Disrupted Prenatal Maternal Cortisol, Maternal Obesity, and Childhood Wheeze

Insights into Prenatal Programming

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Rationale: Exploring prenatal factors influencing childhood wheeze may inform programming mechanisms.

Objectives: We examined associations among prenatal maternal cortisol profiles, maternal obesity, and repeated wheeze up to age 2 years ($n = 261$).

Methods: Salivary cortisol was collected five times per day over 3 days at 29.0 ± 4.9 weeks gestation. Mothers were categorized as obese (body mass index ≥ 30 kg/m²) versus nonobese (body mass index < 30 kg/m²). Using logistic regression, we examined the influence of log-transformed cortisol metrics (level at each time point, morning rise, diurnal and afternoon slopes) and obesity on wheeze adjusting for covariates. Linear mixed models were implemented to examine associations between cortisol trajectories and wheezing. Interactions between maternal cortisol and obesity were considered. **Measurements and Main Results:** Mothers were primarily minority (56.5% Hispanic, 24.1% African American), 61% had less than or equal to 12 years of education, 34% were obese, and 8.4% of children had repeated wheeze. An interquartile range increase in mean log cortisol at bedtime (odds ratio, 2.2; 95% confidence interval, 1.09–4.09) and maternal obesity (odds ratio, 3.43; 95% confidence interval, 1.26–9.35) were independently associated with wheeze. Linear mixed models revealed an association between a flatter afternoon slope (slower decline in log cortisol per hour) and repeated wheeze in children of obese mothers (children with [–0.017 change] and without [–0.061 change] wheeze [$P = 0.009$ for time $\times$ wheeze interaction]), but not in children of nonobese mothers (with [–0.050 change] and without [–0.061 change] wheeze [$P = 0.51$]).

Conclusions: Maternal prenatal cortisol disruption and obesity were independently associated with children's wheeze. Obese women with adverse cortisol profiles were most likely to have children with repeated wheeze.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Disruption of the maternal hypothalamic–pituitary–adrenal (HPA) axis may influence fetal development, but this has not been well studied for pediatric respiratory disorders. The HPA axis may play a role in the link between obesity and wheeze.

What This Study Adds to the Field

This study reveals associations between altered prenatal maternal HPA axis functioning and repeated wheeze in children. Maternal obesity and prenatal cortisol disruption had independent effects on children's repeated wheeze suggesting that these factors contribute through different mechanisms. Obese women with adverse cortisol profiles were most likely to have children with repeated wheeze. These findings inform the understanding of programming mechanisms contributing to child wheeze beginning in pregnancy.

Keywords: prenatal programming; obesity; maternal cortisol; childhood wheeze

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Evidence links prenatal stress (1) and stress correlates (i.e., maternal psychological functioning) to childhood wheeze and asthma (2, 3). Research identifying underlying mechanisms remains sparse. Based largely on animal data, it is postulated that maternal prenatal stress influences fetal development through disruption of key regulatory systems with significant focus on the hypothalamic–pituitary–adrenal (HPA) axis indexed by altered cortisol production (4). Maternal HPA axis disruption may contribute to programming of infant respiratory disease directly through trans-placental passage of maternal hormones (e.g., cortisol) and/or indirectly by impacting maternal-fetal immune function (5, 6). Consequent immunomodulation, in particular an enhanced proinflammatory state, may influence fetal development and disease risk.

Altered prenatal cortisol production, particularly elevated levels later in the day, is associated with adverse child outcomes including low birthweight and poorer cognition (7–9). Beijers and colleagues (10) recently linked prenatal maternal anxiety and infant respiratory illnesses (a composite measure including asthma, cough, upper respiratory infection, and so forth). At 37 weeks gestation, mothers provided saliva to assess cortisol rhythms, a correlate of the maternal stress response. Higher evening cortisol and a flattened diurnal rhythm (i.e., smaller decline in cortisol over the day) were related to more infant respiratory illness.

Maternal obesity before and during pregnancy is also associated with infant wheeze (11) and childhood asthma (12–14). Although underlying mechanisms are poorly understood, altered neuroendocrine and immune function in the context of the obese state may be central (15). Numerous studies demonstrate relationships between HPA axis functioning, cortisol metabolism, and obesity (16–18). Enhanced systemic and placental inflammation is seen in obese pregnant women (19, 20). Plasma C-reactive protein and interleukin-6 are elevated in obese pregnant women and obesity is associated with increased proinflammatory cytokine expression in the placenta (20). Adipose tissue produces proinflammatory adipokines and chemokines that may influence fetal development including immunomodulation (21) and contribute to airway inflammation and hyperresponsiveness in children (22). These data suggest a role for HPA axis functioning in the link between obesity and wheeze phenotypes or possibly an interactive effect if both contribute to a proinflammatory immune milieu, albeit through different mechanisms (23–25).

