A lower maternal cortisol to cortisone ratio precedes clinical diagnosis of preterm and term preeclampsia by many weeks

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Abstract

Context: Previous studies have shown reduced placental levels of 11-beta-hydroxysteroid dehydrogenase-type 2 (11βHSD2) in preeclampsia. However, it is not known if the maternal cortisol to cortisone ratio is predictive of placental complications of pregnancy.

Objective: To determine the relationship between the maternal serum cortisol to cortisone ratio at different stages of pregnancy and the risk of preeclampsia or fetal growth restriction (FGR).

Design: Women from the Pregnancy Outcome Prediction (POP) study experiencing preeclampsia (n=194) or FGR (n=185) plus a random sample of healthy controls (n=279) were studied. Steroids were measured at ~12, ~20, ~28 and ~36 weeks of gestational age (wkGA). Separate analyses were performed for outcomes with term or preterm delivery. Associations were modelled using logistic regression.

Results: At 28 wkGA, the cortisol to cortisone ratio was negatively associated (odds ratio (OR) per one standard deviation increase, 95% confidence interval (CI)) with preterm preeclampsia (OR 0.33, 95% CI 0.19-0.57), term preeclampsia (OR 0.61, 95% CI 0.49-0.76) and preterm FGR (OR 0.50, 95% CI 0.29-0.85). At 36 wkGA, the cortisol to cortisone ratio was negatively associated with term preeclampsia (OR 0.42, 95% CI 0.32-0.55) but not term FGR (OR 1.07, 95% CI 0.87-1.31). Associations were not materially affected by adjustment for maternal characteristics.

Conclusions: A lower maternal serum cortisol to cortisone ratio precedes clinical manifestation of preeclampsia and preterm FGR by many weeks, despite previous reports of reduced levels of placental 11βHSD2 in these conditions. Our observations implicate enhanced maternal 11βHSD2 activity or reduced 11βHSD1 activity in the pathophysiology of preeclampsia.

Precis: A lower maternal cortisol to cortisone ratio precedes clinical diagnosis of preterm and term preeclampsia.

Abbreviations

11βHSD1- 11-beta-hydroxysteroid dehydrogenase-type 1

11βHSD2 – 11-beta-hydroxysteroid dehydrogenase-type 2

FGR – Fetal growth restriction

FTE – Full time education

MoM – Multiples of the Median

PE – Preeclampsia

POP – Pregnancy Outcome Prediction Study

wkGA – Gestational age in weeks

Introduction

Preeclampsia (PE) and fetal growth restriction (FGR) are poorly understood conditions of pregnancy associated with substantial perinatal morbidity and mortality. PE is a disorder of placental origin, characterized by hypertension and proteinuria that develops during the antenatal or immediate postpartum period (1). It affects between 2 and 8 percent of pregnancies and is a major cause of direct maternal death worldwide (2,3). It is often accompanied by FGR, defined as a fetus not reaching its genetically determined growth potential (4), due to poor placental function. Currently, there are no effective management options available, apart from delivery, and accurate early prediction of the disease is not possible.

One marker of placental function which might be affected in PE is the maternal serum cortisol to cortisone ratio (5). 11-beta-hydroxysteroid dehydrogenase-type 2 (11βHSD2) is a steroid enzyme highly expressed in the placenta and, by metabolizing cortisol to cortisone, protects the fetus from exposure to excessive maternal cortisol during pregnancy, while 11-beta-hydroxysteroid dehydrogenase-type 1 (11βHSD1) performs the reverse of 11βHSD2 and reactivates cortisol by converting cortisone to cortisol (6). Several studies have reported that both PE and FGR are associated with reduced levels of placental 11βHSD2 (7-15) and reduced expression and activity of placental 11βHSD1 have been demonstrated in cases of small for gestational age (SGA) (16,17). Reduced 11βHSD2 activity would be expected to result in an increased maternal serum cortisol to cortisone ratio; hence these findings suggest that the maternal serum cortisol to cortisone ratio may be used as a predictor or marker of disease. A small number of studies previously addressed this question and paradoxically, reported that women with PE exhibited lower cortisol to cortisone ratios, suggesting increased maternal 11βHSD2 activity (18-20). However, these analyses were limited to samples obtained following diagnosis and consisted of studies that had not included more than 42 cases of confirmed PE and 14 cases of confirmed FGR per study. The aim of the present study was to investigate the relationship between maternal cortisol and cortisone levels with PE and FGR, before the onset of disease, using measurements taken at different time points in pregnancy in a large number of optimally characterized cases and controls.

Materials and methods

Study design

We utilized a case-cohort design from the Pregnancy Outcome Prediction (POP) study. Details of the study are provided elsewhere (21), but in brief, it was a prospective cohort study that collected blood, ultrasound scan and pregnancy outcome data from 4212 nulliparous women at ~12, 20, 28, and 36 weeks of gestational age (wkGA) between January 14th 2008 and July 31st 2012 (22,23). The case-cohort included 379 women with cases of PE and FGR and a random sample of the total cohort (n=325) (24). Out of the 325 women in the random sub-cohort, 279 did not experience any adverse outcome and are referred to as "healthy".

Definitions of outcomes studied.

For this analysis, we studied women with PE or FGR, and divided them into preterm and term sub-groups based on gestational age at delivery (<37 wkGA and ≥37 wkGA, respectively). PE was diagnosed according to American College of Obstetricians and Gynecologists (ACOG) 2013 guidelines (25) and the cases in this analysis included all severe PE at term and non-severe, non-superimposed PE at term, and all preterm PE. PE cases with preterm delivery were compared with all the women in the sub-cohort who did not experience PE leading to preterm birth. Term PE cases were compared with women from the sub-cohort who delivered at term without experiencing any type of PE. Preterm FGR was defined as SGA <10th customized percentile (26) with delivery <37 wkGA. Preterm FGR cases were compared with women in the sub-cohort who did not deliver a preterm infant with FGR. We excluded preterm cases of PE from analyses of preterm FGR. FGR at term was defined as severe SGA (birth weight <3rd customized percentile) or SGA with growth restriction (birth weight <10th customized percentile and reduced growth velocity of the abdominal circumference on serial ultrasound scans) with delivery ≥37 wkGA. Term FGR cases were compared with women in the sub-cohort who delivered an infant at term without FGR. In addition to preterm births, we excluded all types of term PE from analyses of term FGR. Exclusion of preterm births from the analysis of term outcome was done as women delivering preterm are, necessarily,

not at risk of complications at term, and the exclusion of PE from the analysis of FGR was made to better separate the two complications which can be overlapping, especially at preterm GAs.

Outcomes were collected from paper-based hospital records and relevant electronic databases. Maternal characteristics (age, marital status, ethnicity, smoking, age at leaving full-time education) were self-reported, except for height and body mass index (BMI), which were measured at the time of recruitment, and deprivation, which was measured using the Index of Multiple Deprivation 2007 (27), calculated from the woman's postcode.

Steroid measurements

Steroid levels were measured in maternal serum at Metabolon, Inc. (Durham, NC) by non-targeted ultra-high performance liquid chromatography and tandem mass spectrometry. Samples were run in batches of 36 and all batches contained an equal proportion of cases and controls. All samples from a given participant were run in the same batch. Peaks were quantified using area under the curve of primary MS ions. Missing values were assumed to be the result of falling below the detection sensitivity, and thus were imputed with the minimum detection value based on each metabolite. In the entire POP study, where 3,196 samples were successfully processed, only one cortisol value and two cortisone values were missing (<0.1%). For this analysis, only one woman in the random sub-cohort had a missing cortisol and cortisone value and this was at 12 wkGA. To adjust for instrument batch effects for each run day, the raw ion counts for each steroid were divided by the median value for the run day. Internal standards were not available for all metabolites, so values were not quantified in standard, SI units. Hence, cortisol and cortisone levels were expressed as multiples of the median (MoMs) (28).

