# Influence of Race on the Effect of Premature Birth on Salivary Cortisol Response to Stress in Adolescents

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## Abstract

**Objectives:** To compare pre- and post-stress salivary cortisol levels in adolescents born preterm to those born term, and to assess the influence of race and sex on this relationship.

**Methods:** We measured salivary cortisol before and 20 minutes after a maximal-exercise stress test and calculated the cortisol stress response. We used linear regression to compare cortisol stress responses between preterm-term groups, adjusting for birth weight z-score and maternal hypertension, and examined effect modification by race and sex.

**Results:** We evaluated 171 adolescents born preterm with very low birth weight and 50 born term. Adolescents born preterm had reduced cortisol stress response compared to term (0.03 vs. 0.08 μg/dL, p=0.04). This difference was race-dependent; non-Black adolescents born preterm had significantly reduced cortisol stress response compared to those born at term (adjusted $\beta$: −0.74; 95% CI −1.34,−0.15) while there was no difference in Black adolescents (0.53; −0.16,1.22). Sex did not modify the relationship.

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**Conclusions:** Adolescents born preterm exhibit a reduced salivary cortisol response to exercise stress, suggesting long term alterations in the hypothalamic-pituitary-adrenal axis. This relationship was evident in non-Black but not Black adolescents, suggesting that race may modify the influence of preterm birth on stress alterations of the hypothalamic-pituitary-adrenal axis.

## INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis regulates many physiologic processes in response to stress. Persistent stress results in long-term hyperactivation of the HPA axis, causing sustained release of elevated basal concentrations of cortisol, one of the primary glucocorticoid hormones that regulates several metabolic functions.(1) Persistent stress can also lead to altered HPA axis reactivity, response to stress, illness, or exercise. Elevations in basal cortisol are known to increase lipogenesis,(2) abdominal fat deposition,(3) and blood pressure,(4) and can result in a dietary shift towards more palatable, energy-dense foods,(5) all of which can contribute to increasing the long-term risk of obesity, diabetes, hypertension, and cardiovascular disease.

*In utero* conditions and early postnatal events may contribute to maladaptive programming of the HPA axis, such that overall HPA axis function is altered.(6) While the placental enzyme 11-β hydroxysteroid dehydrogenase type 2 protects the fetus from high levels of maternal cortisol, the enzyme's activity can be weakened by maternal anxiety, infection, or inflammation, leading to sustained increased fetal cortisol exposure.(7-9) This fetal overexposure to cortisol is associated with numerous adverse outcomes, including low birth weight, cardiometabolic disease, and mental illness.(10, 11) Preterm birth and related factors such as undernutrition, infection, exogenous glucocorticoid administration, and painful medical procedures have all been associated with a blunted response to acute stress as compared to term-born age-matched peers.(12-14) Although HPA axis function is thought to be largely controlled by stress levels, it may also be influenced by growth,(15) sex,(16, 17) race,(15, 18) socioeconomic status,(15, 18) and exogenous glucocorticoid exposure.(16, 17, 19) For example, females have increased HPA axis activity as compared to males,(16, 17) and in adults, Black race is associated with altered patterns of cortisol release throughout the day compared to non-Black individuals.(18)

However, significant gaps remain in our understanding of how these factors influence the HPA axis in individuals born preterm. Many of the previously studied preterm cohorts were born in the 1970's and 1980's prior to current advances in perinatal care, and many lack racial diversity, which limits their generalizability. Therefore, our study aimed to assess the association between preterm birth and salivary cortisol response to stress in a racially diverse cohort of adolescents born preterm in the 1990's as compared to those born at term. Secondarily, we aimed to assess whether race or sex modify this association. We hypothesized that adolescents born preterm have a higher pre-stress salivary cortisol and a decreased cortisol response to exercise-induced stress compared to their term-born peers. We also hypothesized that race and sex would modify this relationship such that females born preterm would have a higher pre-stress salivary cortisol and a higher stress response than males and that Black participants born preterm would have a lower cortisol response.

