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Platelet activation and placenta-mediated adverse pregnancy outcomes: an ancillary study to the EAGeR trial

Lauren H. THEILEN, MD, MS^{1,2}, Heather D. CAMPBELL, MD^{1,2}, Sunni L. MUMFORD, PhD³, Alexandra C. PURDUE-SMITHE, PhD³, Lindsey A. SJAARDA, PhD³, Neil J. PERKINS, PhD³, Jeannie G. RADO, BS³, Robert M. SILVER, MD^{1,2}, Enrique F. SCHISTERMAN, PhD³

¹University of Utah Health, Department of Obstetrics & Gynecology, Salt Lake City, UT

²Intermountain Healthcare, Women & Newborns Clinical Program, Salt Lake City, UT

³Eunice Kennedy Shriver National Institute of Child Health and Human Development, Epidemiology Branch, Division of Intramural Population Health Research, Bethesda, MD

Abstract

Background: Platelet activation may play a role in the pathophysiology of placenta-mediated obstetric complications, as evidenced by the efficacy of aspirin for preventing preeclampsia, but published data regarding the relationship between biomarkers for platelet activation and adverse obstetric outcomes are sparse. Specifically, it is unknown whether pre-pregnancy biomarkers of platelet activation are associated with adverse pregnancy outcomes.

Objectives: To determine: 1) whether maternal plasma concentrations of platelet factor 4 are associated with risk of placenta-mediated adverse obstetric outcomes, and 2) whether these associations are modified by low-dose aspirin.

Study Design: This ancillary study included measurement of platelet factor 4 among 1,185 of the 1,228 reproductive-age women enrolled in the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial with available plasma samples, with relevant outcomes assessed among 584 women with pregnancies lasting at least 20 weeks' gestation. We measured platelet factor 4 in plasma samples obtained at the pre-pregnancy study visit (prior to randomization to low-dose aspirin or placebo), 12 weeks of gestation, and 28 weeks of gestation. The primary outcome was a composite of hypertensive disorders of pregnancy, placental abruption, and small-for-gestational age neonate. We estimated relative risks and 95% confidence intervals for the association between platelet factor 4 and the composite and individual outcomes at each time point using log-binomial regression that was weighted to account for potential selection bias and adjusted for age, BMI, education, income, and smoking. To evaluate potential effect modification by aspirin, we stratified analyses by aspirin treatment assignment.

Corresponding author: Lauren Theilen| 30 N 1900 E, Suite 2B200, Salt Lake City, UT, 84132| phone 801 581 8425| fax 801 585 2594| lauren.theilen@hsc.utah.edu.

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Results: During follow-up, 95 women experienced the composite adverse obstetric outcome, with 57 cases of hypertensive disorders of pregnancy, 35 of small-for-gestational age (SGA), and 6 of placental abruption. Overall, pre-pregnancy platelet factor 4 was positively associated with the composite outcome (tertile 3 vs. tertile 1 RR 2.36, 95% CI 1.38, 4.03) and with hypertensive disorders of pregnancy (tertile 3 vs. tertile 1 RR 2.14, 95% CI 1.08, 4.23). In analyses stratified by treatment group, associations were stronger in the placebo (tertile 3 vs. tertile 1 RR 3.36, 95% CI 1.42, 7.93) versus the aspirin group (tertile 3 vs. tertile 1 RR 1.78, 95% CI 0.90, 3.50).

Conclusions: High concentrations of platelet factor 4 prior to pregnancy are associated with increased risk of placenta-mediated adverse pregnancy outcomes, particularly for hypertensive disorders of pregnancy. Aspirin may mitigate the increased risk of these outcomes among women with higher plasma concentrations of preconception platelet factor 4, but low-dose aspirin non-responders may require higher doses of aspirin or alternate therapies to achieve obstetric risk reduction.

Keywords

abruption; aspirin; biomarker; fetal growth restriction; gestational hypertension; platelet factor 4; preeclampsia; small-for-gestational age

Introduction

Abnormalities in placentation have been implicated in the pathophysiology of several adverse obstetric outcomes, including hypertensive disorders of pregnancy,^{1–4} placental abruption,⁵ and delivery of a small-for-gestational age (SGA) infant.^{6,7} For the most part, effective strategies for the prediction and prevention of these complications have not been identified, with the notable exception of aspirin for reducing the risk of preeclampsia.^{8–11}

Platelets may be involved in the abnormal placentation process underlying the aforementioned pregnancy complications through several mechanisms including maternal vascular remodeling, placental angiogenesis, and thrombosis. Platelet activation releases bioactive peptides, such as RANTES and MIP-1 α , which promote the migration of extravillous trophoblasts toward maternal spiral arteries.^{12–15} Inappropriately timed or prolonged platelet activation, however, may have detrimental effects on endothelial function. Platelets also store and release a number of angiogenic (e.g., SDF1- α and VEGF) and antiangiogenic (e.g., platelet factor 4 and endostatin) factors,¹⁶ with differential packaging and selective release of these chemokines in response to various stimuli.^{17–19} During normal placentation, trophoblasts inhibit platelet aggregation,²⁰ but this inhibitory function is deficient in pregnancies complicated by placental abruption or fetal growth restriction.²¹

A number of bioactive peptides released from platelet α -granules *in vivo* have been studied as markers of platelet activation, but it is unknown whether variation in platelet activation prior to or during pregnancy affects placentation and, ultimately, pregnancy outcomes. Platelet factor 4 is a chemokine stored within platelet α -granules and released upon activation.^{22–26} It can be easily measured in plasma by ELISA, and it is a specific marker of *in vivo* platelet activation and secretion.^{27–29} Platelet factor 4 concentrations are less affected by renal failure than other markers for platelet activation (such as beta-

thromboglobulin).³⁰ Previously published studies describing the relationship between maternal plasma platelet factor 4 concentrations and preeclampsia only measured platelet factor 4 in the third trimester,^{31–33} though preconception and early pregnancy concentrations may be particularly relevant during early placental development.

To address knowledge gaps regarding the association between platelet activation before and throughout pregnancy with adverse obstetric outcomes, we aimed to determine whether high platelet factor 4 concentrations before pregnancy, in the first trimester, and in the third trimester are associated with placenta-mediated adverse obstetric outcomes. We further examined whether this association was modified by low-dose aspirin initiated prior to conception.