Statistical Analyses

The cortisol to cortisone ratio for a given sample was generated by dividing the MoM for cortisol by the MoM for cortisone. Initial descriptive analyses were then conducted by summarizing cortisol, cortisone and the cortisol to cortisone ratio for each time of measurement (12, 20, 28 and 36 wkGA) in healthy

pregnancies and women with PE or FGR. Linear regression analyses for cortisone against cortisol were performed in cases and controls and *P* values for interaction between these groups were calculated. We excluded the 36 wkGA measurement from all the analyses of preterm delivery. The MoM values for the given hormone and their ratio at the given time of sampling were converted to Z scores, using the sub-cohort as the reference. Unadjusted logistic regressions were performed to analyze their associations with the outcomes. These analyses were repeated adjusting for maternal characteristics (age at recruitment, age left FTE, height, BMI, smoking status, deprivation, ethnicity, marital status). Missing values were imputed using the mode for categorical variables and mean for continuous variables.

Time to event analyses (29) were then conducted for term PE from the 36 week measurement onwards and preterm PE from the 28 wkGA measurement onwards. We studied the whole case-cohort sample, weighting the non-cases of the comparator group by the inverse of the sampling fraction (30). We compared the cumulative incidence of PE between women with a cortisol to cortisone ratio in the 1st decile (using a threshold derived from the random sub-cohort) to women in the 2nd to 10th deciles. Delivery without PE was considered a competing risk.

Finally, we performed a sensitivity analysis confined to women where it was documented that they had not received steroids antenatally (either for fetal lung maturation or medical conditions such as asthma). As the research database only started recording information on whether women had received steroids to accelerate fetal lung maturation from 27/11/2009 onwards, the majority of women excluded in the sensitivity analysis were omitted because the data were absent rather than they were documented as receiving steroids. Where the apparent statistical significance of an association between an outcome and the cortisol to cortisone ratio differed in the sensitivity analysis, we tested for an interaction between the ratio and antenatal steroid use to determine if there was a true difference between the groups, or whether the P value became non-significant due to the reduced sample size in the sub-group.

Statistical software

All analyses were performed in Stata version 14.0 (StataCorp, College Station, TX, USA).

Ethics approval

All patients gave their informed, written consent to participate and the study was approved by the Cambridgeshire 2 Research Ethics Committee (reference number 07/H0308/163)

Results

Maternal characteristics

There were 878, 873, 854 and 591 women with steroid measurements at approximately mean (±SD) gestational ages 89 (±6), 143 (±3), 198 (±3) and 254 (±3) days, respectively. These are reported as measurements at 12, 20, 28 and 36 wkGA. Maternal characteristics of the women studied are summarized in Table 1. There were 29 cases of preterm PE, 160 cases of PE at term, 25 cases of preterm FGR and 165 cases of FGR at term in the case-cohort for the main analysis. The sensitivity analysis included 12 cases of preterm PE, 123 cases of PE at term, 15 cases of preterm FGR and 125 cases of FGR at term, and 251 women remained in the random sub-cohort.

Patterns of cortisol, cortisone and cortisol to cortisone ratio throughout pregnancy

In healthy women, cortisol and cortisone both increased as pregnancy progressed (Figs. 1a and 1b, respectively), whereas the cortisol to cortisone ratio showed an upward trend between 12 and 20 wkGA, plateaued until 28 wkGA and then fell at 36 wkGA (Fig. 1c). This pattern was similar in cases of FGR, but in PE, the cortisol to cortisone ratio started to decline at 28wkGA onwards (Fig. 1c).

Associations between cortisol, cortisone and cortisol to cortisone ratio with PE and FGR

There was a positive association between cortisol and cortisone in cases and controls, but for a given level of cortisol, the cortisone level was greater in cases of preterm PE at 28 wkGA and term PE at 36 wkGA (Figs. 2a and 2b, respectively). The cortisol to cortisone ratio was negatively associated with PE (Table 2 and Fig. 3a) at 28wkGA and at 36wkGA, and the association was not materially affected by adjustment for maternal characteristics (Table 2). At 28 wkGA, the cortisol to cortisone ratio showed a negative association with the risk of preterm PE, term PE and preterm FGR. At 36 wkGA, the cortisol to cortisone ratio was also negatively associated with term PE but not with term FGR (Fig. 3a). The associations were broadly similar when the analysis was restricted to women who were not treated with steroids antenatally (Table 3). The association between the cortisol to cortisone ratio at 28 wkGA and preterm FGR was not

202 statistically significant at an alpha of 0.05, but there was no evidence of an interaction between the groups
203 with and without antenatal steroid use (*P* value = 0.15 for the unadjusted analysis and *P* value = 0.13 for
204 the adjusted analysis). Hence, the apparent loss of statistical significance likely reflected reduced
205 statistical power due to a smaller sample size.

206 Cortisol was negatively associated with the risk of term PE from 28 wkGA onwards (Fig. 3b)
207 while the association between cortisone and the risk of term PE was negative in the first trimester and
208 changed to positive in the third trimester (Fig. 3c). A similar association was also seen with cortisone and
209 preterm PE (Fig. 3c), but not cortisol (Fig. 3b). The associations seen were stronger as pregnancy
210 progressed for both the cortisol to cortisone ratio and cortisone in term and preterm PE (Figs. 3a and 3c).
211 Cortisol was negatively associated with preterm FGR at 28 wkGA (Fig. 3b), but there were no clear
212 associations between cortisone and preterm FGR (Fig. 3c), and, there was no clear association between
213 cortisol or cortisone and term FGR (Figs. 3b and 3c).

214

215 <u>Cumulative incidence of PE according to cortisol to cortisone ratio</u>

216 There was a higher cumulative incidence of preterm PE and term PE for women in the lowest decile of
217 cortisol to cortisone ratio compared with deciles 2-10 at the 28 and 36 wkGA measurements, respectively;
218 approximately 2.2% and 11% cases in lowest decile vs 0.4% and 3% in deciles 2-10. For preterm PE, the
219 curves started to deviate 2 weeks after the 28 wkGA measurement (Fig. 4a), and for term PE, the curves
220 began to deviate at least a week after the 36 wkGA measurement (Fig. 4b). Similar results were obtained
221 when women treated with steroids antenatally were excluded.

Discussion

In this study, we found a clear negative association between the maternal serum cortisol to cortisone ratio and the risk of preterm and term PE. In women with term PE, the cortisol to cortisone ratio was significantly lower more than eight weeks before delivery. In term PE cortisol levels were lower at 28 wkGA and cortisone levels were higher at 36 wkGA, and in preterm PE cortisone levels were higher at 28 wkGA. These changes could represent higher 11βHSD2 activity or lower 11βHSD1 activity in women with PE, which occurs prior to the manifestation of clinical signs and symptoms.