## METHODS

### Study Participants

This study included a subset of participants from a larger cohort of adolescents who were recruited at 14 years of age to participate in a longitudinal study of the effects of preterm birth on the development of cardiometabolic outcomes.(20) Participants were eligible if they were a singleton birth born between 1992 and 1996 at a regional perinatal center (Forsyth Medical Center in Winston-Salem, NC) with very low birth weight (VLBW; <1500 g), without a major congenital anomaly, and returned for evaluation at one year adjusted age. Term-born adolescents who had a birth weight 2500 g with no congenital anomalies or antenatal steroid exposure and were delivered at the same medical center from 1994-1996 were recruited by newspaper ads, fliers, and word of mouth (Figure 1). The term-born participants were recruited in accordance with race and sex distribution of the VLBW cohort. More detailed information on study protocols and inclusion/exclusion criteria has been published previously.(21-23) Subjects were compensated for participation. Written informed consent was obtained from the parent or legal guardian and assent was obtained from the participant. The Institutional Review Boards of Wake Forest School of Medicine and Forsyth Medical Center approved the study.

### Neonatal Characteristics

Neonatal characteristics, including birth weight, gestational age, indication for delivery, antenatal corticosteroid exposure, and postnatal corticosteroid exposure were retrieved from research databases and from the medical records of mothers and neonates by a trained research nurse. Gestational age was determined in order of availability, either the first trimester ultrasound, maternal report of last menstrual period, or clinical assessment of the neonate. Birth weight z-scores and percentiles were determined from gestational age- and sex-specific reference standards.(24)

### Adolescent Characteristics

Participants' weight and height were measured in triplicate without shoes and in light clothing using a digital platform scale and wall-mounted stadiometer, respectively. The child's body mass index (BMI) and BMI z-score were calculated.(25) Parents reported their child's race and Medicaid eligibility. Race was reported as Black, white, Asian, or Native American and parents could select multiple options; participants reporting any Black race were categorized for this analysis as Black and all others were categorized as non-Black. Both parent and child reported medications taken on a regular basis, including inhaled corticosteroids, over-the-counter medications, and supplements. Participants privately self-reported their sexual maturity on a questionnaire with a scale of 1 to 5 for both pubic hair and breast development (females) and pubic hair and external genitalia development (males). (26) For this analysis we report the proportion of participants with a score of 5 in either of the secondary sexual characteristics.

We measured salivary cortisol levels prior to and 20 minutes after a graded exercise test in which participants were verbally encouraged to exercise to exhaustion. Exercise testing was performed on a cycle ergometer (CPX Metabolic Cart and Corival cycle ergometer, Medical

Graphics, St. Paul, MN). The increment in workload was based on height following the Godfrey Protocol (10, 15, or 20 W each minute for height of <125, 125-150, or > 150 cm, respectively) and designed to reach volitional maximum within 8-10 minutes.(27) In order to standardize the time of salivary cortisol collection, exercise testing was performed mid-day in the non-fasting state. After rinsing their mouths, participants chewed a piece of gum for 1 minute and then spit into a sterile plastic vial.(28) The salivary cortisol levels were measured by Esoterix labs (Calabasas Hills, California) using an FDA-approved radioimmunoassay prior to May 19, 2008 and subsequently by high-performance liquid chromatography with tandem mass spectrometry after solvent extraction. Using reference data provided by Esoterix, radioimmunoassay levels were transformed to be comparable to mass spectrometry results.(29) The salivary cortisol response to stress was defined as the difference between the post- and pre-stress cortisol levels.

### Statistical Analysis

We performed descriptive statistics to examine measures of central tendency and dispersion and used the Shapiro-Wilk test to examine normality. The cortisol variables (pre-stress, post-stress, and stress response) were not normally distributed and therefore natural log-transformations were used to improve distributional characteristics. We used the t-test and Mann–Whitney $U$ test to compare continuous variables as appropriate and the chi-square test to compare categorical variables between the preterm and term groups.

We used general linear regression analysis to examine the association between preterm birth status and log-transformed salivary cortisol variables (pre-stress, post-stress, and stress response). For adjusted analyses, we used a directed acyclic graph approach to determine the minimally sufficient set of covariates to include in the adjusted models.(30) Adjusted models included the covariates birth weight z-score and maternal hypertension during pregnancy. Of note, the method of cortisol determination (immunoassay vs. mass spectrometry) was also considered as a possible confounder and found not to be significantly associated with the outcome or predictor, so was not included in the final analysis. Beta-coefficients can be interpreted by taking the $\exp(\beta)$ to obtain odds ratios.