Materials and Methods

Study population

This was an ancillary study to the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, a multi-center, block-randomized, double-blind, placebo-controlled trial to investigate the effect of daily low-dose aspirin on reproductive outcomes in women with 1-2 prior pregnancy losses. Full details of the study design and findings of the EAGeR trial have been published previously.^{34,35}

The EAGeR trial included 1,228 women attempting to conceive who were 18-40 years of age, had menstrual cycles 21-42 days in length, a history of 1-2 pregnancy losses, and no history of infertility who were attempting spontaneous conception. Exclusion criteria included the presence of major medical disorders regardless of severity, clinical indication for anticoagulation or use of chronic nonsteroidal antiinflammatory drugs, self-reported history of physician-diagnosed infertility or subfertility, pelvic inflammatory disease, tubal occlusion, endometriosis, anovulation, polycystic ovary syndrome, and uterine abnormality. Study recruitment took place at four clinical sites (Salt Lake City, Utah; Denver, Colorado; Buffalo, New York; Scranton, Pennsylvania) from 2007-2011.

Participants were block-randomized to receive daily low-dose aspirin (LDA, 81 mg) or placebo prior to conception and followed for up to six months while attempting pregnancy, and then throughout pregnancy for women who conceived. The intervention was double-blinded, and the placebo tablets were manufactured to match the aspirin tablets on size, color, taste, and weight; all study staff, physicians, and participants were blinded to treatment allocation. All participants received folic acid (400 mcg daily). Adherence to treatment was self-reported daily and additionally assessed by weighing medication bottles at each study visit. High adherence to treatment assignment was reported among women in both groups.³⁶

Information regarding baseline demographic characteristics and reproductive history were obtained via questionnaires and medical record abstraction. Women were provided with fertility monitors (Clearblue Easy Fertility Monitor; Iverness Medical Innovations, Waltham, MA, USA) to time intercourse and schedule clinic visits. Following a positive urine pregnancy test, women visited the clinic at 6-7 weeks gestation to confirm an intrauterine

pregnancy, as visualized by a gestational sac upon ultrasonography. Methods for determining pregnancy and pregnancy loss diagnoses in the EAGeR trial have been previously published.³⁷

The institutional review boards at each study site and the data coordinating center approved the protocol for the trial. All participants provided their written consent prior to enrolling in the study. The trial was registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00467363) (#NCT00467363).

Platelet factor 4 assessment

Blood samples were obtained at the baseline preconception study visit, 12 weeks of gestation, and 28 weeks of gestation. Preconception samples were collected on day 2-3 of the menstrual cycle and prior to initiation of aspirin or placebo. Venipuncture was performed using a 21-gauge needle and a light tourniquet. Specimens were processed within 30 minutes of collection and were centrifuged at $1500 \times g$ for 10 minutes with no break to obtain platelet-poor plasma. These plasma samples were then stored at -80°C until analysis.

Citrate plasma samples were thawed at 37°C and then centrifuged at $13,000 \times g$ for 2 minutes to remove any remaining platelets, because the presence of residual platelets may affect the accuracy of platelet factor 4 concentration.³⁸ Platelet factor 4 concentrations were measured using a commercially-available ELISA (R&D Systems, Minneapolis, MN). The lower limit of detection for platelet factor 4 concentration was 78 ng/mL. The intra-assay coefficient of variation was 6.9%, and the inter-assay coefficient of variation was 11.8% at 26 ng/mL.

Outcome assessment

The outcomes of interest were placenta-mediated adverse obstetric outcomes, including hypertensive disorders of pregnancy (gestational hypertension and preeclampsia), placental abruption, and delivery of a small-for-gestational age neonate. Owing to the rarity of these conditions in our study population, the primary outcome was a composite of these outcomes, and we considered each individual outcome in secondary analyses. Diagnoses of hypertensive disorders of pregnancy, placental abruption, and SGA (defined as birth weight $<10^{\text{th}}$ percentile) were obtained prospectively by maternal report and abstracted from participant delivery records by trained research staff.³⁶

Statistical analysis

Of 1,228 women enrolled, 1,185 women had available measures of platelet factor 4 at preconception. Of these, 584 women had a pregnancy lasting at least 20 weeks' gestation, with 566 having available platelet factor 4 measures at 12 weeks' gestation and 550 with measures at 28 weeks' gestation. For each time point (i.e., preconception, 12 weeks' and 28 weeks' gestation), we categorized women into tertiles of platelet factor 4 based on the distribution of all women with available platelet factor 4 measures at that time. We compared demographic and pregnancy characteristics across preconception platelet factor 4 tertiles using general linear models and χ^2 tests for continuous and categorical variables, respectively.

For our analysis, the exposure of interest was plasma platelet factor 4 tertile (using the first tertile as the reference group) at each time point. We estimated unadjusted and adjusted relative risks (RR) and 95% confidence intervals (CI) for composite and individual placenta-mediated adverse obstetric outcomes. This analysis was done comparing higher versus lower tertiles of preconception, 12 weeks' gestation, and 28 weeks' gestation platelet factor 4 using log-binomial regression models with robust standard errors. We selected potential confounders *a priori* for inclusion in multivariable models (maternal age, body mass index, maternal education, household income, and smoking).

The placenta-mediated adverse obstetric outcomes of interest could only be observed among the EAGeR trial participants who conceived and carried a pregnancy to at least 20 weeks' gestation, but exposure information from the entire cohort of EAGeR participants was used in the stabilized inverse-probability weights in regression models to account for potential bias introduced by this selection in addressing the questions of exposure at each time point. Weights were generated using variables associated with pregnancy in this cohort (i.e., preconception platelet factor 4, age, BMI, maternal education, household income, smoking, and treatment assignment). Models evaluating platelet factor 4 plasma concentrations at 28 weeks' gestation were conducted among women with pregnancies lasting at least 28 weeks. These analyses were further weighted to account for the conditional probability of a pregnancy lasting to 20 weeks and then continuing to 28 weeks, when the final blood sample could be collected.

Because aspirin is a potent inhibitor of platelet activation, we hypothesized effect modification *a priori* and therefore stratified all analyses by treatment group (LDA, placebo).

Statistical analyses were performed using SAS software v9.4 (Cary, NC). P-values <0.05 from two-sided tests were considered statistically significant.

Results

Characteristics of the study population

Within the EAGeR trial study population, 1,185 women had available measures of preconception platelet factor 4. See Figure 1 for a flow diagram of the study population. On average, women with high compared to low preconception platelet factor 4 were younger, had higher BMI, lower income, were more likely to be married and more likely to achieve pregnancy during study follow-up (Table 1). Over the duration of follow-up, 95 women experienced the composite placenta-mediated adverse pregnancy outcome, with 57 cases of hypertensive disorders of pregnancy, 35 of SGA, and 6 of placental abruption. Overall, we found that circulating platelet factor 4 decreased from the preconception visit throughout pregnancy in both treatment groups (Figure 2).