Multiple studies have shown lower placental 11βHSD2 activity after vaginal and cesarean delivery in women with PE and FGR (7-15), but our work and that of other studies investigating maternal blood and urine cortisol to cortisone ratio in women with established disease (18-20), suggests that maternal systemic 11βHSD2 activity is increased. The observation of reduced 11βHSD2 activity in placentas from women with PE may reflect general placental failure and local regulation of cortisol metabolism by the feto-placental unit which is independent of the mother. Aufdenblatten *et al.* (35) found that placental cortisol was almost completely inactivated in normotensive pregnancies, indicating an effective 11βHSD2 barrier from high levels of cortisol. This was in contrast to pregnancies with PE and low birth weight which had reduced placental 11βHSD2 activity and increased placental cortisol levels (31). Therefore, as 11βHSD2 is also expressed outside the placenta, the association between PE and FGR with a lower cortisol to cortisone ratio could be related to extra-placental 11βHSD2 activity, such as maternal renal 11βHSD2 (32,33).

PE is frequently accompanied by renal dysfunction, but impaired renal function and hypertension in diseases outside of pregnancy have been associated with decreased renal 11βHSD2 expression and activity (34,35). Thus, maternal 11βHSD2 activity may increase as a compensatory response in PE, acting to reduce systemic cortisol. The systemic stress and inflammatory response which occurs in PE (36,37) may result in higher maternal cortisol secretion, which would suggest that 11βHSD2 activity increases to the extent that conversion of cortisol to cortisone exceeds cortisol secretion. However, cortisol secretion is

not definitively known to rise in PE, and there is limited evidence that cortisol itself stimulates 11βHSD2 activity; exogenous corticosteroids have been shown to stimulate 11βHSD2 activity in bronchial epithelial cells (38), but the effects of cortisol on renal 11βHSD2 activity are not known, and since we observed a linear relationship between cortisol and cortisone levels in women with and without PE, it is likely that there are other factors responsible for stimulating 11βHSD2 action.

Another explanation for the changes observed in cortisol and cortisone levels might be reduced 11βHSD1 activity, as opposed to increased 11βHSD2 activity. There are studies showing reduced placental and chorionic 11βHSD1 activity in infants born SGA (birth weight less than the 10^{th} percentile) (10,16), which could be a compensatory mechanism to reduce fetal cortisol exposure due to increased placental crossover of cortisol. But there are no studies in the literature which have studied activity of 11βHSD1 in placentas from women with PE. Furthermore, 11βHSD1 is present in the chorio-decidua (39,40) but it is unclear whether reduced 11βHSD1 activity at the feto-maternal interface would affect maternal cortisol and cortisone levels. Reduced maternal systemic 11βHSD1 activity is another possible explanation for the findings, but this is also yet to be investigated and would be an important area of future research.

Our study had several methodological strengths. First, to our knowledge, this is the first study to look at measurements of cortisol and cortisone in maternal serum preceding the onset of disease in both PE and FGR. Second, the case-cohort study design meant that we were able to study a large number of cases of PE and FGR, making this the largest study to date. Moreover, due to the large size of the cohort and the availability of serial ultrasound scans, we were able to confine our analysis of FGR to either infants which were extremely small for gestational age ($<3^{rd}$ percentile) or infants $<10^{th}$ percentile with other features indicating a likely pathological cause (preterm birth or reduced fetal growth velocity). Many other studies simply study small for gestational age infants ($<10^{th}$ percentile) and a large proportion of these will be healthy. The case-cohort design also meant that we were able to compare cases of PE and FGR with a population representative of the whole cohort. This is more likely to demonstrate a true

difference in participants with and without the outcomes studied than a case-control study, as in case-control studies the comparison group are often healthy, and differences seen may just reflect the lack of other outcomes in the control group rather than the outcome of interest in cases. Details of the methodology underlying case-cohort studies and potential statistical and reporting issues are described by Sharp *et al.* (41).

However, our study also has some limitations. Cortisol and cortisone were quantified as multiples of the median rather than SI units, so values cannot be directly compared with traditional measures of cortisol and cortisone in nmol/L. This is because the metabolomics assay was untargeted, and internal standards were not available for all metabolites. But, the quantification method is accurate and the ratio is unitless, so the results correctly reflect cortisol and cortisone levels measured using standard methods. Additionally, human cortisol secretion follows a circadian rhythm which was not accounted for in this study (42). But, Kosicka *et al*. (19) controlled for diurnal variation by taking early morning blood samples and drew the same conclusion that the maternal serum cortisol to cortisone ratio was reduced in PE. Cortisol secretion is influenced by external factors, including stress and anxiety (43). Although, we adjusted for some characteristics such as deprivation, marital and smoking status it was difficult to quantify any other external stresses that the study participants may have been experiencing. However, confounding by maternal stress is unlikely to have caused the associations as it would tend to be associated with increased rather than decreased cortisol levels. Furthermore, we also considered whether our findings could have been influenced by exogenous antenatal steroid use, and there were no clear differences in our results when these women were excluded. Whilst the association between the cortisol to cortisone ratio and preterm FGR at 28 wkGA where cases of antenatal steroid use had been excluded was not significant at an alpha of 0.05, the point estimates (OR) were within the 95% CIs of the analyses where they had not been excluded, and there was no evidence of an interaction between the ratio and exposure to steroids, thus the higher *P* values reflected reduced statistical power from a smaller sample size.

In conclusion, this study demonstrates that a lower maternal serum cortisol to cortisone ratio precedes the clinical manifestation of PE and preterm FGR by many weeks despite previous reports of elevated levels of placental 11βHSD2 in these conditions. Our observations implicate enhanced maternal 11βHSD2 activity in the pathophysiology of PE. However, further investigation into the potential utility of the cortisol to cortisone ratio as a predictive marker of PE would be beneficial, given there is no current accurate predictive test for the condition. In addition, research into maternal and placental 11βHSD1 and 11βHSD2 activity in women with PE and FGR and links to fetal and newborn cortisol metabolism would be useful in gaining a deeper understanding of the pathophysiology of the diseases.

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Author contributions

GCSS had the original idea. NAJ performed the analyses, and wrote the manuscript. AEH performed the analyses, supervised the analysis and contributed to the manuscript. US assisted with statistical analyses, supervised the analysis and contributed to the manuscript. EC assisted with data collection and contributed to the manuscript. GCSS and DSCJ conceived the study, supervised the analysis and contributed to the manuscript.

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Figure legends

Figure 1. Mean with 95% confidence intervals for cortisol (a), cortisone (b) and the cortisol to cortisone ratio (c) at 12, 20, 28 and 36 wkGA in healthy pregnancies, women that developed PE and women who delivered a FGR infant. Cortisol and cortisone are reported as multiples of the median. Healthy pregnancies included women who did not have FGR, PE, gestational diabetes or spontaneous preterm delivery. Cases of PE were defined by the ACOG 2013 guidelines (24) and included all severe and non-severe, non-superimposed PE at term and all preterm PE. Cases of term FGR included severe SGA (birth weight <3rd customized percentile at term) and SGA with growth restriction (birth weight <10th customized percentile at term with reduced growth velocity of the abdominal circumference on serial ultrasound scans) without PE. Cases of preterm FGR included SGA with preterm delivery (birth weight <10th customized percentile and delivery prior to 37 completed wkGA) without PE.

