also strongly supports a causative relationship between inflammation and reduced fetal growth. The largest study in humans to address this research question was within the Generation R birth cohort, in which CRP levels were measured in the first trimester of pregnancy.[7] Increased levels were associated with lower estimated fetal weight, measured by ultrasound in the third trimester, and also with lower birth weight.[7] Other cross-sectional studies have similarly observed inverse associations between CRP, measured at various time points during pregnancy, and birth weight.[26,27] Two small studies (N≤200) with repeated measures of CRP did not analyze associations by trimester, but also observed inverse associations with birth weight.[6,13] Our studies are somewhat consistent with these findings, although we observed null associations with CRP measured at ~10 weeks gestation, and the most precise effect estimates with levels measured at ~35 weeks gestation. These data suggest that inflammation later in pregnancy—whether consequence or cause—may be characteristic of decreased fetal growth as well.

Few studies have examined cytokines in relation to birth weight or fetal growth,[8,10] and to our knowledge none has done so with repeated biomarkers or ultrasound measurements. Our largely null findings for inflammatory cytokines suggest these markers may not be useful in the study of fetal growth. This may be due to poor correlation between plasma cytokines and

inflammation in the compartment of interest (e.g., placenta or fetus). Additional work to examine this question in more detail is warranted.

Oxidative stress is an imbalance between reactive oxygen species and antioxidant capacity that may result from or cause inflammation. While an elevation of oxidative stress in early pregnancy relative to pre-pregnancy is normal, levels that are too high could interfere with normal placentation. Few studies in humans have investigated associations between prenatal oxidative stress biomarkers and fetal growth. Studies measuring 8-OHdG levels in urine from early[11,12] as well as late[9,14] pregnancy have demonstrated associations with decreased birth weight or increased risk of small for gestational age. Our findings are consistent with these data, as we observed associations between repeated measures of 8-OHdG over pregnancy and decreased head circumference and femur length z-scores. Interestingly, the association with weight was strongest in girls in our stratified analysis by fetal sex, which was also observed by Lindstrom et al.[11]

Levels of 8-isoprostane in amniotic fluid collected during pregnancy have been strongly associated with fetal growth restriction.[28] However, the Lindstrom study, which examined urinary 8-isoprostane concentrations at both 14 and 30 weeks gestation, found no association with birth weight or other metrics at delivery.[11] In fact, they observed that elevated levels early in pregnancy were associated with increased weight. We found that 8-isoprostane was inversely associated with repeated measures of head circumference and femur length, and that levels at ~26 weeks gestation were associated with decreased weight. This may suggest that oxidative stress levels in pregnancy have a stronger influence on some anthropometric parameters (e.g., head size) compared to others.

Our study of inflammation and oxidative stress markers in relation to fetal growth was limited in part by our study population. This was a secondary analysis using existing data from a nested case-control study of preterm birth. This population was chosen for this analysis because of the availability of the rich set of biomarkers of inflammation and oxidative stress. However, it was not designed specifically to investigate the associations between these biomarkers and fetal growth. Because of inverse probability weights applied to all analyses, the results are adjusted for the case-control design and the findings do not overly represent associations that are unique to cases of preterm birth. The primary limitations of using this study population are due to the fact that the ultrasound data utilized in the present analysis was collected clinically and not for

research purposes. This could limit the quality of the data collected. Additionally, because scans later in pregnancy are more likely to be performed among women who are suspected to have pregnancy complications, our findings may be characteristic of events occurring in higher risk pregnancy. Also for this reason, our sample size was limited for analyses examining ultrasound measurements only (head and abdominal circumference and femur length). Nevertheless, this data provide additional power beyond what we could muster using birth measurements alone. Furthermore, they provide the ability to examine individual anthropometric parameters like head circumference and femur length, which are rarely captured in these types of studies.

Because of the limited availability of repeat ultrasound measurements in pregnancy, we were unable to capture associations with rates of growth during gestation, which may be particularly important. In our other studies of inflammation and oxidative stress measures in relation to preterm birth and preeclampsia we were able to separate cases based on presentations that may have more homogeneous etiologies (including spontaneous vs. placentally-mediated for preterm birth and early vs. late onset for preeclampsia). Distinguishing pathologic from normal fetal growth is a more difficult challenge.

