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Natural variation in gestational cortisol is associated with patterns of growth in marmoset monkeys (*Callithrix geoffroyi*)

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Abstract

High levels of prenatal cortisol have been previously reported to retard fetal growth. Although cortisol plays a pivotal role in prenatal maturation, heightened exposure to cortisol can result in lower body weights at birth, which have been shown to be associated with adult diseases like hypertension and cardiovascular disease. This study examines the relationship between natural variation in gestational cortisol and fetal and postnatal growth in marmoset monkeys. Urinary samples obtained during the mother's gestation were analyzed for cortisol. Marmoset body mass index (BMI) was measured from birth through 540 days in 30- or 60-day intervals. Multi-level modeling was used to test if marmoset growth over time was predicted by changes in gestational cortisol controlling for time, sex, litter, and litter size. The results show that offspring exposed to intra-uterine environments with elevated levels of cortisol had lower linear BMI rates of change shortly after birth than did offspring exposed to lower levels of cortisol, but exhibited a higher curvilinear growth rate during adolescence. Average daily change in gestational cortisol during the first trimester had a stronger relationship with postnatal growth than change during the third trimester. Higher exposure to cortisol during gestation does alter developmental trajectories, however there appears to be a catch-up period during later post-natal growth. These observations contribute to a larger discussion about the relationship of maternal glucocorticoids on offspring development and the possibility of an earlier vulnerable developmental window.

Keywords

glucocorticoids; pregnancy; body mass index (BMI); intra-uterine programming; developmental trajectories; multi-level modeling

1. Introduction

Growing evidence across many disciplines suggests that excessive prenatal exposure to glucocorticoids can retard prenatal growth and potentially postnatal growth into adulthood. Moreover, exposure to glucocorticoids during gestation may have other behavioral

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consequences throughout the development of the offspring. These effects, including hindered growth, have been found in many species including, but not limited to, humans [15, 19, 31], primates [11, 44] pigs [33], sheep [14], rodents [3, 29], and fish [7]. Cortisol, the primary glucocorticoid of the primate hypothalamic-pituitary-adrenal (HPA) axis, is essential for normal fetal development. Cortisol is required for the maturation and preparation of the lungs for the extra-uterine environment [41], fetal central nervous system development [48], and the growth of the fetus and induction of enzymes important for the preparation for extra-uterine life [10]. Glucocorticoids play a pivotal role in lung maturation, and glucocorticoids have long been used clinically to enhance/alter the maturation of tissues and organs, often to develop the lungs in cases where mothers are at high risk for preterm deliveries [23].

The relationship between intra-uterine glucocorticoids and growth has been well examined. Many studies have shown that pharmacological doses of synthetic glucocorticoids, such as dexamethasone, retard fetal growth resulting in lower birth-weights of offspring [13, 27, 54]. The effect of pharmacological doses of synthetic glucocorticoids on growth does not appear to be limited to birth weights. There is evidence suggesting that human fetuses, whose mothers received synthetic glucocorticoids during pregnancy showed a 'catch-up' period after initial fetal retardation of growth in early adolescence [32], but much less is known about the long-term effects of glucocorticoids on growth and growth trajectories from birth into early adulthood.

The association of endogenous intra-uterine glucocorticoids and extra-uterine outcomes like growth has been examined with increasing interest. The interest in endogenous glucocorticoids is useful because it reflects a more natural intra-uterine environment without the addition of synthetic glucocorticoids. For example, researchers may use indirect maternal reports of stress as measurements of prenatal glucocorticoid exposure [16, 20], and like increase artificial glucocorticoid exposure, high levels of maternal depression have also been associated with lower birth weights. It is worth noting that even indirect measurements of glucocorticoids like elevated maternal depression are associated with lower birth weights. This evidence suggests that natural variations in glucocorticoid production during gestation can be sufficient to alter fetal growth, and that these effects can vary by the nature of the stress, timing during gestation, genetic strain of the animal, and activity of the placental barrier [24].