FGR, fetal growth restriction; MoM, multiples of the median; PE, pre-eclampsia; SGA, small for gestational age; wkGA, gestational age in weeks

Figure 2. Scatter plots for cortisol vs cortisone at 28 wkGA in cases of preterm PE and controls (a) and 36 wkGA in cases of term PE and controls (b). Cortisol and cortisone are reported as multiples of the median. Cases of PE were defined by the ACOG 2013 guidelines (24) and included all severe and non-severe, non-superimposed PE at term and all preterm PE. Controls included women who did not have PE. Preterm delivery included pregnancies delivered < 37 wkGA and term delivery included pregnancies delivered ≥37 wkGA. For the measurement at 28 wkGA, the equation for the regression line in cases of preterm PE is y = 0.829 + 0.442 * x (*P* value for association = 0.01) and in controls is y = 0.526 + 0.469 * x (*P* value for association <0.001). The *P* value for interaction between preterm PE cases and controls at 28 wkGA is 0.80. For the measurement at 36 wkGA, the equation for the regression line in cases of term PE is y = 0.909 + 0.612 * x (*P* value for association <0.001) and in controls is y = 0.887 + 0.479 * x (*P* value for association <0.001). The *P* value for interaction between term PE cases and controls at 36 wkGA is 0.10.

MoM, multiples of the median; PE, pre-eclampsia; wkGA, gestational age in weeks

Figure 3. Unadjusted odds ratios with 95% confidence intervals at 12, 20, 28 and 36 wkGA for term PE, preterm PE, term FGR and preterm FGR for (a) cortisol to cortisone ratio, (b) cortisol and (c) cortisone.

Odds ratios are for cases referent to controls, associated with a one standard deviation higher cortisol to cortisone ratio, cortisol or cortisone value. Error bars represent the 95% confidence intervals. Cases of PE were defined by the ACOG 2013 guidelines (24) and included all severe and non-severe, non-superimposed PE at term and all preterm PE. Cases of term FGR included severe SGA (birth weight <3rd customized percentile at term), SGA with growth restriction (birth weight <10th customized percentile at term with reduced growth velocity of the abdominal circumference on serial ultrasound scans), and all cases of PE were excluded from analyses of term FGR. Cases of preterm FGR included SGA (birth weight <10th customized percentile) with preterm delivery, and cases of preterm PE were excluded from analyses of preterm FGR. Preterm delivery included pregnancies delivered <37 wkGA and term delivery included pregnancies delivered ≥37 wkGA.

FGR, fetal growth restriction; PE, pre-eclampsia; PTD, pre-term delivery; wkGA, gestational age in weeks

Figure 4. Cumulative incidence of PE comparing the 1st decile of the cortisol to cortisone ratio with the 2nd-10th deciles of cortisol to cortisone ratio from 28 wkGA onwards in cases of preterm PE (a), and 36 wkGA onwards in cases of term PE (b). Figure 4a is reported in births per 1000 women and 4b is reported in births per 100 women. Cases of PE were defined by the ACOG 2013 guidelines (24) and included all severe and non-severe, non-superimposed PE at term and all preterm PE. Preterm delivery included pregnancies delivered < 37 wkGA and term delivery included pregnancies delivered ≥37 wkGA. Delivery without PE was considered as the competing risk.

488 FGR, fetal growth restriction; PE, pre-eclampsia; wkGA, gestational age in weeks; PTD, pre-term

489 delivery; SHR, Subhazard Ratio; CI, confidence interval

Tables

Table 1. Maternal characteristics and birth outcomes in women with healthy pregnancies, PE and FGR[a].

	Healthy[b]	Preeclampsia[c]		Fetal growth restriction[d]	
		Preterm	Term	Preterm	Term
N (% of case- cohort)	279 (30.2%)	29 (3.1%)	165 (17.9%)	25 (2.7%)	160 (17.3%)
Maternal characteristics					
Age in years	30 (5)	28 (6)	30 (6)	30 (5)	30 (6)
Age left FTE in years	21 (4)	20 (4)	20 (4)	20 (3)	21 (4)
Missing (%)	1 (0.3%)	1 (3.4%)	5 (3.0%)	0 (0.0%)	3 (1.9%)
Height in cm	165 (6)	162 (8)	164 (6)	165 (8)	165 (7)
BMI	24.9 (4.7)	29.7 (5.9)	27.8 (6.3)	25.0 (4.9)	25.6 (5.0)
Booking MAP	78 (9)	87 (10)	83 (10)	81 (9)	79 (8)
Missing (%)	9 (3.2%)	0 (0.0%)	4 (2.4%)	1 (4.0%)	6 (3.8%)
Married (%)	203 (72.8%)	22 (75.9%)	105 (63.6%)	16 (64.0%)	98 (61.3%)
Smoker (%)	13 (4.7%)	1 (3.5%)	6 (3.6%)	3 (12.0%)	29 (18.1%)
Alcohol (%)	10 (3.6%)	0 (0.0%)	7 (4.2%)	1 (4.0%)	7 (4.4%)
Deprivation quartile (%)					
1 (lowest)	60 (21.5%)	8 (27.6%)	41 (24.8%)	3 (12.0%)	42 (26.3%)
2	81 (29.0%)	2 (6.9%)	38 (23.0%)	6 (24.0%)	27 (16.9%)
3	57 (20.4%)	14 (48.3%)	40 (24.2%)	6 (24.0%)	41 (25.6%)
4 (highest)	71 (25.4%)	5 (17.2%)	38 (23.0%)	9 (36.0%)	43 (26.9%)
Missing (%)	10 (3.6%)	0 (0.0%)	8 (4.8%)	1 (4.0%)	7 (4.4%)
White ethnicity (%)	263 (94.3%)	26 (89.7%)	157 (95.2%)	21 (84.0%)	151 (94.4%)
Non-white ethnicity (%)	11 (3.9%)	2 (6.9%)	7 (4.2%)	4 (16.0%)	7 (4.4%)
Missing (%)	5 (1.8%)	1 (3.4%)	1 (0.6%)	0 (0.0%)	2 (1.3%)
Characteristics of Delivery					
Mode of delivery (%)					
Vaginal	140 (50.2%)	7 (24.1%)	36 (21.8%)	6 (24.0%)	94 (58.8%)
Assisted vaginal	58 (20.8%)	0 (0.0%)	51 (30.9%)	5 (20.0%)	30 (18.8%)
Intrapartum caesarean	54 (19.4%)	3 (10.3%)	55 (33.3%)	1 (4.0%)	16 (10.0%)
Pre-labour caesarean	25 (9.0%)	19 (65.6%)	22 (13.3%)	13 (52.0%)	20 (12.5%)
Missing (%)	2 (0.7%)	0 (0.0%)	1 (0.6%)	0 (0.0%)	0 (0.0%)
Labour induced (%)	88 (31.5%)	7 (24.1%)	106 (64.2%)	4 (16.0%)	54 (33.8%)

BMI, body mass index; FGR, fetal growth restriction; FTE, full-time education; MAP, mean arterial blood pressure; PE, preeclampsia; SD, standard deviation; wkGA, gestational age in weeks

[a]Data is expressed as mean (±SD) or n (%) with number of missing values below each characteristic. Where there is no missing category, data was complete.

[b]Women without FGR, PE, gestational diabetes or spontaneous preterm delivery.

[c]Diagnosed according to ACOG 2013 guidelines (24) and divided into preterm (delivery <37 wkGA) and term outcomes (delivery ≥37 wkGA). Cases of PE include all severe and non-superimposed, non-severe PE at term and all preterm PE.

[d]Divided into preterm (delivery <37 wkGA) and term outcomes (delivery ≥37 wkGA). Cases of FGR at term include severe SGA (birth weight <3rd customized percentile) and SGA with growth restriction (birth weight <10th customized percentile and reduced growth velocity of the abdominal circumference on serial ultrasound scans) without PE. Cases of preterm FGR include SGA (birth weight <10th customized percentile) with preterm delivery and without PE. Maternal characteristics and the summary of cortisol and cortisone levels were summarized for cases and healthy women due to the small overlap in cases in the random sub-cohort.