This study benefited from the availability of four measurements of a panel of both inflammation and oxidative stress measures during pregnancy, which allowed us to examine windows during gestation when these levels may be particularly influential. We also were able to utilize ultrasound measurements of fetal growth, which gave us greater power in repeated measures models and also allowed us to identify associations with anthropometric parameters that had not been examined in relation to these markers in the past.

In conclusion, we observed inverse associations between CRP and fetal weight and between the oxidative stress markers 8-OHdG and 8-isoprostane and head circumference and femur length. Effect estimates for CRP were strongest (i.e., most negative) with levels measured later in pregnancy, and the same was true for 8-isoprostane. This represents the first study to our knowledge to examine associations between inflammation and oxidative stress biomarkers measured at multiple time points within the same participants in relation to fetal growth. These findings inform not only the understanding of biological changes in pregnancy that are related to perturbations in fetal growth, but also could help to explain why perturbations in fetal growth are linked to consequences in childhood and later in life.

337

338 REFERENCES

339

340 1. Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, Golan A, Network VO. Morbidity and
341 mortality among very-low-birth-weight neonates with intrauterine growth restriction.
342 *American journal of obstetrics and gynecology.* 2000;182(1):198-206.
343 2. McCormick MC. The contribution of low birth weight to infant mortality and childhood
344 morbidity. *New England journal of medicine.* 1985;312(2):82-90.
345 3. Barker DJ, Godfrey KM, Gluckman PD, Harding JE, Owens JA, Robinson JS. Fetal
346 nutrition and cardiovascular disease in adult life. *The Lancet.* 1993;341(8850):938-941.
347 4. Moormann AM, Sullivan AD, Rochford RA, et al. Malaria and pregnancy: placental
348 cytokine expression and its relationship to intrauterine growth retardation. *Journal of*
349 *Infectious Diseases.* 1999;180(6):1987-1993.
350 5. Cotechini T, Komisarenko M, Sperou A, Macdonald-Goodfellow S, Adams MA, Graham
351 CH. Inflammation in rat pregnancy inhibits spiral artery remodeling leading to fetal
352 growth restriction and features of preeclampsia. *Journal of Experimental Medicine.*
353 2014:jem. 20130295.
354 6. de Oliveira LC, Franco-Sena AB, Farias DR, Rebelo F, Kac G. Maternal C-reactive
355 protein concentrations during pregnancy and birth weight in a prospective cohort in Rio
356 de Janeiro, Brazil. *The Journal of Maternal-Fetal & Neonatal Medicine.* 2016:1-8.
357 7. Ernst GD, de Jonge LL, Hofman A, et al. C-reactive protein levels in early pregnancy,
358 fetal growth patterns, and the risk for neonatal complications: the Generation R Study.
359 *American journal of obstetrics and gynecology.* 2011;205(2):132. e131-132. e112.
360 8. Guven MA, Coskun A, Ertas IE, Aral M, Zencırcı B, Oksuz H. Association of maternal
361 serum CRP, IL-6, TNF-α, homocysteine, folic acid and vitamin b12 levels with the
362 severity of preeclampsia and fetal birth weight. *Hypertension in pregnancy.*
363 2009;28(2):190-200.
364 9. Hsieh Ts-Ta, Chen S-F, Lo L-M, Li M-J, Yeh Y-L, Hung T-H. The association between
365 maternal oxidative stress at mid-gestation and subsequent pregnancy complications.
366 *Reproductive Sciences.* 2012;19(5):505-512.