To examine for the presence of effect modification we independently evaluated interaction terms for preterm status with race and with sex. We stratified our models where there was evidence of statistical interaction (p<0.05).

Finally, because exposure to exogenous glucocorticoids is associated with HPA axis dysfunction, we performed a sensitivity analysis to examine the relationship between preterm birth status and log-transformed salivary cortisol variables among adolescents not exposed to exogenous glucocorticoids (in utero, postnatally, or inhaled corticosteroid use during adolescence). All p-values were on the basis of 2-tailed tests with a significance level of 0.05. All statistical analysis was performed using Stata v14.2.

## RESULTS

Compared to those eligible (N=479) but not enrolled in the parent study, the 188 participants included in the parent study had lower gestational age (mean ± SD 27.7 ± 2.6 v 28.3 ± 2.5

weeks) and higher birth weight z-score (−0.26 ± 0.81 versus −0.43 ± 0.81) but were similar in birth weight, race, sex, and mode of delivery (results not shown). We evaluated 171 adolescents born preterm with VLBW (41% Black, 43% male) and 50 born at term with normal birth weight   2500 g (40% Black, 46% male). Participants in the parent study with incomplete data for this analysis (N=19) were mostly similar to participants with complete data except that they were more likely to be male, have a younger gestational age, and receive Medicaid insurance. Characteristics for preterm and term participants at birth and 14 years of age are shown in Table 1. There were no preterm-term group differences observed according to race or sex; however the preterm group had a lower birth weight z-score.

## Salivary Cortisol

In bivariate analyses of untransformed cortisol values at age 14 years using Mann-Whitney U tests we observed no preterm-term group differences in pre-stress salivary cortisol. However, post-stress cortisol levels and the cortisol stress response were both lower in the preterm group compared to those born term (Table 2).

Values for pre- and post-exercise stress cortisol measurements and the cortisol stress response were log-transformed to improve normality. Bivariate analyses of the log-transformed cortisol variables demonstrated similar patterns to the untransformed variables, however, the preterm-term differences for post-stress cortisol and cortisol stress response did not reach statistical significance (Table 3, Model 1). Adjustment for the potentially confounding factors of birth weight z-score and maternal hypertension during pregnancy did not substantively change the results (Table 3, Model 2).

## Effect Modification by Race and Sex

We observed effect modification of the association between preterm birth status and cortisol response to stress according to race (interaction term p-value 0.02). The unadjusted cortisol stress response stratified by race is depicted in Figure 2. In adjusted analysis, preterm birth was associated with a significantly lower cortisol response to stress in non-Black participants (β: −0.74; 95% CI: −1.34, −0.15), which corresponds to a 52% lower cortisol response compared to those born at term. There was no association between preterm birth status and cortisol stress response in Black participants, (Table 3, Model 3). We observed no effect modification by sex.

## Sensitivity Analyses

We separately restricted our analyses to adolescents who were not exposed to exogenous corticosteroids, including antenatal corticosteroids, neonatal high-dose corticosteroids, or inhaled corticosteroids during adolescence. There were no preterm-term differences observed in pre-stress cortisol levels. Preterm birth was associated with a significantly lower post-stress cortisol level (aβ: −0.53; 95% CI −0.99, −0.09) and a decreased cortisol response to stress that did not reach statistical significance (Table 3, Model 4).

## DISCUSSION

This study examined salivary cortisol response to exercise-induced stress in adolescents born preterm with VLBW as compared to those born term with normal birth weight in a racially diverse cohort born in the 1990s. Adolescents born preterm had similar pre-stress salivary cortisol levels but cortisol stress response was race-dependent, with greater preterm-term differences in non-Black adolescents. We did not observe effect modification in the preterm-term relationships by sex.