Primary outcome

Among women trying to conceive, preconception platelet factor 4 was positively associated with the composite outcome in both unadjusted and adjusted analyses (Table 2, Figure 3). At 12 weeks' gestation, the highest tertile of platelet factor 4 was associated with a modest increase in risk for the composite outcome as compared to those with platelet factor 4 in the

lowest tertile (adjusted tertile 3 vs. 1 RR 1.63, 95% CI 1.01, 2.62). Among the 550 women who carried a pregnancy to at least 28 weeks' gestation and had measured platelet factor 4 concentrations, platelet factor 4 in the second or third tertile at 28 weeks was not significantly associated with risk for the composite outcome.

Consistent with the hypothesis that LDA treatment may modify associations between platelet factor 4 and adverse obstetric outcomes, we observed stronger associations for preconception platelet factor 4 and the composite adverse obstetric outcome among women in the placebo versus LDA group (Table 2, Figure 3). However, in the placebo group, higher platelet factor 4 values at 12 weeks and 28 weeks' gestation were not associated with the composite adverse obstetric outcome. In the LDA group, platelet factor 4 concentrations in the highest tertile at 12 weeks were associated with the composite adverse obstetric outcome, but higher platelet factor 4 values at 28 weeks were not.

Secondary outcomes

In analyses evaluating individual adverse obstetric outcomes, higher preconception platelet factor 4 was positively associated with risk of hypertensive disorders of pregnancy (Table 3, Figure 3). Platelet factor 4 concentrations measured at 12 and 28 weeks' gestation were not associated with risk for hypertensive disorders of pregnancy in the overall study population.

In analyses stratified by LDA for individual outcomes, higher preconception platelet factor 4 was more strongly associated with risk of hypertensive disorders of pregnancy in the placebo group than in the LDA treatment group (Table 3, Figure 3). Conversely, platelet factor 4 concentrations at 12 weeks' and 28 weeks' gestation were more strongly associated with risk of hypertensive disorders of pregnancy in the aspirin versus placebo group. For these analyses stratified by treatment group, many of the comparisons yielded wide confidence intervals without statistical significance.

Estimates from analyses evaluating SGA were similar in magnitude to those for the composite outcome, though sample size was limited and none of the associations were statistically significant (for preconception platelet factor 4 in tertile 3 vs tertile 1, adjusted RR 2.31, 95% CI 0.88, 6.08). Similarly, small numbers of placental abruption limited power to evaluate risk of this outcome overall (for preconception platelet factor 4 in tertile 3 vs. tertile 1, adjusted RR 2.39, 95% CI 0.18, 31.32) or within strata of LDA treatment/placebo (unable to run stratified models due to small numbers).

Comment

Principal findings

In this prospective study, higher concentrations of circulating platelet factor 4 prior to and in early pregnancy were associated with an increased risk for composite placenta-mediated adverse pregnancy outcomes including hypertensive disorders of pregnancy, placental abruption, and SGA infant.

Results

The association between maternal plasma platelet factor 4 concentration and the composite outcome of placenta-mediated adverse pregnancy outcomes was primarily driven by the association between elevated preconception platelet factor 4 plasma concentrations and hypertensive disorders of pregnancy. Among women with the highest plasma concentrations of preconception platelet factor 4, treatment with aspirin diminished the association of platelet factor 4 with the composite outcome, and hypertensive disorders of pregnancy in particular. Although multiple investigators have previously found that platelet activation is increased at the time hypertensive disorders of pregnancy are diagnosed,^{39–42} our findings expand on prior work by evaluating changes in platelet factor 4 from preconception throughout pregnancy and whether the association was modified by aspirin.^{33,43}

Clinical implications

Platelet activation occurs when receptors on the platelet surface are engaged by a variety of agonists, triggering a complex signaling cascade that results in mobilization of intracellular calcium and change in cytoskeletal elements. Granules centralize and fuse with the plasma membrane, secreting their contents. In addition, receptors stored in platelet cytoplasm, such as CD40 ligand and P-selectin, are relocated to the platelet surface, where they promote interaction with monocytes, macrophages, and endothelial cells.⁴⁴ Thus, platelet activation triggers a local inflammatory response characterized by release of chemokines, upregulation of surface receptors, and increased cell-cell interactions. The presence of platelet activation prior to and in early pregnancy may promote an altered endothelial cell phenotype, increased recruitment of leukocytes, and inflammation at the maternal-fetal interface, resulting in altered placentation and eventually greater risk of adverse pregnancy outcomes.

Among women assigned to the aspirin group, the association between high preconception platelet factor 4 and higher risk for adverse placenta-mediated obstetric outcomes was negated. Interestingly, however, women with persistently high platelet factor 4 during pregnancy despite aspirin assignment had increased risk for these adverse outcomes. It is possible that these women with persistently high platelet factor 4 despite randomization to aspirin were noncompliant with their treatment. It is also possible that these women represent a low-dose aspirin non-response phenotype, for whom alternative dosing regimens for aspirin (or alternative treatments) may be more effective at reducing obstetric risks.

Research implications

These observations may be due to aspirin's role in irreversibly inhibiting cyclooxygenase-1 (COX-1) and production of thromboxane A2 (TxA2), which markedly amplifies platelet activation.⁴⁵ Although even low doses of aspirin (as low as 30 mg daily) effectively reduce production of TxA2 by more than 95% after 5 days of use, other routes of platelet activation are not affected by inhibition of COX-1, including activation by collagen, thrombin, and ADP.⁴⁶ One previous publication demonstrated that TxA2 is less likely to be completely inhibited in obese pregnant women compared to normal weight pregnant women.⁴⁷ In addition, persistent platelet reactivity despite aspirin treatment may be dependent upon the dose, frequency of administration, and platelet turnover.⁴⁵ For example, newly synthesized platelets produced in settings of increased turnover express COX-2 in addition to COX-1,

and COX-2 is not inhibited by low doses of aspirin. These caveats to suppression of platelet activity by aspirin may explain, in part, the modest effect of low dose aspirin for prevention of preeclampsia and controversial effects in prevention of recurrent fetal growth restriction.
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It is important to note that, because of cost constraints, we have only assessed one of many biomarkers for platelet activation. We chose platelet factor 4 because of its reliability as a marker of *in vivo* platelet activation, inherent antiangiogenic properties,^{17,22–25} and ease of measurement via a commercially-available ELISA kit. Previously published studies of other biomarkers have demonstrated associations between other platelet-derived chemokines (e.g., sFlt-1,⁴⁹ endostatin,⁵⁰ RANTES^{51,52}) and adhesion molecules (e.g., CD40 ligand,^{52–54} P-selectin^{55,56}) with adverse obstetric outcomes. However, the majority of these studies determined circulating concentrations of these biomarkers cross-sectionally at the time the adverse pregnancy outcome had already been diagnosed. Assessing the pre-conception concentrations of these other biomarkers of platelet activation and changes in these biomarkers over the course of healthy versus complicated pregnancies may shed more light on the complex role that platelets may play in human placentation. Understanding these processes may then allow for optimization of preventive strategies, such as aspirin.