Because it is known that some quantity of glucocorticoids can pass through the placenta, one might expect that elevation in maternal glucocorticoids would increase fetal glucocorticoid exposure [45]. Maternal glucocorticoids can be elevated for numerous reasons. Glucocorticoids are involved intimately in the 'fight or flight' response, meaning instances of high stress would result in a mostly acute, though sometimes chronic, elevation of plasma cortisol. Causes of high stress depend on the animal's situational needs, but they can often include malnutrition, predation, and many other components of survival. Also, as referred to previously, glucocorticoids can be pharmacologically elevated for various clinical reasons, including assisting lung maturation for fetuses at high risk of preterm delivery. The placenta does have an enzymatic barrier—11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD-2)—which prevents natural glucocorticoids from crossing the placenta unimpeded by catabolizing cortisol or corticosterone into biologically inert components [45]. This barrier is important because glucocorticoids can have deleterious effects on neuron proliferation if too much glucocorticoids reach the fetus [28]. However, 11 β -HSD-2 is not a complete barrier. About 10 to 20% of natural glucocorticoids cross the placenta and enter the fetal blood stream, while, in contrast, synthetic glucocorticoids like dexamethasone and betamethasone can cross the placenta nearly unimpeded [46]. This explains, in part, why these synthetic glucocorticoids are potent inhibitors of fetal growth. There remains a potentially

unavoidable prenatal vulnerability with exposure to high levels of maternal cortisol on fetal and postnatal development.

Low birth weights have been linked to many adult diseases suggesting the possibility of a 'fetal programming hypothesis.' Barker's hypothesis [4] posits that during critical periods of organogenesis and tissue growth, alterations in the intra-uterine environment, such as heightened exposure to glucocorticoids, can permanently alter organ structure and function, which, in turn, may compromise later-life functioning. For instance, low birth weights have been associated with hypertension and cardiovascular diseases even after controlling for any effect of other risk factors like diet, smoking, or alcohol consumption [5, 6, 26]. Consequently, both the level and severity of glucocorticoid exposure and the timing of fetal development when the glucocorticoid exposure occurs are critical for developmental outcomes.

Marmosets, like humans, have naturally elevated glucocorticoids during pregnancy, so this species can provide a useful model for examining the association between intra-uterine glucocorticoids and growth. Marmosets are a small, monogamously breeding, New-World primate that commonly give birth to twin offspring. Marmosets also exhibit an interesting family unit where both the mother and the father, as well as available siblings, assist in the rearing the offspring for the first few months of development. Birth weights and growth are affected by other variables, like maternal nutrition [25]; likewise, differences in growth, including birth weight in small primates like marmosets, are linked to both the size of the mother and litter size. Tardif et al. [53] found that singleton infants of large marmoset mothers were on average larger than twins of small marmoset mothers with singletons of small mothers and twins of large mother being intermediate between the two in average birth weight. Furthermore, maternal age in marmosets is associated with higher birth weight as well as higher litter sizes, which all appear to facilitate larger adult weights [51]. This picture is further complicated by the fact that maternal and early infant behavior (i.e. early life locomotion and weaning) can also influence early life growth, but not later growth rate [52]. Though maternal conditions appear to play an important role in infant growth, maternal conditions like maternal body size do not fully account for the differences in growth rate both within and between primate species [30].

This study aims to explore whether natural variation in cortisol influences composite body growth trajectories through early adulthood in marmosets. Increases in glucocorticoids during pregnancy have repeatedly shown to be associated with low birth weights. Therefore, it is anticipated that marmoset pregnancies with higher changes in cortisol will result in offspring with lower birth BMIs. The rate of offspring postnatal growth is anticipated to be lower for offspring exposed to higher concentrations of cortisol within their intra-uterine environments. To the extent that glucocorticoids contribute significantly to variation in fetal and postnatal growth, we would expect that the relationship between gestational cortisol and the rate of BMI change should be independent of offspring sex, litter size, and conditions of the marmoset mother like maternal age and size.