10. Kumarathasan P, Vincent R, Das D, et al. Applicability of a high-throughput shotgun plasma protein screening approach in understanding maternal biological pathways relevant to infant birth weight outcome. *Journal of proteomics.* 2014;100:136-146.
11. Lindström E, Persson L-Å, Raqib R, Arifeen SE, Basu S, Ekström E-C. Associations between oxidative parameters in pregnancy and birth anthropometry in a cohort of women and children in rural Bangladesh: The MINIMat-cohort. *Free radical research.* 2012;46(3):253-264.
12. Potdar N, Singh R, Mistry V, et al. First-trimester increase in oxidative stress and risk of small-for-gestational-age fetus. *BJOG: An International Journal of Obstetrics & Gynaecology.* 2009;116(5):637-642.
13. Pringle KG, Rae K, Weatherall L, et al. Effects of maternal inflammation and exposure to cigarette smoke on birth weight and delivery of preterm babies in a cohort of indigenous Australian women. *Frontiers in immunology.* 2015;6.
14. Scholl T, Stein T. Oxidant damage to DNA and pregnancy outcome. *Journal of Maternal-Fetal Medicine.* 2001;10(3):182-185.
15. Ferguson KK, McElrath TF, Chen Y-H, Loch-Caruso R, Mukherjee B, Meeker JD. Repeated measures of urinary oxidative stress biomarkers during pregnancy and preterm birth. *American journal of obstetrics and gynecology.* 2015;212(2):208. e201-208. e208.
16. Ferguson KK, McElrath TF, Chen YH, Mukherjee B, Meeker JD. Longitudinal profiling of inflammatory cytokines and C-reactive protein during uncomplicated and preterm pregnancy. *American journal of reproductive immunology.* 2014;72(3):326-336.
17. Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. *JAMA pediatrics.* 2014;168(1):61-68.
18. Jiang Y, Scott AJ, Wild CJ. Secondary analysis of case-control data. *Statistics in medicine.* 2006;25(8):1323-1339.
19. Obstetricians ACo, Gynecologists. Method for estimating due date. Committee Opinion No. 611. *Obstet Gynecol.* 2014;124(4):863-866.
20. Casas M, Valvi D, Ballesteros-Gomez A, et al. Exposure to bisphenol A and phthalates during pregnancy and ultrasound measures of fetal growth in the INMA-Sabadell cohort. *Environmental health perspectives.* 2016;124(4):521.

21. Hindmarsh PC, Geary MP, Rodeck CH, Kingdom JC, Cole TJ. Intrauterine growth and its relationship to size and shape at birth. *Pediatric Research.* 2002;52(2):263-268.
22. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. *Am J Obstet Gynecol.* 1985;151(3):333-337.
23. Cantonwine DE, Ferguson KK, Mukherjee B, et al. Utilizing longitudinal measures of fetal growth to create a standard method to assess the impacts of maternal disease and environmental exposure. *PloS one.* 2016;11(1):e0146532.
24. Buckley JP, Doherty BT, Keil AP, Engel SM. Statistical Approaches for Estimating Sex-Specific Effects in Endocrine Disruptors Research. *Environmental Health Perspectives.* 2017;67013:1.
25. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *American journal of obstetrics and gynecology.* 2006;195(1):40-49.
26. Savvidou MD, Lees CC, Parra M, Hingorani AD, Nicolaides KH. Levels of C-reactive protein in pregnant women who subsequently develop pre-eclampsia. *BJOG: An International Journal of Obstetrics & Gynaecology.* 2002;109(3):297-301.
27. Darling AM, McDonald CR, Conroy AL, et al. Angiogenic and inflammatory biomarkers in midpregnancy and small-for-gestational-age outcomes in Tanzania. *American journal of obstetrics and gynecology.* 2014;211(5):509. e501-509. e508.
28. Longini M, Perrone S, Kenanidis A, et al. Isoprostanes in amniotic fluid: a predictive marker for fetal growth restriction in pregnancy. *Free Radical Biology and Medicine.* 2005;38(11):1537-1541.