While we did not find an association between pre-stress cortisol and preterm birth, previous studies examining basal cortisol expression in children (31-33) and adults (13, 34-36) born preterm compared to those born at term have produced mixed results. One study of 128 school-aged children born preterm found that hair cortisol levels were lower in children born preterm compared to children born term. The etiology for this difference was gender-specific; in preterm boys who had the minor allele for NFKBIA rs2233409, lower hair cortisol was associated with greater neonatal pain, and this was not true for preterm girls. (31) One explanation for this finding is that the morning cortisol levels observed in these studies may actually represent a stress response due to fasting and the novel and potentially stressful clinic setting in which the blood samples were obtained. This hypothesis is supported by the link between lower birth weight and increased free cortisol levels during a fasting state(35). Our study did not show a difference in non-fasting pre-stress cortisol levels between the preterm and term groups, similar to other studies showing no association between birth weight and cortisol secretion in the unstressed state.(33)

Our finding that non-Black adolescents born preterm had a blunted cortisol stress response compared to those non-Black adolescents born term is consistent with previous work in non-Black persons born preterm that demonstrated dysregulation of the HPA axis.(15, 36) Kaseva et al reported blunted cortisol levels in response to a psychological stressor in VLBW young adults compared to their normal birth weight peers.(36) Another study by Buske-Kirschbaum et al showed that children ages 8-12 born at any degree of prematurity showed attenuated salivary cortisol responses to a psychological stress test, although this difference did not reach statistical significance, likely due to the study's small sample size (18 preterm children and 18 same-sex controls).(33) We observed that the cortisol stress response was race-specific. The preterm birth-associated blunted cortisol response to stress was detected in non-Black adolescents but not Black adolescents whose post-stress cortisol levels were lower irrespective of preterm/term status. This has been noted in other studies wherein race, regardless of socioeconomic status, is associated with variable HPA axis expression.(18, 37) Self-reported race is a complicated factor to accurately measure because it represents the cumulative effect of genetic background and epigenetics, participant and parental physical phenotypes (e.g. skin color), culture, historical factors, and psychosocial exposures.(38) It has been hypothesized that African Americans are exposed to more discrimination than whites, which leads to chronically elevated levels of stress hormones that may blunt the cortisol stress response.(39) Alternatively, there may be heritable differences in HPA axis components such as the cortisol receptor.(37) Further work is needed to explore the relationships among preterm birth, race, and the HPA axis.

Our study has several limitations including its observational design. The modest sample size in the term birth group indicates that the estimates may be less precise, thus a lack of association among Black term-preterm participants does not preclude the possibility that an association might be observed with a larger sample size. Additionally, there are many other exposures during early life (fetal, perinatal, and early childhood) and adolescence (physical activity, sleep, menstrual cycle phase, etc.) that may influence the cortisol stress response but were not assessed in our study. There is also the potential for effect modification of the observed associations by preterm management strategies. Only 193 participants were enrolled out of 479 patients who met inclusion criteria, which may have introduced some selection bias into the original study sample. Our categorization of race was based on one question in which parents reported their adolescent's race and our cohort had few children of Hispanic ethnicity. While our preterm population is generally similar to other preterm cohorts in respect to birth weight, gestational age, neonatal morbidity, and age at follow-up it is possible that heterogeneity exists; however, the primary focus of this paper was a term-preterm comparison and we were not adequately powered to evaluate for heterogeneity based on preterm characteristics. Additionally, the laboratory changed the measurement of cortisol in the midst of the study, and although they provided an equation to transform the radioimmunoassay levels to be comparable to mass spectrometry results, it is possible that error was introduced in this transformation. However, as there was no difference by cortisol methodology and race, we believe that our results are valid. Finally, we used salivary cortisol as a biomarker for HPA axis activity; however, the processes involved in the response to physiologic stress are complex and may not be fully defined by salivary cortisol.(40) Our study was strengthened by a more racially diverse cohort than previous studies, thus this study may be more generalizable to the current preterm population. Additionally, many of the previous adult cohorts were born prior to medical advances such as surfactant and antenatal steroid therapies for preterm and VLBW infants.

In conclusion, adolescents born preterm demonstrated similar pre-stress cortisol levels and a blunted cortisol response to stress when compared to their term-born peers, an effect that was only identified in non-Black adolescents. As dysregulation of the HPA axis is associated with increased risk for mental health problems, obesity, diabetes, hypertension, and cardiovascular disease,(16, 17) our findings suggest that preterm birth-related HPA axis alterations may contribute in part to the increased risk for these chronic diseases that has been described in those born preterm.