2. Methods

2.1 Subjects

The study included 30, (16 male and 14 female) white-faced marmoset (*Callithrix geoffroyi*) offspring from 18 pregnancies of six different mothers. All animals were socially housed in colony rooms at the University of Nebraska at Omaha (UNO) Callitrichid Research Center (CRC). Colony rooms were maintained at 19.7–22.1° C and on a 12:12 light–dark cycle. Wire-mesh enclosures varied in size depending on the number of individuals in the social group, about 0.8 m³ per animal and included various enrichment items. All animals were fed

twice each day first at 0800 h and later in the afternoon between 1300 and 1500 h. All animal use procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska at Omaha (IACUC 07-033-05-FC). Further details of animal housing and husbandry have been previously reported [43].

2.2 Somatic Measurements

The 30 subjects went through a series of somatic measurements at specific times of development (Day 2, 30, 60, 120, 180, 240, 300, 360, 420, 480, 540), totaling 11 time points of somatic measurements. The somatic measurements included various body measurements, but only two are reported in this study to represent one conventional composite measure of growth accounting for both length and weight in the form of a Body Mass Index (BMI) ($\text{weight/torso length}^2$) (mg/mm^2). Torso length was estimated by measuring suprasternal-pubic length (SSPL) in millimeters, and weight was a measurement of body mass in grams. BMI has been previously used to study growth trajectories [1]. Marmoset offspring were removed from their homecages to a designated location in the CRC and returned immediately to their homecages upon completion of measurements. Young infants were maintained in an incubator maintained at 35°C while their littermate underwent the measurement procedure. Each measurement was performed three times by trained personnel to the nearest 0.1 g or mm and these replicate measures were averaged.

2.3 First-void Urine Collections

First-void urine samples from breeding females were collected using noninvasive, stress-free collection techniques. One to three urine samples a week were collected in hand-held pans in exchange for a food reward between 0730 and 0800 h. Samples were centrifuged for approximately two minutes to separate the urine from any sediment contamination. The urine was transferred into a test tube and stored at -20°C until assayed. Urine sampling reflects total circulating hormones levels since the last time the bladder was emptied, and first void urine samples are the best and most readily available representation for the composite daily average of cortisol [22]. All urine samples (one to three samples per week) collected over the duration of the pregnancy were assayed for cortisol and used in determining overall maternal cortisol concentration for full gestation and trimester gestational stages.

2.4 Pregnanediol glucuronide (PdG) enzyme immunoassay

The date of conception was determined by enzyme immunoassay (EIA) of urinary progesterone metabolite (pregnanediol glucuronide; PdG) concentrations. The average length of gestation in this study was 148.72 $\pm$ 5.75 (range = 144 – 166 days). Concentrations of PdG in 6 female marmosets were measured by EIA from urinary samples available over the course of 18 pregnancies. Urinary PdG levels were monitored by EIA following protocol established by Munro et al. [37] and adapted for marmosets [22]. High and low concentration quality control pools were assayed on each plate. Intra- and inter-assay coefficients of variation were 22.0 and 6.1% (high, n = 53) and 28.0 and 6.8% (low, n = 53), respectively. Variation in fluid intake and output was indexed by measuring concentrations of urinary creatinine (Cr). The creatinine assay utilized a modified Jaffé end-point assay, previously described and validated for use in this species [22]. Pregnancy was confirmed by persistently high levels of PdG (> 10 $\mu\text{g/mg Cr}$) for at least three consecutive samples.

2.5 Cortisol enzyme immunoassay

Urinary cortisol concentrations were determined using an enzyme immunoassay (EIA) previously described and validated for use in *C. kuhlii* [50]. Two quality control urine pools,

one that averaged approximately 70% binding (Low QC) and one that averaged 30% binding (High QC) were assayed on every plate to insure reliability and stability. Cortisol assays were conducted over a period of eight years, and intra-assay coefficients of variation (CV) for high and low pools ranged from 3.7 – 7.3% and 3.3 – 6.1%, respectively. Inter-assay CVs for the high and low pools ranges from 8.7 – 14.7%, and 13.3 – 18.2%, respectively. To minimize procedural variability, all samples for a single individual (i.e., at all three time points) were assayed at the same time whenever possible.