Table 1. Weighted percentages of characteristics of the study population (N=482)

Characteristic	%
Child sex	
Male	45%
Female	55%
Maternal education	

High school or less	14%
Technical school or some college	15%
College graduate	29%
Graduate school	40%
Missing	*3%*
Maternal race	
White	59%
Black	16%
Other	26%
Maternal age	
18-25	14%
26-30	24%
31-34	32%
35+	29%
Missing	*1%*
Body mass index at visit 1	
<25 kg/m^2	53%
25-30 kg/m^2	26%
>30 kg/m^2	20%
Missing	*1%*
Smoking during pregnancy	
Some	6%
None	93%
Missing	*2%*
Assisted reproductive technology	
Yes	9%
No	91%

Table 2. Adjusted[a] change in repeated[b] z-score measures of fetal growth in association with an interquartile range difference in inflammation biomarker from repeated measures models

	Weight (n=443 subjects, 935 observations)	Abdominal circumference (n=310 subjects, 467 observations)	Head circumference (n=309 subjects, 464 observations)	Femur length (n=310 subjects, 467 observations)
	Δ in z-score (95% CI)	Δ in z-score (95% CI)	Δ in z-score (95% CI)	Δ in z-score (95% CI)
CRP	-0.12 (-0.21, -0.02)	-0.08 (-0.19, 0.02)	-0.09 (-0.19, 0.02)	-0.03 (-0.14, 0.09)
IL-1β	-0.05 (-0.14, 0.03)	-0.03 (-0.12, 0.06)	-0.08 (-0.17, 0.00)	-0.04 (-0.13, 0.05)
IL-6	-0.02 (-0.09, 0.04)	-0.01 (-0.09, 0.06)	-0.05 (-0.12, 0.02)	-0.01 (-0.09, 0.07)
IL-10	-0.03 (-0.09, 0.04)	-0.03 (-0.10, 0.05)	-0.03 (-0.10, 0.04)	-0.01 (-0.08, 0.07)
TNF-α	-0.01 (-0.10, 0.08)	0.04 (-0.06, 0.14)	-0.01 (-0.11, 0.09)	-0.03 (-0.13, 0.08)

[a]All associations modeled with random intercept for participant and random slope for gestational age at ultrasound measurement and include fixed effects terms for child sex, and maternal age, race, education level, and body mass index at visit 1. [b]For estimated fetal weight, repeated measures models include measures from ultrasound and delivery. For other parameters, repeated measures models include measures from ultrasound only. Abbreviations: CRP, C-reactive protein.

Table 3. Adjusted[a] change in repeated[b] z-score measures of fetal growth in association with an interquartile range difference in oxidative stress biomarker from repeated measures models

	Fetal weight (n=448 subjects,	Abdominal circumference (n=314 subjects,	Head circumference (n=313 subjects,	Femur length (n=314 subjects,

	937 observations)	468 observations)	465 observations)	468 observations)
	Δ in z-score (95% CI)	Δ in z-score (95% CI)	Δ in z-score (95% CI)	Δ in z-score (95% CI)
8-OHdG	-0.09 (-0.25, 0.06)	-0.03 (-0.21, 0.15)	-0.20 (-0.37, -0.02)	-0.16 (-0.36, 0.03)
8-isoprostane	-0.03 (-0.13, 0.06)	-0.07 (-0.18, 0.05)	-0.13 (-0.24, -0.02)	-0.13 (-0.24, -0.01)

[a]All associations modeled with random intercept for participant and random slope for gestational age at ultrasound measurement and include fixed effects terms for urinary specific gravity (time-varying) child sex, and maternal age, race, education level, and body mass index at visit 1. [b]For estimated fetal weight, repeated measures models include measures from ultrasound and delivery. For other parameters, repeated measures models include measures from ultrasound only.
Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine.