Strengths and limitations

Limitations of our study include the small number of pregnancies with placental abruption or SGA infants, which limits our ability to draw conclusions regarding the association between platelet factor 4 concentration and these specific adverse outcomes. Further, we grouped women with gestational hypertension and preeclampsia into the category of hypertensive disorders of pregnancy, so there is phenotypic heterogeneity within this outcome. Additionally, our study population is predominantly white with relatively high income, which limits generalizability. We only assessed one of many markers of platelet activation, and we did not examine platelet factor 4 plasma concentrations beyond 28 weeks.

Strengths of our study include the measurement of platelet factor 4 at multiple time points, including prior to conception. This allowed us to demonstrate that platelet activation prior to and early in pregnancy is associated with adverse obstetric outcomes. We also determined the longitudinal changes in platelet factor 4 concentration from prior to conception through the third trimester in women on LDA and placebo. The exclusion of women with certain comorbid conditions limits potential confounding. Very few women were lost to follow-up, minimizing potential for selection bias. Adherence to aspirin therapy was assessed as part of the EAGeR trial, and similarly high rates of adherence were reported between treatment and placebo groups. Additionally, the randomization of women to aspirin or placebo therapy in the EAGeR trial allows us to report additional hypothesis-generating data regarding the mechanism of obstetric risk reduction via aspirin. For example, aspirin may be an effective risk reduction strategy for hypertensive disorders of pregnancy for some women with high pre-pregnancy platelet activation. With regard to these outcomes, further study is needed to determine why some women with elevated platelet factor 4 may respond better to aspirin treatment, and whether a personalized approach to obstetric risk reduction based on degree of pre-pregnancy platelet activation may be warranted.

Notably, the EAGeR trial preceded the Aspirin for Evidence-based Preeclampsia Prevention (ASPRe) trial, which found significant reduction in risk for preterm preeclampsia following treatment of high-risk women with 150 mg of aspirin daily.¹⁰ While international enthusiasm for a higher dose of aspirin for preeclampsia prevention seems to be growing, the United States Preventive Services Task Force,⁸ American College of Obstetrics and Gynecology,⁴⁸ and World Health Organization⁵⁷ still recommend an aspirin dose of 75-81 mg/day. The National Institute for Health and Care Excellence recommends a dose of 75-150 mg/day.⁵⁸ The International Federation of Gynecology and Obstetrics is currently the only organization recommending a dose of 150 mg daily.⁵⁹ Another multi-country randomized controlled trial recently demonstrated benefit of an 81 mg dose for the prevention of preterm preeclampsia, preterm birth, and perinatal mortality.¹¹ Thus, our findings remain relevant to contemporary obstetric practice.

Conclusions

In summary, higher plasma concentrations of platelet factor 4 prior to pregnancy are associated with increased risk of placenta-mediated adverse outcomes, particularly for hypertensive disorders of pregnancy. Although low-dose aspirin modifies the association between adverse obstetric outcomes and high preconception platelet factor 4, this modification was not observed among women with persistently high platelet factor 4 during pregnancy despite randomization to aspirin treatment. These results illuminate potential mechanisms by which adverse placenta-mediated obstetric outcomes occur. Additionally, these results suggest that platelet factor 4 response to aspirin treatment outside of pregnancy may identify which women will benefit from aspirin as a risk-reducing strategy during pregnancy.

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References

1. Conrad KP, Rabaglino MB, Post Uiterweer ED. Emerging role for dysregulated decidualization in the genesis of preeclampsia. *Placenta*. 2017;60:119–129. doi:10.1016/j.placenta.2017.06.005 [PubMed: 28693893]
2. Lecarpentier E, Tsatsaris V. Angiogenic balance (sFlt-1/PlGF) and preeclampsia. *Ann Endocrinol*. 2016;77(2):97–100. doi:10.1016/j.ando.2016.04.007
3. Velicky P, Knöfler M, Pollheimer J. Function and control of human invasive trophoblast subtypes: Intrinsic vs. maternal control. *Cell Adhes Migr*. 2016;10(1-2):154–162. doi:10.1080/19336918.2015.1089376
4. Brosens I, Pijnenborg R, Vercruysse L, Romero R. THE “GREAT OBSTETRICAL SYNDROMES” ARE ASSOCIATED WITH DISORDERS OF DEEP PLACENTATION. *Am J Obstet Gynecol*. 2011;204(3):193–201. doi:10.1016/j.ajog.2010.08.009 [PubMed: 21094932]