2.6 Data Analysis

Multi-level modeling was used to assess the relationship of gestational cortisol and somatic growth from birth through adolescence in marmoset offspring. The primary advantage of multi-level modeling over traditional hierarchical regression is the ability to account for non-independence of longitudinal data. Multi-level modeling, or hierarchical modeling, allows researchers to model nested data. In this case, nested data, or non-independence of data, exists for offspring measurements of same offspring over time and littermates (i.e. offspring who shared intra-uterine exposure to gestational cortisol). We were interested in how offspring measurements may vary because of factors associated with each pregnancy. Growth measurements of individual offspring throughout development ($n = 330$) were nested within offspring ($n = 30$), which were further nested within pregnancy ($n = 18$). In other words, these data are represented in three levels: BMI is accounted for by both linear and curvilinear change over time (level 1); sex of the offspring (level 2); and pregnancy characteristics including litter size, maternal baseline levels of cortisol at the estimated day of conception, and average amount of change in maternal cortisol through gestation (level 3). Thus, information about higher levels, such as pregnancy characteristics, specifically cortisol exposure, can be used to predict the intercept (starting point) and slope (change) of variables in lower levels of the model, such as individual BMI growth (cross-level interaction). Figure 1 illustrates the variability of urinary cortisol over the course of gestation by individual pregnancies. Analyses were conducted for the whole gestation and per trimester of pregnancy as well to determine if the relationship between gestational cortisol and post-natal growth was stronger earlier or later in the pregnancy. All multi-level analyses were conducted using HLM version 6.08 [8]. The criterion for statistical significance for all analyses was set at $p < .05$. Preliminary analyses ruled out any significant association of maternal age and size with offspring BMI growth, and these predictors were excluded from the model.

3. Results

The average BMI at birth was $M = 17.58$, $SE = .52$ and at day 540, the last measurement, the average BMI was $M = 25.71$, $SE = .97$. To see a distribution of BMI over time, see figure 2. There were no differences between males and females in BMI at birth through day 540. The average change in cortisol (per day of gestation) was $M = .56$ units, $SE = .33$ (trimester 1), $M = 1.12$, $SE = 1.06$ (trimester 2), and $M = 1.92$, $SE = 1.36$ (trimester 3). The unconditional model, where no variables were regressed on BMI, resulted in an intra-class correlation wherein 88.5%, .001%, and 11.5% of the variance in BMI was attributable to variability over time (level 1), variability between offspring (level 2), and variability between pregnancies (level 3) respectively. The final estimation of BMI variance resulted in a significant amount of variance left to be explained at level 3 (i.e. differences in growth from pregnancy characteristics) $\chi^2_{(17)} = 58.38$, $p < .05$. This significant variance implies that there are potential differences in pregnancy characteristics, i.e., gestational cortisol, are related to BMI independent of the relationship of time and sex with growth to be accounted for. See Figure 2 for a representation of variance in BMI across different pregnancies and mothers.

3.1 Full Gestation

3.1.1 Differences in growth by level 1 predictors (time)—After modeling the effect of linear change over time on growth, time significantly predicted an increase in growth ($\beta = .44$, $b = 1.38$, $t_{(17)} = 6.93$, $p < .05$). The modeling of linear time on growth results in a reduction in error of 27.41%, a statistically significant improvement of model prediction ($\Delta \chi^2_{(7)} = 58.38$, $p < .05$), compared to the unconditional model where no variables were used to estimate BMI. To explain, there was a significant linear relationship between time and BMI. As time increased so did BMI. Furthermore, curvilinear time did not significantly predict growth above and beyond the effect of linear time ($\beta = -.35$, $b = -.20$, $t_{(17)} = 1.64$, $p > .05$), but curvilinear time reduced error by 6.59% resulting in a marginally significant improvement in model prediction ($\Delta \chi^2_{(7)} = 12.73$, $p = .08$). Because there was a near significant reduction in error ($p = .08$), allowing curvilinear time in the model is useful for explaining the nature of the relationship between time and BMI.