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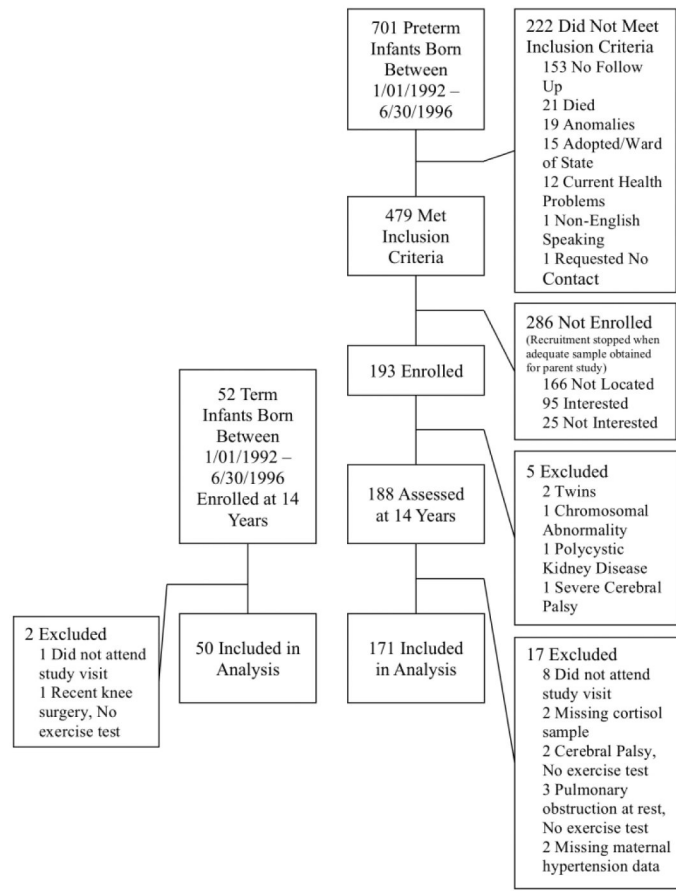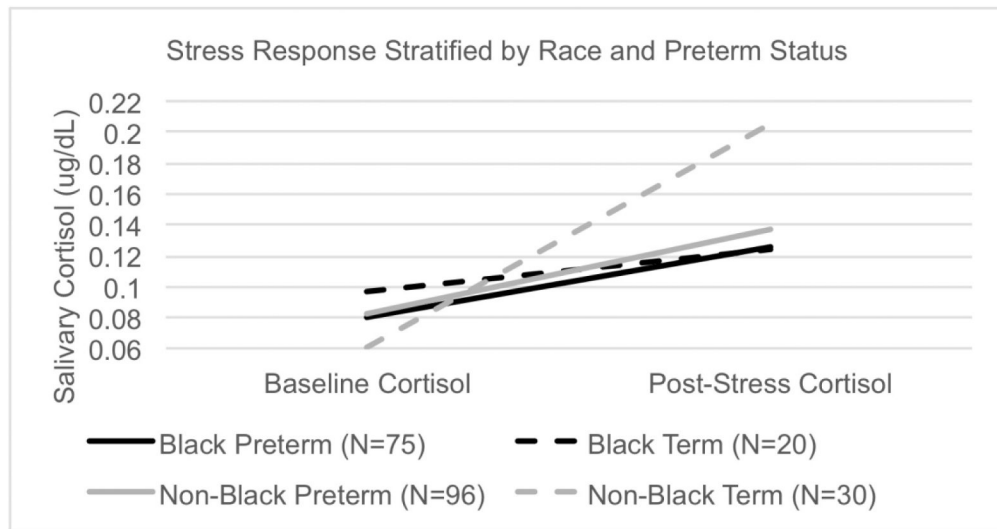
**Figure 1:**
Cohort Flow Diagram

**Figure 2:**
Unadjusted Salivary Cortisol Levels Before and After Exercise Stress, According to Preterm Birth Status and Race