Figure Legends

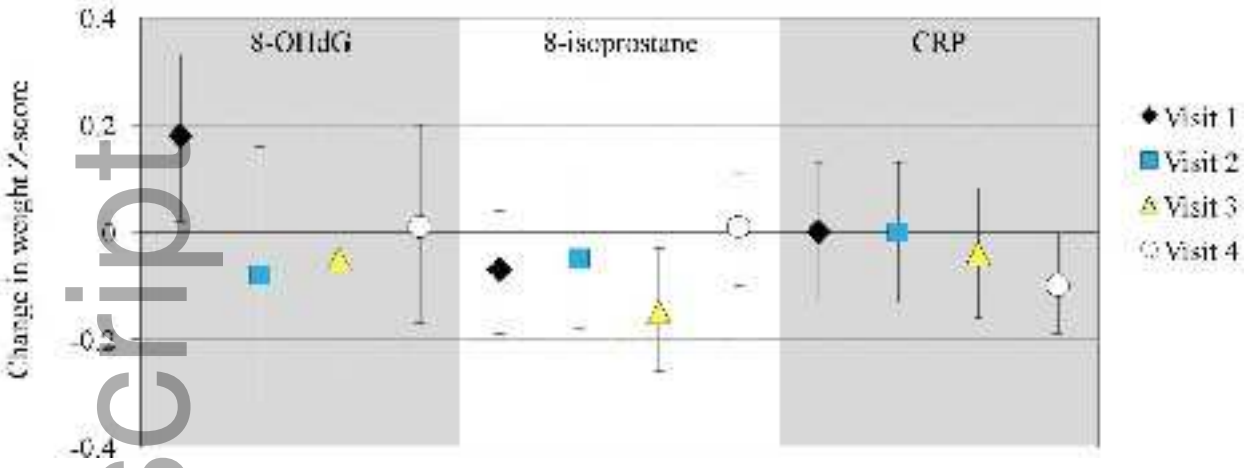
Figure 1. Assessing windows of vulnerability during pregnancy: adjusted[a] change in repeated weight z-score measures in association with an interquartile range difference in oxidative stress or inflammation biomarker measurement in models stratified by visit of sample collection.

[a]All associations modeled with random intercept for participant and random slope for gestational age at ultrasound measurement and include fixed effects terms for visit-specific urinary specific gravity (8-OHdG and 8-isoprostane models only), child sex, and maternal age, race, education level, and body mass index at visit 1. Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; CRP, C-reactive protein.

Figure 2. Assessing sex differences in associations: adjusted[a] change in repeated weight z-score measures in association with an interquartile range difference in repeated measures of oxidative stress or inflammation biomarkers in models stratified by fetal sex.

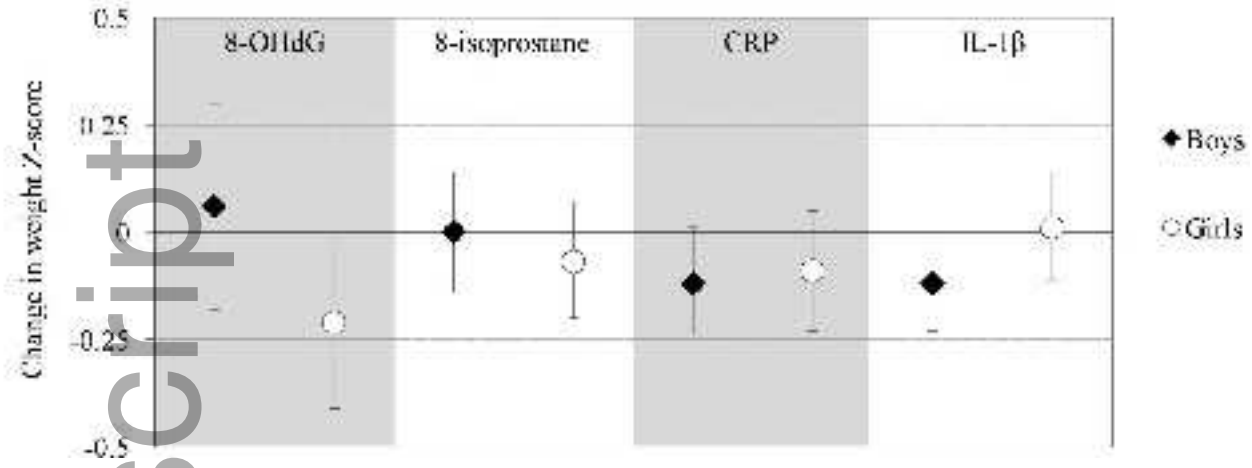
[a]All associations modeled with random intercept for participant and random slope for gestational age at ultrasound measurement and include fixed effects terms for urinary specific gravity (time-varying, 8-OHdG and 8-isoprostane models only) and maternal age, race, education level, and body mass index at visit 1. Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; CRP, C-reactive protein.

Figure 1.



aji_13017_f1.jpg

Figure 2.



aji_13017_f2.jpg