3.1.2 Differences in growth by level 2 predictors (sex)—Sex did not affect fetal growth (intercept of BMI at birth) ($\beta = .03$, $b = -1.57$, $t_{(28)} = 1.53$, $p > .05$) or rate of linear ($\beta = .17$, $b = .01$, $t_{(28)} = 1.24$, $p > .05$; curvilinear $\beta = -.07$, $b = -.08$, $t_{(28)} = 0.51$, $p > .05$) rate of growth. Overall, modeling sex resulted in a proportional reduction in prediction error of level 2 variance of 88%, but because the level 2 variance was so small (.001%), the overall model prediction improvement was not significant compared to the variables modeled at level 1, ($\Delta \chi^2_{(2)} = 4.12$, $p > .05$). Offspring sex did not significantly account for any unique variance in the change of BMI over time suggesting that there is no relationship between the sex of the offspring and BMI in marmosets.

3.1.3 Differences in growth by level 3 predictors (litter size and gestational cortisol)—Litter size did not significantly associate with any differences in BMI at birth ($\beta = .22$, $b = .87$, $t_{(16)} = .67$, $p > .05$), or any differences in postnatal linear ($\beta = .32$, $b = .01$, $t_{(28)} = 1.11$, $p > .05$) or curvilinear ($\beta = .36$, $b = -.25$, $t_{(28)} = 1.44$, $p > .05$) rate of growth. Although litter size did not affect growth at birth or postnatal growth rate from one pregnancy to another, it did improve the overall model prediction compared to a model free of level 3 predictors (i.e. above and beyond time alone) ($\Delta \chi^2_{(3)} = 9.26$, $p < .05$). Therefore, litter size is a variable that is appropriate to control when accounting for BMI.

The addition of baseline gestational cortisol did not significantly improve the model, ($\Delta \chi^2_{(3)} = 4.51$, $p > .05$), and only resulted in a very modest proportional reduction in prediction error of 0.37%. Cortisol baseline was not associated with any differences in BMI at birth, ($\beta = .25$, $b = .76$, $t_{(15)} = .45$, $p > .05$), or in rate of BMI change (linear, $\beta = -.10$, $b = -.01$, $t_{(15)} = 1.60$, $p > .05$; curvilinear, $\beta = .02$, $b = .01$, $t_{(28)} = .17$, $p > .05$). Cortisol baseline did not reduce prediction error (actually resulted in a slight increase for change in time linearly, $\Delta \chi^2_{(1)} = .00$, $p > .05$, and curvilinearly, $\Delta \chi^2_{(1)} = .00$, $p > .05$). Overall, the cortisol baseline level at conception accounts for little variance of offspring growth.

Maternal average daily cortisol change was assessed (i.e. as cortisol increases over the course of the pregnancy, a higher change cortisol reflects an overall higher increase in cortisol exposure) to evaluate the relationship between gestational cortisol and growth. Mother's average daily change in cortisol was not associated with BMI at birth ($\beta = -.31$, $b = .11$, $t_{(14)} = 0.14$, $p > .05$), which suggests that differences in exposure to gestational cortisol did not influence BMI at birth. There is a marginally significant relationship between the mother's average daily cortisol change and growth over time ($\beta = -.48$, $b = -.02$, $t_{(14)} = 2.05$, $p = .06$). This relationship was reversed and not significant for the curvilinear BMI change seen during the later portion of postnatal growth measurement ($\beta = .36$, $b = .20$, $t_{(14)} = 1.69$, $p = .11$). Daily change in cortisol significantly improved the model prediction

compared to the previous model ($\Delta \chi^2_{(3)} = 10.07, p < .05$) suggesting that is an important predictor when accounting for variance of postnatal BMI change. Daily change in cortisol as a predictor resulted in a negligible proportional reduction in prediction error of .013% for BMI at birth ($\Delta \chi^2_{(1)} = .006, p > .05$) and a very strong 48.61% reduction in prediction error for postnatal BMI growth (21.42% on the linear change in BMI, $\Delta \chi^2_{(1)} = 4.16, p < .05$; 17.19% curvilinear change in BMI, $\Delta \chi^2_{(1)} = 2.29, p < .05$). On the whole, pregnancies with higher changes in cortisol resulted in less BMI change initially (during the linear growth phase) and an increase in BMI rate of change during the later life (where BMI change was curvilinear). The improvement in model prediction demonstrates that change in cortisol is a predictor of later BMI change and the patterns of results are consistent. These effects are independent and beyond the effect of time, sex, litter size, and baseline cortisol on BMI. The association of all pregnancy characteristics (i.e. litter size, baseline cortisol, and daily change in cortisol) together account for significantly more variance in BMI than does time and offspring sex alone ($\Delta \chi^2_{(9)} = 23.85, p < .05$). Differences in BMI over time for offspring exposed to either high and low average daily change in gestational cortisol is shown in Figure 3.