5. Lockwood CJ, Paidas M, Murk WK, et al. Involvement of human decidua cell-expressed tissue factor in uterine hemostasis and abruption. *Thromb Res.* 2009;124(5):516–520. doi:10.1016/j.thromres.2009.07.017 [PubMed: 19720393]
6. Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. *Am J Obstet Gynecol.* 2018;218(2S):S745–S761. doi:10.1016/j.ajog.2017.11.577 [PubMed: 29422210]
7. Groom KM, David AL. The role of aspirin, heparin, and other interventions in the prevention and treatment of fetal growth restriction. *Am J Obstet Gynecol.* 2018;218(2S):S829–S840. doi:10.1016/j.ajog.2017.11.565 [PubMed: 29229321]
8. LeFevre ML US Preventive Services Task Force. Low-dose aspirin use for the prevention of morbidity and mortality from preeclampsia: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med.* 2014;161(11):819–826. doi:10.7326/M14-1884 [PubMed: 25200125]
9. Tolcher MC, Chu DM, Hollier LM, et al. Impact of USPSTF recommendations for aspirin for prevention of recurrent preeclampsia. *Am J Obstet Gynecol.* 2017;217(3):365.e1–365.e8. doi:10.1016/j.ajog.2017.04.035 [PubMed: 28455079]
10. Rolnik DL, Wright D, Poon LC, et al. Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *N Engl J Med.* 2017;377(7):613–622. doi:10.1056/NEJMoa1704559 [PubMed: 28657417]
11. Hoffman MK, Goudar SS, Kodkany BS, et al. Low-dose aspirin for the prevention of preterm delivery in nulliparous women with a singleton pregnancy (ASPIRIN): a randomised, double-blind, placebo-controlled trial. *Lancet Lond Engl.* 2020;395(10220):285–293. doi:10.1016/S0140-6736(19)32973-3
12. Sato Y, Fujiwara H, Zeng B-X, Higuchi T, Yoshioka S, Fujii S. Platelet-derived soluble factors induce human extravillous trophoblast migration and differentiation: platelets are a possible regulator of trophoblast infiltration into maternal spiral arteries. *Blood.* 2005;106(2):428–435. doi:10.1182/blood-2005-02-0491 [PubMed: 15797992]
13. Li Z, Delaney MK, O'Brien KA, Du X. Signaling during platelet adhesion and activation. *Arterioscler Thromb Vasc Biol.* 2010;30(12):2341–2349. doi:10.1161/ATVBAHA.110.207522 [PubMed: 21071698]
14. Ramsey EM. The story of the spiral arteries. *J Reprod Med.* 1981;26(8):393–399. [PubMed: 7024541]
15. Brosens I, Puttemans P, Benagiano G. Placental bed research: I. The placental bed: from spiral arteries remodeling to the great obstetrical syndromes. *Am J Obstet Gynecol.* 2019;221(5):437–456. doi:10.1016/j.ajog.2019.05.044 [PubMed: 31163132]
16. Chatterjee M, Huang Z, Zhang W, et al. Distinct platelet packaging, release, and surface expression of proangiogenic and antiangiogenic factors on different platelet stimuli. *Blood.* 2011;117(14):3907–3911. doi:10.1182/blood-2010-12-327007 [PubMed: 21330475]
17. Italiano JE, Richardson JL, Patel-Hett S, et al. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood.* 2008;111(3):1227–1233. doi:10.1182/blood-2007-09-113837 [PubMed: 17962514]
18. Battinelli EM, Thon JN, Okazaki R, et al. Megakaryocytes package contents into separate α -granules that are differentially distributed in platelets. *Blood Adv.* 2019;3(20):3092–3098. doi:10.1182/bloodadvances.2018020834 [PubMed: 31648331]
19. Nylander M, Osman A, Ramstrom S, Aklint E, Larsson A, Lindahl TL. The role of thrombin receptors PAR1 and PAR4 for PAI-1 storage, synthesis and secretion by human platelets. *Thromb Res.* 2012;129(4):e51–58. doi:10.1016/j.thromres.2011.12.021 [PubMed: 22283974]
20. Hutton RA, Chow FP, Craft IL, Dandona P. Inhibitors of platelet aggregation in the fetoplacental unit and myometrium with particular reference to the ADP-degrading property of placenta. *Placenta.* 1980;1(2):125–130. doi:10.1016/s0143-4004(80)80020-8 [PubMed: 6255456]
21. O'Brien WF, Knuppel RA, Saba HI, Angel JL, Benoit R, Bruce A. Platelet inhibitory activity in placentas from normal and abnormal pregnancies. *Obstet Gynecol.* 1987;70(4):597–600. [PubMed: 2819799]
22. Bikfalvi A Platelet factor 4: an inhibitor of angiogenesis. *Semin Thromb Hemost.* 2004;30(3):379–385. doi:10.1055/s-2004-831051 [PubMed: 15282661]

23. Gengrinovitch S, Greenberg SM, Cohen T, et al. Platelet factor-4 inhibits the mitogenic activity of VEGF121 and VEGF165 using several concurrent mechanisms. *J Biol Chem*. 1995;270(25):15059–15065. doi:10.1074/jbc.270.25.15059 [PubMed: 7797488]
24. Hansell P, Maione TE, Borgstrom P. Selective binding of platelet factor 4 to regions of active angiogenesis in vivo. *Am J Physiol*. 1995;269(3 Pt 2):H829–836. doi:10.1152/ajpheart.1995.269.3.H829 [PubMed: 7573524]
25. Maurer A-M, Zhou B, Han ZC. Roles of platelet factor 4 in hematopoiesis and angiogenesis. *Growth Factors Chur Switz*. 2006;24(4):242–252. doi:10.1080/08977190600988225
26. Harrison P, Cramer EM. Platelet alpha-granules. *Blood Rev*. 1993;7(1):52–62. [PubMed: 8467233]
27. Kaplan KL, Owen J. Plasma levels of platelet secretory proteins. *Crit Rev Oncol Hematol*. 1986;5(3):235–255. [PubMed: 2427239]
28. Gurney D, Lip GYH, Blann AD. A reliable plasma marker of platelet activation: does it exist? *Am J Hematol*. 2002;70(2):139–144. doi:10.1002/ajh.10097 [PubMed: 12111787]
29. Kowalska MA, Rauova L, Poncz M. Role of the platelet chemokine platelet factor 4 (PF4) in hemostasis and thrombosis. *Thromb Res*. 2010;125(4):292–296. doi:10.1016/j.thromres.2009.11.023 [PubMed: 20004006]
30. Endo Y, Mamiya S, Satoh M, Takahashi K, Harada T. Plasma beta-thromboglobulin and platelet factor 4 in patients with chronic renal failure and effect of hemodialysis. *Tohoku J Exp Med*. 1981;135(4):349–358. doi:10.1620/tjem.135.349 [PubMed: 6175048]
31. Socol ML, Weiner CP, Louis G, Rehnberg K, Rossi EC. Platelet activation in preeclampsia. *Am J Obstet Gynecol*. 1985;151(4):494–497. doi:10.1016/0002-9378(85)90276-5 [PubMed: 3156500]
32. Ayhan A, Akkok E, Urman B, Yarali H, Dundar S, Kirazli S. Beta-thromboglobulin and platelet factor 4 levels in pregnancy and preeclampsia. *Gynecol Obstet Invest*. 1990;30(1):12–14. doi:10.1159/000293203 [PubMed: 2146196]
33. Csaicsich P, Deutinger J, Tatra G. Platelet specific proteins (beta-thromboglobulin and platelet factor 4) in normal pregnancy and in pregnancy complicated by preeclampsia. *Arch Gynecol Obstet*. 1989;244(2):91–95. [PubMed: 2523691]
34. Schisterman EF, Silver RM, Perkins NJ, et al. A randomised trial to evaluate the effects of low-dose aspirin in gestation and reproduction: design and baseline characteristics. *Paediatr Perinat Epidemiol*. 2013;27(6):598–609. doi:10.1111/ppe.12088 [PubMed: 24118062]
35. Schisterman EF, Mumford SL, Schliep KC, et al. Preconception low dose aspirin and time to pregnancy: findings from the effects of aspirin in gestation and reproduction randomized trial. *J Clin Endocrinol Metab*. 2015;100(5):1785–1791. doi:10.1210/jc.2014-4179 [PubMed: 25710565]
36. Schisterman EF, Silver RM, Leshner LL, et al. Preconception low-dose aspirin and pregnancy outcomes: results from the EAGeR randomised trial. *Lancet Lond Engl*. 2014;384(9937):29–36. doi:10.1016/S0140-6736(14)60157-4
37. Mumford SL, Silver RM, Sjaarda LA, et al. Expanded findings from a randomized controlled trial of preconception low-dose aspirin and pregnancy loss. *Hum Reprod Oxf Engl*. 2016;31(3):657–665. doi:10.1093/humrep/dev329
38. Soderstrom AC, Nybo M, Nielsen C, Vinholt PJ. The effect of centrifugation speed and time on pre-analytical platelet activation. *Clin Chem Lab Med*. 2016;54(12):1913–1920. doi:10.1515/cclm-2016-0079 [PubMed: 27227708]
39. Holthe MR, Staff AC, Berge LN, Lyberg T. Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women. *Am J Obstet Gynecol*. 2004;190(4):1128–1134. doi:10.1016/j.ajog.2003.10.699 [PubMed: 15118653]
40. Konijnenberg A, Stokkers EW, van der Post JA, et al. Extensive platelet activation in preeclampsia compared with normal pregnancy: enhanced expression of cell adhesion molecules. *Am J Obstet Gynecol*. 1997;176(2):461–469. [PubMed: 9065199]
41. Macey MG, Bevan S, Alam S, et al. Platelet activation and endogenous thrombin potential in preeclampsia. *Thromb Res*. 2010;125(3):e76–81. doi:10.1016/j.thromres.2009.09.013 [PubMed: 19822350]
42. Harlow FH, Brown MA, Brighton TA, et al. Platelet activation in the hypertensive disorders of pregnancy. *Am J Obstet Gynecol*. 2002;187(3):688–695. [PubMed: 12237649]