**Table 1:**

Perinatal and Adolescent Characteristics

| | Preterm n=171 | Term n=50 | p value [b] |
|---|---|---|---|
| Perinatal Characteristics | | | |
| Male, % | 43.3 | 46.0 | 0.7 |
| Black race, % | 43.9 | 40.0 | 0.6 |
| Gestational age, weeks [a] | 27.9 (2.7) | 39.6 (1.1) | <0.001 |
| Birth weight, g [a] | 1060.1 (264.5) | 3457.9 (481.1) | <0.001 |
| Birth weight percentile [a] | 42.6 (25.3) | 50.2 (29.4) | 0.04 |
| Birth weight z-score [a] | −0.29 (0.8) | −0.01 (1.0) | 0.03 |
| SGA, % | 11.7 | 8.0 | 0.5 |
| LGA, % | 1.8 | 12.0 | 0.001 |
| Maternal hypertension, % | 39.8 | 6.0 | <0.001 |
| Antenatal corticosteroids, % | 40.7 | 0 | - |
| Postnatal corticosteroids, % | 17.0 | 0 | - |
| Indication for preterm delivery | | | - |
| Induced, % | 36.5 | | |
| Spontaneous, % | 63.5 | | |
| Adolescent Characteristics | | | |
| Age, years [a] | 14.5 (0.3) | 14.6 (0.3) | 0.2 |
| Weight, kg [a] | 59.5 (16.9) | 64.5 (15.1) | 0.03 |
| Weight z-score [a] | 0.34 (1.30) | 0.82 (0.83) | 0.008 |
| Height, cm [a] | 161.3 (9.2) | 167.9 (7.0) | <0.001 |
| Height z-score [a] | −0.37 (1.10) | 0.52 (0.92) | <0.001 |
| BMI, kg/m² [a] | 22.8 (5.8) | 22.9 (5.0) | 0.5 |
| BMI z-score | 0.47 (0.09) | 0.64 (0.82) | 0.2 |
| BMI 85th percentile, % | 33.4 | 32.0 | 0.9 |
| Sexual maturity rating of 5, % | 59.2 | 53.1 | 0.4 |
| Inhaled corticosteroid use, % | 5.3 | 0 | - |
| Medicaid insurance, % | 40.6 | 16.0 | 0.001 |

[a] Mean (SD)

[b] Difference of characteristics by preterm status using Pearson chi-square or t-test

**Table 2:**

Cortisol Levels at 14 years of age

| | Preterm N=171 | Term N=50 | P value [b] |
|---|---|---|---|
| Cortisol Levels | | | |
| Pre-stress cortisol (μg/dL) [a] | 0.05 (0.03, 0.11) | 0.06 (0.03, 0.09) | 0.7 |
| Post-stress cortisol (μg/dL) [a] | 0.09 (0.04, 0.17) | 0.14 (0.05, 0.25) | 0.03 |
| Cortisol response to stress (μg/dL)[a] | 0.03 (−0.03, 0.12) | 0.08 (−0.01, 0.19) | 0.04 |

[a] median (IQR)

[b] Difference by preterm/term status using Mann-Whitney U test on non-transformed values

**Table 3:**

Association Between Preterm Birth and Salivary Cortisol Levels Before and in Response to Stress

| Outcome Variable | Model 1: Unadjusted<br>N=208<br>β (95% CI) | Model 2[†]: Adjusted<br>N=208<br>β (95% CI) | Model 3[†]: Stratified by race | | Model 4[†]: Steroid unexposed<br>N=112<br>β (95% CI) |
|---|---|---|---|---|---|
| | | | Black<br>N=90<br>β (95% CI) | Non-Black<br>N=118<br>β (95% CI) | |
| Pre-stress cortisol | 0.02 (−0.28, 0.31) | 0.04 (−0.27, 0.35) | −0.32 (−0.84, 0.19) | 0.21 (−0.18, 0.62) | −0.14 (−0.54, 0.26) |
| Post-stress cortisol | −0.34 (−0.69, 0.01) | −0.33 (−0.70, 0.04) | 0.15 (−0.41, 0.72) | −0.52 (−1.02, −0.02)[*] | −0.53 (−0.99, −0.09)[*] |
| Cortisol response to stress | −0.34 (−0.77, 0.09) | −0.34 (−0.80, 0.11) | 0.53 (−0.16, 1.22) | −0.74 (−1.34, −0.15)[*] | −0.33 (−0.89, 0.22) |

General linear regression models with natural-log transformed outcome variables. Beta-coefficients can be interpreted by taking the $\exp(\beta)$ to derive odds ratios.

[†] Models 2, 3, and 4 adjusted for maternal hypertension and birth weight z-score

[*] p<0.05