3.2 Differences in Growth by Trimester

To investigate if the relationship between daily change in gestational cortisol and postnatal BMI are associated with a particular trimester, the same model building approach was applied using data only from either the first, second, and third trimester. Average daily change in cortisol during the first, second, and third trimester were not significantly correlated with each other ($r(18) = [.21 - .38], p > .05$), and by determining the amount of variance accounted for, one can begin to assess the relative importance of daily change in cortisol during each trimester on BMI growth. Whether the relationship is being driven by early pregnancy, later pregnancy, or whole pregnancy daily change in cortisol has important theoretical implications.

Higher average daily change in cortisol during the first trimester marginally predicted lower BMI rates of change during the early linear growth phase, ($\beta = -.003, b = -4.62, t_{(14)} = 2.04, p = .061$), and higher BMI rates of change during the curvilinear growth phase ($\beta = .04, b = 0.65, t_{(14)} = 2.06, p = .06$). This finding was not as strong and not statistically significant for the second trimester ($\beta = -.001, b = -.51, t_{(14)} = 1.01, p > .05$, for linear and $\beta = .02, b = .06, t_{(14)} = .81, p > .05$, for curvilinear) and similarly for the third trimester ($\beta = -.0001, b = -.10, t_{(14)} = .21, p > .05$, for linear and $\beta = .01, b = .03, t_{(14)} = .40, p > .05$, for curvilinear). This suggests that early pregnancy change in cortisol has a relatively strong association with BMI than later pregnancy. In all three trimesters, however, the same direction of effect was observed; higher cortisol fluctuation resulted in lower linear BMI rates of change (early life BMI change) and higher curvilinear BMI rates of change (later life BMI change). Figures 3 show differences in BMI growth over time by way of high and low daily change in gestational cortisol.

4. Discussion

The data suggest that above and beyond the effects of litter size, offspring sex, and change in growth over time, fluctuations in gestational cortisol predict patterns of postnatal changes in BMI. Offspring that come from pregnancies characterized by higher increases in cortisol levels through the pregnancy have a lower rate of BMI change through adolescence. More specifically, pregnancies with higher change in cortisol were associated with a lower BMI rate of change during the early postnatal growth, i.e., linear portion, and a higher BMI rate of change during the later postnatal growth, i.e. curvilinear portion. This change in growth rate appears to reflect a catch-up period for those offspring who experienced a reduced period of growth rate during early postnatal growth. Furthermore, the relationship between

daily change in gestational cortisol and postnatal BMI appears to be more strongly associated with daily change during early pregnancy, and not late pregnancy. Although the relationships between change in cortisol and BMI was marginally significant, the overall model significantly reduced prediction error. This demonstrates that daily change in cortisol is a predictor of postnatal BMI rate of change, and all the patterns of results were consistent. These data lend support for the hypothesis that higher exposure to cortisol is associated with retarded growth during early life, but a later life catch-up appears to occur. This suggests there was no permanent alteration in BMI.

Considerable evidence shows that pharmacological doses of glucocorticoids including dexamethasone impair fetal growth in sheep and rodents [2] and primates [13]; however, much less is known about whether natural variation and biologically relevant levels of cortisol can influence fetal and postnatal growth. Our data support the notion that normative levels of cortisol can differentially influence postnatal growth rates. Interestingly, there does not appear to be any observable effect on fetal growth because the BMI scores at birth did not differ by exposure to natural variation in cortisol. Since previous research generally focused on growth as a function of weight, this null finding of fetal growth could be due to a potential floor effect in BMI. There is a biologically relevant limitation to how low BMI can go, especially at birth, but also throughout the lifespan. As the infants begin to grow, differences in BMI begin to emerge. The implications of our findings seem to suggest that natural variation in cortisol may not be significant enough to predict patterns of fetal BMI change, but there may be enough natural fluctuation in cortisol to begin observing differential effects of post-natal BMI change.