43. Hayashi M, Kiumi F, Mitsuya K. Changes in platelet ATP secretion and aggregation during pregnancy and in preeclampsia. *Am J Med Sci.* 1999;318(2):115–121. [PubMed: 10452571]
44. Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med.* 2007;357(24):2482–2494. doi:10.1056/NEJMr071014 [PubMed: 18077812]
45. Pettersen AA, Arnesen H, Seljeflot I. A brief review on high on-aspirin residual platelet reactivity. *Vascul Pharmacol.* 2015;67-69:6–9. doi:10.1016/j.vph.2015.03.018 [PubMed: 25869498]
46. Hankey GJ, Eikelboom JW. Aspirin resistance. *Lancet Lond Engl.* 2006;367(9510):606–617. doi:10.1016/S0140-6736(06)68040-9
47. Finneran MM, Gonzalez-Brown VM, Smith DD, Landon MB, Rood KM. Obesity and laboratory aspirin resistance in high-risk pregnant women treated with low-dose aspirin. *Am J Obstet Gynecol.* 2019;220(4):385.e1–385.e6. doi:10.1016/j.ajog.2019.01.222 [PubMed: 30786253]
48. ACOG Committee Opinion No. 743: Low-Dose Aspirin Use During Pregnancy. *Obstet Gynecol.* 2018;132(1):e44–e52. doi:10.1097/AOG.0000000000002708 [PubMed: 29939940]
49. Major HD, Cambell RA, Silver RM, Branch DW, Weyrich AS. Synthesis of sFlt-1 by platelet-monocyte aggregates contributes to the pathogenesis of preeclampsia. *Am J Obstet Gynecol.* 2014;210(6):547.e1–547.e7. doi:10.1016/j.ajog.2014.01.024 [PubMed: 24440566]
50. Wikström A-K, Larsson A, Akerud H, Olovsson M. Increased circulating levels of the antiangiogenic factor endostatin in early-onset but not late-onset preeclampsia. *Reprod Sci Thousand Oaks Calif.* 2009;16(10):995–1000. doi:10.1177/1933719109339348
51. Hentschke MR, Krauspenhar B, Guwzinski A, et al. PP040. Expression of RANTES (CCL5) in maternal plasma, fetal plasma and placenta in pre-eclampsia and normotensive controls. *Pregnancy Hypertens.* 2012;2(3):263. doi:10.1016/j.preghy.2012.04.151
52. Mellembakken JR, Solum NO, Ueland T, Videm V, Aukrust P. Increased concentrations of soluble CD40 ligand, RANTES and GRO-alpha in preeclampsia--possible role of platelet activation. *Thromb Haemost.* 2001;86(5):1272–1276. [PubMed: 11816717]
53. Erez O, Romero R, Hoppensteadt D, et al. PREMATURE LABOR: A STATE OF PLATELET ACTIVATION? *J Perinat Med.* 2008;36(5):377–387. doi:10.1515/JPM.2008.082 [PubMed: 18958919]
54. Erez O, Gotsch F, Mazaki-Tovi S, et al. Evidence of maternal platelet activation, excessive thrombin generation, and high amniotic fluid tissue factor immunoreactivity and functional activity in patients with fetal death. *J Matern-Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet.* 2009;22(8):672–687. doi:10.1080/14767050902853117
55. Docheva N, Romero R, Chaemsaitong P, et al. The Profiles of Soluble Adhesion Molecules in the “Great Obstetrical Syndromes.” *J Matern-Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet.* 2019;32(13):2113–2136. doi:10.1080/14767058.2018.1427058
56. Laskowska M, Laskowska K, Oleszczuk J. Elevated maternal serum sP-selectin levels in preeclamptic pregnancies with and without intrauterine fetal growth restriction, but not in normotensive pregnancies complicated by isolated IUGR. *Med Sci Monit Int Med J Exp Clin Res.* 2013;19:118–124. doi:10.12659/MSM.883780
57. WHO Recommendations for Prevention and Treatment of Pre-Eclampsia and Eclampsia. Geneva: World Health Organization; 2011 <http://www.ncbi.nlm.nih.gov/books/NBK140561/>. Accessed February 6, 2020.
58. Recommendations Hypertension in pregnancy: diagnosis and management Guidance NICE. <https://www.nice.org.uk/guidance/ng133/chapter/Recommendations>. Accessed February 6, 2020.
59. Poon LC, Shennan A, Hyett JA, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. *Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet.* 2019;145 Suppl 1:1–33. doi:10.1002/ijgo.12802

Condensation:

High pre-pregnancy plasma concentrations of platelet factor 4 are associated with increased risk for placenta-mediated adverse pregnancy outcomes, and low-dose aspirin may be protective.