Table 2. Unadjusted and adjusted ORs (95% CI) for PE and FGR by one standard deviation higher cortisol to cortisone ratio measured at different time points in pregnancy. *P value <0.05 **P value <0.001

Approximate gestation of measurement (wkGA)	Preeclampsia[a]				Fetal growth restriction[b]			
	Preterm		Term		Preterm		Term	
	Unadjusted OR (95% CI)	Adjusted OR[c] (95% CI)	Unadjusted OR (95% CI)	Adjusted OR[c] (95% CI)	Unadjusted OR (95% CI)	Adjusted OR[c] (95% CI)	Unadjusted OR (95% CI)	Adjusted OR[c] (95% CI)
12	0.80 (0.54-1.20) *n/N=29/331*	0.68 (0.44-1.04) *n/N=29/331*	1.17 (0.95-1.43) *n/N=150/425*	1.14 (0.92-1.41) *n/N=150/425*	0.90 (0.59-1.36) *n/N=25/327*	0.93 (0.60-1.45) *n/N=25/327*	1.13 (0.93-1.37) *n/N=156/418*	1.15 (0.94-1.41) *n/N=156/418*
20	0.68 (0.43-1.08) *n/N=25/331*	0.74 (0.46-1.21) *n/N=25/331*	1.02 (0.83-1.24) *n/N=152/433*	1.09 (0.88-1.34) *n/N=152/433*	0.74 (0.47-1.16) *n/N=25/331*	0.78 (0.49-1.24) *n/N=25/331*	1.01 (0.83-1.24) *n/N=156/425*	1.09 (0.88-1.35) *n/N=156/425*
28	0.33 (0.19-0.57)** *n/N=24/328*	0.34 (0.19-0.61)** *n/N=24/328*	0.61 (0.49-0.76)** *n/N=151/430*	0.61 (0.49-0.77)** *n/N=151/430*	0.50 (0.29-0.85)* *n/N=21/325*	0.50 (0.29-0.85)* *n/N=21/325*	0.96 (0.79-1.17) *n/N=154/420*	0.96 (0.78-1.19) *n/N=154/420*
36[d]			0.42 (0.32-0.55)** *n/N=134/409*	0.42 (0.32-0.56)** *n/N=134/409*			1.07 (0.87-1.31) *n/N=148/410*	1.14 (0.92-1.41) *n/N=148/410*

CI; confidence interval; FGR, fetal growth restriction; FTE, full-time education; OR, odds ratio; PE, preeclampsia; SGA, small for gestational age; wkGA, gestational age in weeks

[a]Diagnosed according to ACOG guidelines (24) and divided into preterm (delivery <37 wkGA) and term outcomes (delivery ≥37 wkGA). Cases of PE include all severe and non-superimposed, non-severe PE at term and all preterm PE.

[b]Divided into preterm (delivery <37 wkGA) and term outcomes (delivery ≥37 wkGA). Cases of FGR at term include severe SGA (birth weight <3rd customized percentile) and SGA with growth restriction (birth weight <10th customized percentile and reduced growth velocity of the abdominal circumference on serial ultrasound scans) without PE. Cases of preterm FGR include SGA (birth weight <10th percentile) with preterm delivery and without PE.

[c]Odds ratios adjusted for antenatal height, age, BMI, marital status, ethnicity, smoking, age at leaving FTE, and deprivation. In the analysis of preterm preeclampsia, the adjustments for ethnicity and smoking were omitted since these variables predicted the outcome perfectly.

[d]The 36 wkGA measurements have not been analyzed for preterm outcomes.

Table 3. Unadjusted and adjusted ORs (95% CI) for PE and FGR by one standard deviation higher cortisol to cortisone ratio measured at different time points in pregnancy confined to women who were documented as not having received steroids antenatally. *P value <0.05 **P value <0.001

Approximate gestation of measurement (wkGA)	Preeclampsia				Fetal growth restriction			
	Preterm		Term		Preterm		Term	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
12	0.70 (0.37-1.33) *n/N=12/248*	0.57 (0.28-1.16) *n/N=12/248*	1.15 (0.91-1.44) *n/N=113/331*	1.12 (0.88-1.43) *n/N=113/331*	1.00 (0.59-1.68) *n/N=15/251*	0.97 (0.56-1.69) *n/N=15/251*	1.15 (0.93-1.42) *n/N=120/328*	1.13 (0.90-1.42) *n/N=120/328*
20	0.59 (0.26-1.32) *n/N=9/253*	0.59 (0.25-1.41) *n/N=9/253*	1.02 (0.81-1.29) n/N=113/339	1.06 (0.84-1.35) *n/N=113/339*	0.91 (0.52-1.61) *n/N=15/259*	0.95 (0.52-1.73) *n/N=15/259*	1.00 (0.79-1.26) *n/N=119/335*	1.06 (0.83-1.36) *n/N=119/335*
28	0.22 (0.08-0.66)* *n/N=9/246*	0.10 (0.02-0.51)* *n/N=9/246*	0.54 (0.41-0.71)** *n/N=112/330*	0.54 (0.41-0.71)** *n/N=112/330*	0.71 (0.37-1.35) *n/N=12/249*	0.71 (0.35-1.41) *n/N=12/249*	1.00 (0.80-1.26) *n/N=117/325*	0.98 (0.77-1.25) *n/N=117/325*
36			0.42 (0.31-0.57)** *n/N=99/315*	0.41 (0.30-0.57)** *n/N=99/315*			1.09 (0.86-1.39) *n/N=114/320*	1.14 (0.89-1.47) *n/N=114/320*