The fetal programming hypothesis [4] suggests that the human intra-uterine environment can influence phenotypic outcomes above and beyond the influences of the postnatal environment. For example, one might suspect that changes in growth rate could be attributed to postnatal environmental factors like family size and quality of dietary nutrition. Certainly, these factors play a critical role in the development of the offspring. However, the fetal programming hypothesis may offer an alternative view in offspring development. Because litter size and the mother's pregnancies were controlled for statistically, and nutrition controlled for methodologically, insofar as the offspring were all on equal diets, it appears that fluctuations in exposure to prenatal cortisol are independently predicting patterns of BMI rate of change. We found that the change in cortisol during the first trimester was the strongest predictor of postnatal growth, while change in cortisol during the second and third trimester were not predictive of postnatal growth. This suggests that fetal development and programming during early gestation are more vulnerable to glucocorticoid exposure on later life outcomes than they are during late gestation. The first trimester of pregnancy is a time of organogenesis, the second trimester is a time a cellular adaptation, and the third trimester is a time of maturation [36]. Our finding that first trimester increases in cortisol are more strongly associated with lower postnatal BMI rates of change and later growth catch-ups than later trimesters provides insight for future studies looking to elucidate mechanisms of glucocorticoid exposure and postnatal growth.

There is conflicting evidence of particular mechanisms and critical periods for glucocorticoid exposure. For example, Sandman et al. [42] found that maternal stress during the third trimester was associated with shortened gestation age of birth and lower birth weights in humans. A second report presented similar results demonstrating that maternal stress was associated with elevated salivary cortisol during late pregnancy but not so during early pregnancy meaning the effects of maternal stress and low birth weights are likely attributed to later gestational increases in cortisol [39]. However, Schneider et al. [44] found that, in primates, early prenatal stress resulted in lower birth weights and motor deficiencies and this relationship was weaker or nonexistent during middle and late prenatal stress. When

it comes to the relationship between gestational glucocorticoids, birth weights, and early-life growth, there appears to be inconsistencies as to whether there is a critical period in early or late gestation or both. These inconsistencies might suggest that there are different mechanisms for the effect of maternal HPA functioning on normative development compared to dealing with aversive circumstances.

Many outcomes appear to be consistent with elevated gestational glucocorticoids including later life programming of blood pressure and hyperglycemia [17]; Lindsay et al. [34], alterations of gene expression, hippocampal size and increases in offspring anxiety-life behavior [12, 55] and low birth weight [47]. Given that the marmosets in our study did not exhibit any differences in birth BMI, it is difficult to reconcile the observed later life differences by means of these traditional glucocorticoid-programming hypotheses, because we would expect to find differences at birth. It is conceivable that differences in later life BMI change could be affected by differential behavior profiles of the offspring or mother, like heightened offspring anxiety and emotional reactivity, which are both symptoms of heightened glucocorticoid exposure to gestation. Marmosets that received exposure to dexamethasone early during gestation (gestational days 42–48 of a 145 day gestation) exhibited higher rates of food intake, and greater levels of activity and solitary play, relative to late-gestational exposure to dexamethasone or vehicle treatment [27]. This finding suggests that a link between early glucocorticoid exposure and modified behavioral profiles is plausible. Future studies could incorporate whether offspring whom exhibited lower BMI rates of change are associated with higher anxiety, stress reactivity, or overall behavioral profiles.

The mechanisms through which glucocorticoids exert their effect on BMI are complicated. This complication results from the fact that HPA activity does not happen independent of other endocrine systems. There is a strong co-dependence of hormones during typical stress responses. This co-dependence is more strongly entangled during pregnancy, where hormone activity related to fetal development is highest. Glucocorticoids are known to induce permanent changes in physiological systems. This occurs through influencing hormone bioavailability and the expression of receptors, enzymes, and many other critical cellular proteins in the fetal tissues, [21]. Therefore, endocrine changes that occur can be both a cause and a consequence of intra-uterine programming. There are findings of maternal stress interacting with many other factors including lowering progesterone and prolactin during early pregnancy, which also affect fetal programming [40]. In fact, there is increasing evidence suggesting that birth outcomes are affected by hormones like progesterone and prolactin, but, also, immune factors like cytokines, which also influence intra-uterine programming, which can be related to glucocorticoid levels [18]. Furthermore, increases in glucocorticoids can decrease androgens [9], which have been shown to be associated with fetal and postnatal growth in primates. However, it has been found in marmosets that only increased exposure to early androgens are associated with retarded fetal growth and post-natal catch-up growth [49], so there is little reason to believe potentially suppressed androgens from elevated cortisol are affecting postnatal growth.