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AJOG at a Glance:**A. Why was the study conducted?**

- Platelet activation may play a role in the pathophysiology of placenta-mediated obstetric outcomes, as evidenced by the efficacy of aspirin for preventing preeclampsia, though there are few studies investigating markers of platelet activation prior to conception or in early pregnancy.

B. What are the key findings?

- Higher concentrations of platelet factor 4 prior to pregnancy are associated with increased risk of placenta-mediated adverse pregnancy outcomes, particularly hypertensive disorders of pregnancy.
- Aspirin may mitigate the increased risk of these outcomes among women with higher plasma concentrations of preconception platelet factor 4.

C. What does this study add to what is already known?

- We have identified platelet factor 4 as a potential biomarker for adverse pregnancy outcomes prior to conception.
- Platelet factor 4 may identify low-dose aspirin non-responders in order to personalize obstetric risk reduction strategies.

Figure 1.
Flow diagram of study population.

Figure 2. Mean platelet factor 4 (PF4) concentration (ng/mL) and 95% CI over time among women assigned to low-dose aspirin (LDA) or placebo.

Figure 3. Adjusted relative risk and 95% CI for placenta-mediated adverse pregnancy outcomes (composite and hypertensive disorders alone) among women with platelet factor 4 (PF4) values in the 3rd tertile compared to the 1st tertile at each time point.

Table 1.

Demographic and pregnancy characteristics by baseline platelet factor 4 (PF4) tertile.

	PF4 112–900 ng/mL (n = 395)	PF4 1000–1783 ng/mL (n = 395)	PF4 1785–7596 ng/mL (n = 395)	<i>p-value</i>
Maternal age, years	29.3 (4.8)	28.6 (4.7)	28.2 (4.8)	0.005
Race/ethnicity (n, %)				
White	369 (93.4)	371 (93.9)	382 (96.7)	0.09
Non-white	26 (6.6)	24 (6.1)	13 (3.3)	
BMI	25.6 (6.3)	26.4 (6.3)	26.7 (6.6)	0.03
Smoker (n, %)	50 (12.8)	44 (11.3)	53 (13.5)	0.63
Maternal marital status (n, %)				
Married	350 (88.6)	367 (92.9)	370 (93.7)	0.007
Living with partner	29 (7.3)	25 (6.3)	16 (4.1)	
Other	16 (4.1)	3 (0.8)	9 (2.3)	
More than high school education (n, %)	337 (85.5)	341 (86.3)	346 (87.6)	0.69
Household income (annual) (n, %)				
>\$100,000	156 (39.6)	161 (40.8)	151 (38.2)	0.009
\$75,000-99,999	56 (14.2)	57 (14.4)	35 (8.9)	
\$40,000-74,999	69 (17.5)	55 (13.9)	53 (13.4)	
\$20,000-39,999	89 (22.6)	99 (25.1)	113 (28.6)	
<\$19,999	24 (6.1)	23 (5.8)	43 (10.9)	
Employed (n, %)	287 (76.1)	292 (76.4)	289 (74.9)	0.86
Previous live birth (n, %)	210 (53.2)	211 (53.4)	211 (53.4)	1.00
Aspirin intervention group (n, %)	196 (49.6)	204 (51.6)	198 (50.1)	0.84
Pre-pregnancy PF4, ng/mL	551.6 (207.1)	1283.7 (247.2)	3019.4 (1168.3)	<0.0001
Achieved pregnancy (n, %)	247 (62.5)	249 (63)	279 (70.6)	0.03
Early pregnancy loss (n, %)	18 (7.3)	17 (6.9)	19 (7.0)	1
Live birth (n, %)	185 (75.2)	187 (76.3)	211 (77.3)	0.86
Gestational age at delivery, wks	38.9 (1.5)	38.6 (2.2)	38.8 (1.9)	0.36
Prior history of preeclampsia				
0	218 (98.2)	222 (98.7)	246 (97.6)	0.44
1	4 (1.8)	2 (0.9)	6 (2.4)	
2	0 (0)	1 (0.4)	0 (0)	

Values are mean (SD) unless otherwise indicated.

Table 2.

Unadjusted and adjusted* relative risks and 95% confidence intervals of the composite placenta-mediated adverse obstetric outcome according to circulating platelet factor 4 (PF4) tertiles at each time point for the overall study population and stratified by treatment group.

		PF4 Tertile		
		1	2	3
Overall	Preconception (n=584)			
	Cases (n, %)	17 (9.2)	38 (20.2)	40 (18.9)
	Median (range), ng/mL	558 (122, 894)	1336 (903, 1783)	2621 (1785, 7234)
	Unadjusted	1.00 (ref)	2.35 (1.38, 4.01)	2.25 (1.32, 3.83)
	Adjusted*	1.00 (ref)	2.46 (1.44, 4.18)	2.44 (1.43, 4.15)
	12 weeks (n=566)			
	Cases (n, %)	25 (13.4)	29 (15.3)	39 (20.4)
	Median (range), ng/mL	433 (84, 815)	1130 (818, 1531)	2237 (1535, 7548)
	Unadjusted	1.00 (ref)	1.12 (0.66, 1.89)	1.66 (1.03, 2.66)
	Adjusted*	1.00 (ref)	1.19 (0.7, 2.03)	1.63 (1.01, 2.62)
	28 weeks (n=550)			
Aspirin	Cases (n, %)	27 (14.8)	34 (18.6)	33 (17.9)
	Median (range), ng/mL	381 (31, 769)	1092 (772, 1411)	1949 (1423, 8488)
	Unadjusted	1.00 (ref)	1.52 (0.77, 3.01)	1.47 (0.74, 2.93)
	Adjusted*	1.00 (ref)	1.55 (0.78, 3.08)	1.38 (0.69, 2.78)
	Preconception (n=305)			
	Cases (n, %)	11 (12.4)	18 (18.4)	23 (19.5)
	Median (range), ng/mL	548 (122, 894)	1359 (903, 1783)	2495 (1785, 7234)
	Unadjusted	1.00 (ref)	1.59 (0.78, 3.27)	1.81 (0.92, 3.58)
	Adjusted*	1.00 (ref)	1.54 (0.74, 3.22)	1.83 (0.93, 3.62)
	12 weeks (n=292)			
Placebo	Cases (n, %)	12 (13.3)	19 (18.8)	21 (20.8)
	Median (range), ng/mL	437 (84, 815)	1131 (843, 1512)	2262 (1549, 7548)
	Unadjusted	1.00 (ref)	1.58 (0.73, 3.38)	2.1 (1.03, 4.29)
	Adjusted*	1.00 (ref)	1.72 (0.79, 3.73)	2.16 (1.06, 4.42)
	28 weeks (n=286)			
	Cases (n, %)	14 (15.2)	21 (21.9)	18 (18.4)
	Median (range), ng/mL	417 (81, 767)	1105 (772, 1411)	1936 (1437, 8488)
	Unadjusted	1.00 (ref)	1.93 (0.73, 5.05)	1.68 (0.62, 4.54)
	Adjusted*	1.00 (ref)	1.68 (0.64, 4.42)	1.75 (0.66, 4.63)
	Preconception (n=279)			
	Cases (n, %)	6 (6.3)	20 (22.2)	17 (18.1)
	Median (range), ng/mL	561 (145, 876)	1309 (906, 1775)	2851 (1813, 7214)
	Unadjusted	1.00 (ref)	3.56 (1.57, 8.1)	2.85 (1.22, 6.66)
	Adjusted*	1.00 (ref)	3.49 (1.54, 7.9)	3.41 (1.46, 7.98)