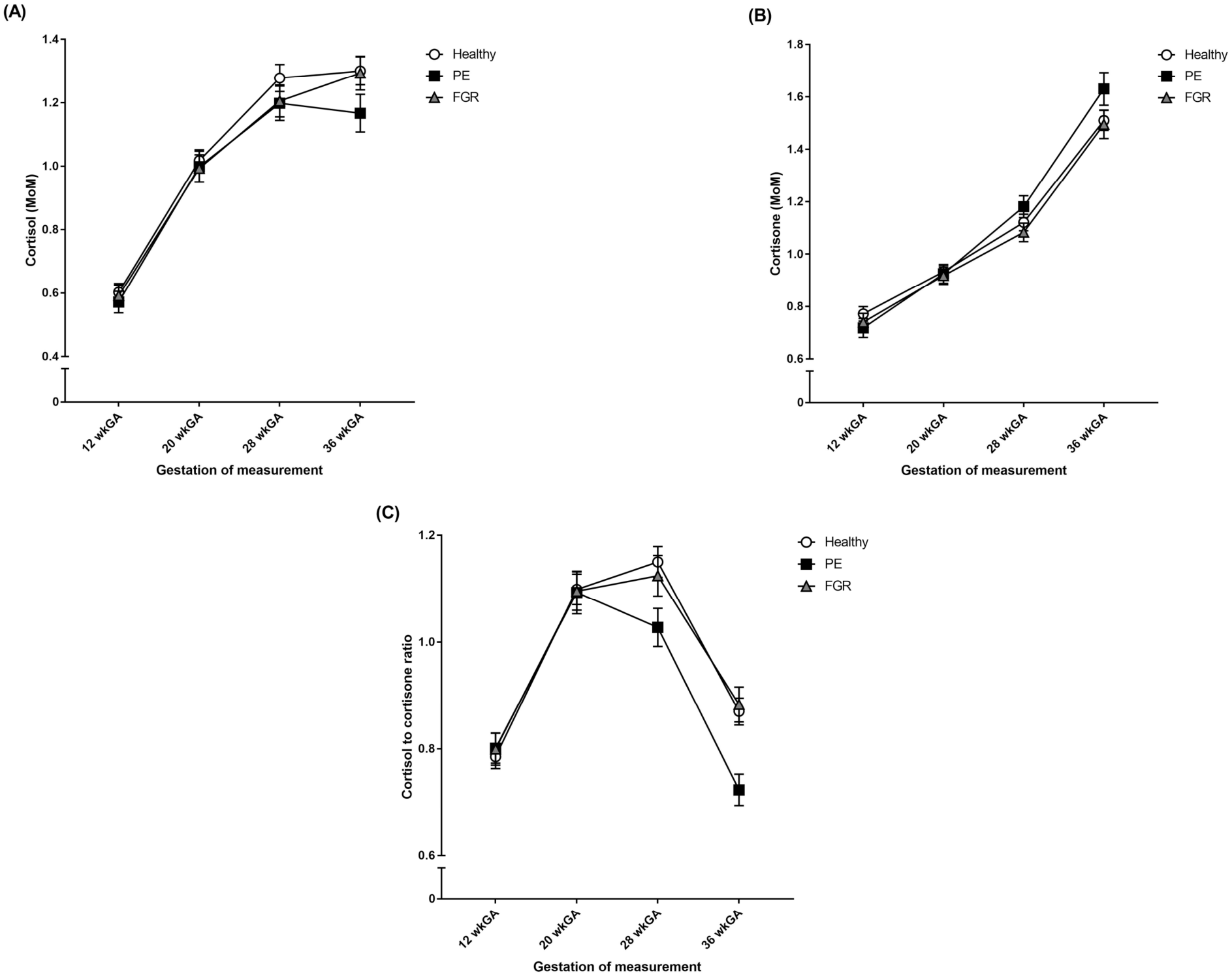
CI; confidence interval; FGR, fetal growth restriction; FTE, full-time education; OR, odds ratio; PE, preeclampsia; SGA, small for gestational age; wkGA, gestational age in weeks

[a]Diagnosed according to ACOG guidelines (24) and divided into preterm (delivery <37 wkGA) and term outcomes (delivery ≥37 wkGA). Cases of PE included all severe and non-superimposed, non-severe PE.

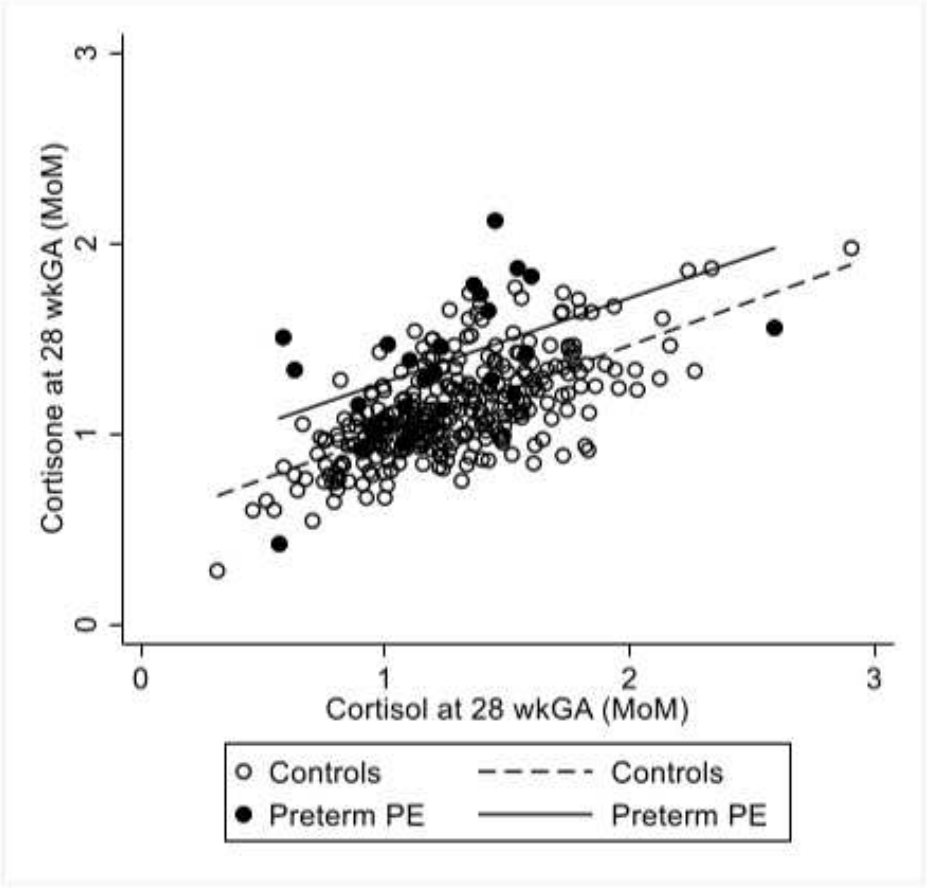
[b]Divided into preterm (delivery <37 wkGA) and term outcomes (delivery ≥37 wkGA). Cases of FGR at term include severe SGA (birth weight <3rd customized percentile) and SGA with growth restriction (birth weight <10th customized percentile and reduced growth velocity of the abdominal circumference on serial ultrasound scans) without PE. Cases of preterm FGR include SGA (birth weight <10th percentile) with preterm delivery and without PE.

[c]Odds ratios adjusted for antenatal height, age, BMI, marital status, ethnicity, smoking, age at leaving FTE, and deprivation. In the analysis of preterm preeclampsia, the adjustments for ethnicity and smoking were omitted since these variables predicted the outcome perfectly.

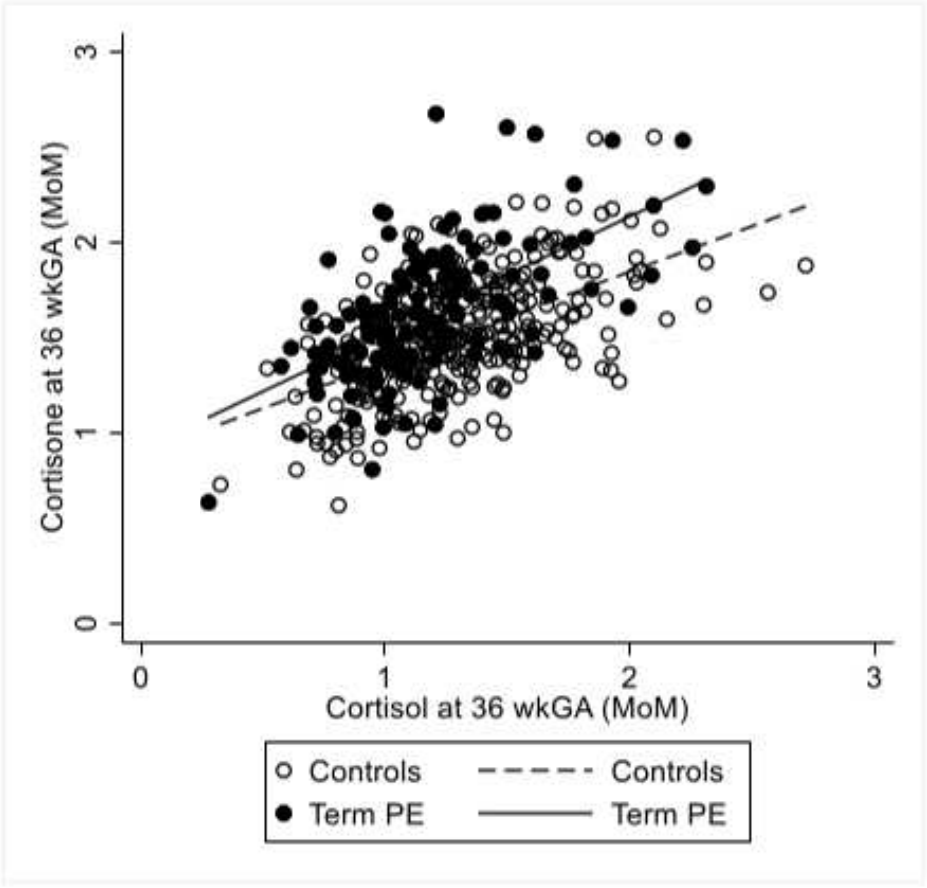
[d]The 36 wkGA measurements have not been analyzed for preterm outcomes.