It is known that cortisol can pass through the placenta and that it can considerably influence the fetal sensitivity to cortisol after the offspring are born [45]. However, prenatal stress has been shown to affect the activity of the 11 β -HSD-2 placental barrier, which is in place to protect the fetus from overexposure to maternal cortisol or corticosterone [38]. The inhibition of the 11 β -HSD-2 placental barrier's ability to prevent glucocorticoids from reaching the fetus is another mechanism through which the potential glucocorticoid intra-uterine programming can occur. In a study looking at prenatal stress in high anxiety and low anxiety rat strains, researchers found that high anxiety rats, but not low anxiety rats, had reduced postnatal neurogenesis when exposed to prenatal stress [35]. They found that

prenatal stress increased 11 β -HSD-2 activity in low anxiety strains, but not high anxiety strains. A heightened sensitivity of hippocampal neurogenesis to prenatal stress in high anxiety strains was accompanied by a failure to increase placental 11 β -HSD-2 activity. For that reason, stress-induced placental 11 β -HSD-2 activity and genetics are two important considerations when explaining potential relationships between prenatal glucocorticoids and post-natal outcomes. In this way, it is possible that higher fluctuations in cortisol are signaling to the fetus that the postnatal environment is a demanding one.

These data do not replicate previous findings that heightened prenatal cortisol exposure resulted in impaired fetal growth, however the data do suggest that heightened prenatal cortisol exposure lessened early postnatal BMI rate of change. Offspring exposed to a high cortisol fluctuating intra-uterine environment, particularly during early gestation, had lower rate of BMI change through the earlier portions of development. During later development, there was a catchup period where the rate of growth was higher compared to offspring from lower cortisol fluctuating intra-uterine environments. Litter size and sex did not appear to be associated with the rate of BMI change or associated any differences in BMI at birth. These observations contribute to a larger discussion about the relationship of maternal glucocorticoids on offspring development and the possibility of an earlier vulnerable developmental window. Finally, these data also suggest that increases in glucocorticoids within naturally occurring ranges can potentially retard early-life growth.

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Fig. 1. Concentrations of urinary cortisol excretion across 18 individual pregnancies from six females in 15-day blocks post-conception. Each line represents one pregnancy.

Fig. 2.

BMI measurements of 30 offspring across 18 individual pregnancies from six females. BMI was measured in offspring at day 2 of age in 30- or 60-day intervals through day 540 of age. Each line represents offspring BMI from one pregnancy. For the 12 pregnancies yielding twin offspring, the BMI of each offspring was averaged to achieve a single BMI trajectory associated with one pregnancy.

Fig. 3.

Mean $\pm$ s.e.m. offspring BMI trajectories for high and low quartiles of full gestation cortisol (high $n = 8$; low $n = 7$), first trimester cortisol (high $n = 6$; low $n = 6$), second trimester cortisol (high $n = 6$; low $n = 6$), and third trimester cortisol (high $n = 6$; low $n = 8$) over 540 days post-natal growth. A total of 18 total pregnancies, 12 pregnancies yielding twin offspring, were used (28 total offspring with 2 offspring removed from incomplete measurements at all time points). High and low quartile sample sizes result from differences in singleton and twin offspring exposure to cortisol at each gestational stage of the pregnancy, so changes in cortisol for individual pregnancies in highest or lowest quartiles may differ from one trimester to the next. Differences at the BMI intercept reflect fetal growth (BMI at day 2), and slope of the lines reflect change in BMI over time. Each line represents mean offspring BMI at each measurement point from pregnancies in the highest and lowest quartiles for average change in cortisol concentration.