	PF4 Tertile		
12 weeks (n=274)			
Cases (n, %)	13 (13.5)	10 (11.4)	18 (20)
Median (range), ng/mL	430 (92, 800)	1126 (818, 1531)	2232 (1535, 5725)
Unadjusted	1.00 (ref)	0.78 (0.37, 1.67)	1.34 (0.7, 2.56)
Adjusted [*]	1.00 (ref)	0.85 (0.4, 1.81)	1.35 (0.7, 2.59)
28 weeks (n=264)			
Cases (n, %)	13 (14.3)	13 (14.9)	15 (17.4)
Median (range), ng/mL	361 (31, 769)	1079 (774, 1395)	1960 (1423, 5976)
Unadjusted	1.00 (ref)	1.16 (0.43, 3.14)	1.3 (0.49, 3.4)
Adjusted [*]	1.00 (ref)	1.26 (0.47, 3.38)	1.32 (0.49, 3.54)

* Adjusted for age, BMI, maternal education, household income, and smoking.

Table 3.

Unadjusted and adjusted* relative risks and 95% confidence intervals for hypertensive disorders of pregnancy according to circulating platelet factor 4 (PF4) tertiles at each time point for the overall study population and stratified by treatment group.

		PF4 Tertile		
		1	2	3
Overall	Preconception (n=580)			
	Cases (n, %)	11 (6)	22 (11.7)	24 (11.4)
	Median (range), ng/mL	558 (122, 894)	1336 (903, 1783)	2621 (1785, 7234)
	Unadjusted	1.00 (ref)	2.2 (1.11, 4.35)	2.12 (1.08, 4.18)
	Adjusted*	1.00 (ref)	2.33 (1.18, 4.59)	2.23 (1.13, 4.4)
	12 weeks (n=562)			
	Cases (n, %)	16 (8.7)	13 (6.9)	28 (14.7)
	Median (range), ng/mL	433 (84, 815)	1130 (818, 1531)	2237 (1535, 7548)
	Unadjusted	1.00 (ref)	0.78 (0.38, 1.61)	1.79 (1.00, 3.21)
	Adjusted*	1.00 (ref)	0.79 (0.39, 1.62)	1.71 (0.95, 3.08)
	28 weeks (n=546)			
	Cases (n, %)	14 (7.8)	20 (10.9)	24 (13.1)
	Median (range), ng/mL	381 (31, 769)	1092 (772, 1411)	1949 (1423, 8488)
	Unadjusted	1.00 (ref)	1.51 (0.62, 3.68)	1.81 (0.76, 4.29)
	Adjusted*	1.00 (ref)	1.39 (0.57, 3.41)	1.49 (0.61, 3.61)
	Aspirin Preconception (n=302)			
	Cases (n, %)	7 (8)	8 (8.2)	15 (12.8)
	Median (range), ng/mL	548 (122, 894)	1359 (903, 1783)	2495 (1785, 7234)
	Unadjusted	1.00 (ref)	1.22 (0.45, 3.27)	1.91 (0.79, 4.59)
	Adjusted*	1.00 (ref)	1.43 (0.53, 3.88)	2.08 (0.88, 4.94)
	12 weeks (n=289)			
	Cases (n, %)	6 (6.8)	8 (8)	15 (14.9)
	Median (range), ng/mL	437 (84, 815)	1131 (843, 1512)	2262 (1549, 7548)
	Unadjusted	1.00 (ref)	1.36 (0.44, 4.27)	3.01 (1.12, 8.15)
	Adjusted*	1.00 (ref)	1.54 (0.49, 4.81)	3.46 (1.28, 9.33)
	28 weeks (n=283)			
	Cases (n, %)	5 (5.6)	12 (12.5)	14 (14.4)
	Median (range), ng/mL	417 (81, 767)	1105 (772, 1411)	1936 (1437, 8488)
	Unadjusted	1.00 (ref)	3.35 (0.68, 16.54)	3.78 (0.78, 18.41)
	Adjusted*	1.00 (ref)	2.54 (0.5, 12.84)	3.77 (0.77, 18.37)
	Placebo Preconception (n=278)			
	Cases (n, %)	4 (4.2)	14 (15.6)	9 (9.7)
	Median (range), ng/mL	561 (145, 876)	1309 (906, 1775)	2851 (1813, 7214)
	Unadjusted	1.00 (ref)	3.68 (1.36, 9.95)	2.26 (0.77, 6.64)
	Adjusted*	1.00 (ref)	3.24 (1.21, 8.69)	2.7 (0.93, 7.9)

	PF4 Tertile		
12 weeks (n=273)			
Cases (n, %)	10 (10.5)	5 (5.7)	13 (14.4)
Median (range), ng/mL	430 (92, 800)	1126 (818, 1531)	2232 (1535, 5725)
Unadjusted	1.00 (ref)	0.53 (0.19, 1.42)	1.25 (0.58, 2.65)
Adjusted [*]	1.00 (ref)	0.53 (0.2, 1.41)	1.23 (0.58, 2.59)
28 weeks (n=263)			
Cases (n, %)	9 (10)	8 (9.2)	10 (11.6)
Median (range), ng/mL	361 (31, 769)	1079 (774, 1395)	1960 (1423, 5976)
Unadjusted	1.00 (ref)	0.86 (0.26, 2.83)	1.13 (0.38, 3.39)
Adjusted [*]	1.00 (ref)	0.86 (0.27, 2.76)	0.94 (0.3, 2.9)

* Adjusted for age, BMI, maternal education, household income, and smoking.