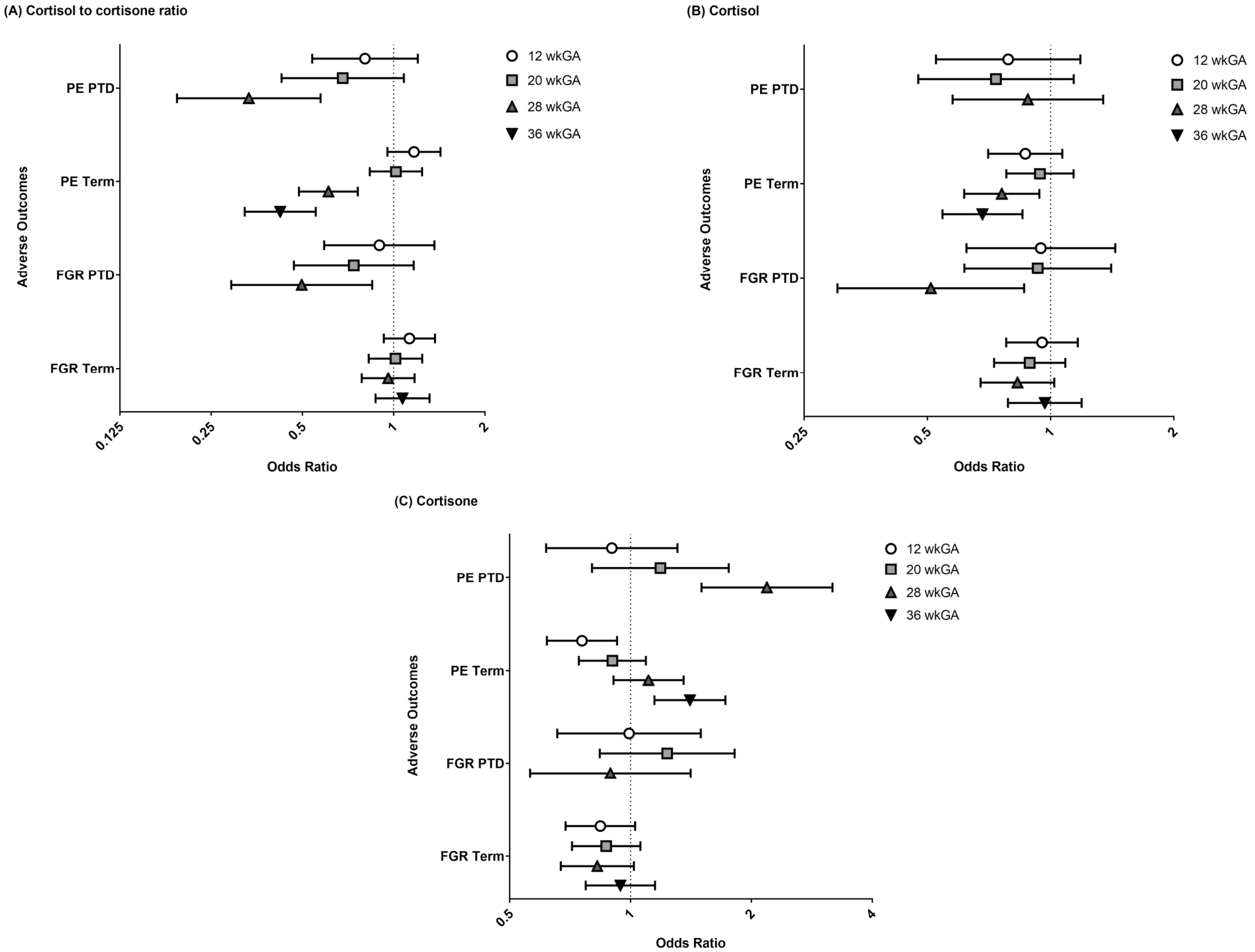

(A)
Cortisol (MoM)
1.4
1.2
1.0
0.8
0.6
0.4
0
12 wkGA
20 wkGA
28 wkGA
36 wkGA
Gestation of measurement
Healthy
PE
FGR
(B)
Cortisone (MoM)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0
12 wkGA
20 wkGA
28 wkGA
36 wkGA
Gestation of measurement
Healthy
PE
FGR
(C)
Cortisol to cortisone ratio
1.2
1.0
0.8
0.6
0
12 wkGA
20 wkGA
28 wkGA
36 wkGA
Gestation of measurement
Healthy
PE
FGR

(A)

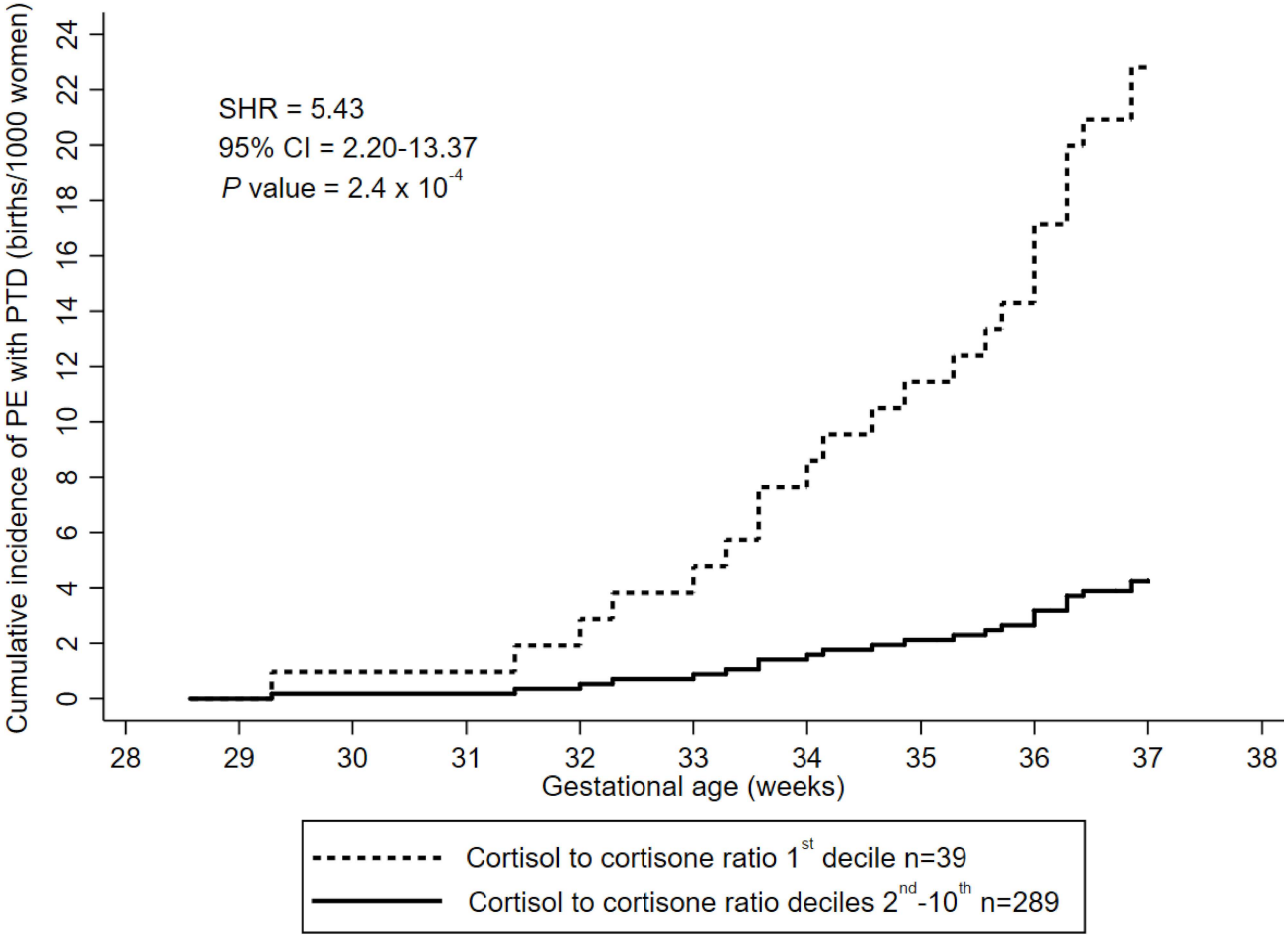

Cortisone at 28 wkGA (MoM)
0
1
2
3
Cortisol at 28 wkGA (MoM)
Controls
Preterm PE
Controls
Preterm PE

(B)

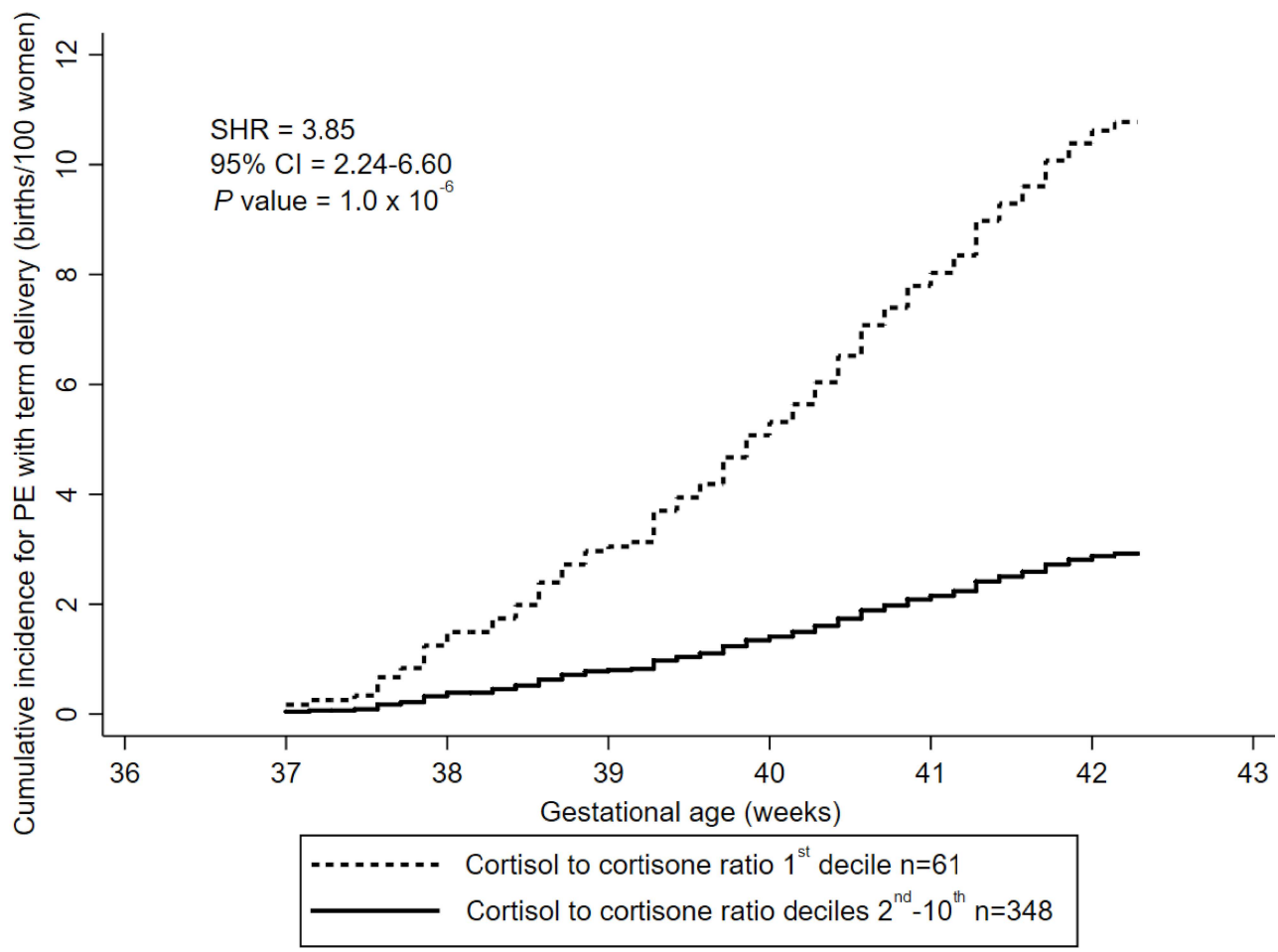

Cortisone at 36 wkGA (MoM)
0
1
2
3
Cortisol at 36 wkGA (MoM)
Controls
Term PE
Controls
Term PE


(A) Cortisol to cortisone ratio
(B) Cortisol
(C) Cortisone
12 wkGA
20 wkGA
28 wkGA
36 wkGA
PE PTD
PE Term
FGR PTD
FGR Term
Adverse Outcomes
Odds Ratio
0.125
0.25
0.5
1
2
4

(A)


SHR = 5.43
95% CI = 2.20-13.37
P value = 2.4 x 10-4
Cumulative incidence of PE with PTD (births/1000 women)
0
2
4
6
8
10
12
14
16
18
20
22
24
28
29
30
31
32
33
34
35
36
37
38
Gestational age (weeks)
Cortisol to cortisone ratio 1st decile n=39
Cortisol to cortisone ratio deciles 2nd-10th n=289

(B)


SHR = 3.85
95% CI = 2.24-6.60
P value = 1.0 x 10-6
Cumulative incidence for PE with term delivery (births/100 women)
0
2
4
6
8
10
12
36
37
38
39
40
41
42
43
Gestational age (weeks)
Cortisol to cortisone ratio 1st decile n=61
Cortisol to cortisone ratio deciles 2nd-10th n=348