We examined associations among maternal prenatal HPA axis functioning, maternal obesity, and repeated wheeze in children followed to age 2 years. We hypothesized that prenatal HPA axis disruption (particularly adverse profiles seen in other studies, such as slower decline over the day and elevated levels late in the day) and maternal obesity would be associated with an increased likelihood of children's repeated wheeze, when considered independently. Because studies link disrupted HPA functioning with obesity, we also examined whether including these factors together would attenuate effects of maternal obesity on wheeze or whether independent associations would remain. Finally, given that mechanisms linking cortisol profiles and obesity to wheeze are complex and may not necessarily completely overlap (26), we tested possible interactive effects of obesity and disrupted cortisol on wheeze. Exploring these relationships may inform mechanisms underlying prenatal programming. Some results from these analyses were presented at the American Thoracic Society International Conference (27).

METHODS

Participants

Participants were from the Asthma Coalition on Community, Environment, and Social Stress project, a prospective pregnancy cohort designed to study effects of prenatal maternal and early life stress and other environmental risk factors on childhood asthma risk (28). Briefly, 500 English- or Spanish-speaking women at least 18 years of age receiving prenatal care at Brigham and Women's Hospital, Boston Medical Center, three community health centers, and affiliated Women, Infants and Children programs were recruited between August 2002 and January 2007. Staff approached women on select clinic days; 78.1% of those eligible agreed to enroll. All women approached to participate completed a screener questionnaire including race and ethnicity, education, and income; there were no significant differences on covariates between eligible women who participated and those who declined. Supplemental funding allowed for cortisol collection in a subset of 375 women invited to participate. Thirty-eight were excluded because of shift work, exogenous steroid use, or nonsingleton pregnancy because these influence cortisol (29). Forty-two were too busy and 20 were unable to complete the collection. After exclusions for missing data on covariates ($n = 14$), 261 women were available for analysis. Women providing cortisol did not differ on covariates from study women who did not participate (see Table E1 in the online supplement). Study activities were approved by the relevant human studies committees; written consent was obtained.

Maternal Cortisol

Pregnant women provided saliva (29.0 ± 4.9 wk gestation) at home by the passive drool technique on 3 typical weekdays within a single week

(30–32). Participants received verbal and written instructions not to eat, drink, or brush their teeth for 15 minutes before sampling and used daily diaries to record protocol adherence. Women provided five samples each day: at awakening (“when your eyes open”), 45 minutes after awakening, approximately 4 and 10 hours after awakening, and “right before getting into bed,” recording time of awakening and date and time for each sample. Samples were refrigerated until pickup and then stored at -70°C until assay. Cortisol was measured using a chemiluminescence assay with a sensitivity of 0.16 ng/ml (IBL, Hamburg, Germany). Interassay and intraassay coefficients of variation were less than 8%.

Repeated Wheeze

Maternal-reported child wheeze was ascertained from birth to age 2 years at approximately 3-month intervals. Mothers were asked, “Since we last spoke with you on (date), has your infant/child had wheezing or whistling in the chest?” Repeated wheeze was defined as two or more episodes (33). Of 261 children, 169 (64.8%) never wheezed, 70 (26.8%) wheezed once, and 9 (3.4%), 9 (3.4%), and 4 (1.5%) had two, three, and four wheeze episodes, respectively.

Maternal Body Mass Index

Maternal body mass index (BMI) was calculated from height and pre-pregnancy weight reported at enrollment and categorized as obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) versus nonobese ($\text{BMI} < 30 \text{ kg/m}^2$) (34). An internal validation study comparing self-report and measured height and weight available in 121 women assessed early in pregnancy (<10 wk) showed good agreement across all levels of height and weight based on Bland-Altman plots (see Figure E1) (35). On average, participants underestimated weight by 0.34 kg and overestimated height by 0.97 cm resulting in underestimation of BMI by 0.097 kg/m^2 .

Covariates

Covariates were categorized as in Table 1 and included variables previously associated with children's wheeze and/or cortisol disruption in women including maternal age, race and ethnicity, education, atopic history (clinician-diagnosed asthma, eczema, and/or hay fever), and smoking status and child's sex and birthweight ascertained by questionnaire. Because cortisol levels increase throughout pregnancy (36), analyses were adjusted for gestational age at time of saliva collection.

Analysis

Relationships between wheeze and covariates were examined using the Pearson chi-square statistic to test differences in proportions and logistic regression for continuous variables.

We examined cortisol levels at each collection time point separately and summary measures including cortisol morning (AM) rise (CAR), diurnal slope over the waking day, and afternoon (PM) slope (37). Cortisol values at each sampling time were log transformed because of nonnormality and then averaged over the 3 sampling days. To limit variation between collection times, we *a priori* restricted analyses to samples taken during defined time windows: at awakening (Time 1); 30 minutes to 1.5 hours after awakening (Time 2); between 4 and 6.5 hours after awakening (Time 3); 7.5–11.5 hours after awakening (Time 4); and before bedtime (>11.5 h after awakening; Time 5) (38). The 261 participants provided 3,643 (93.1%) samples with adequate volume for assay; another 216 were excluded for falling outside sampling time windows, leaving 3,427 (87.5%) samples for analyses. CAR was calculated as the difference between Time 2 and Time 1 measurements. Diurnal slope was calculated as the least squares regression line fit through the cortisol measures versus hours since awakening after removing the Time 2 measurement because of its sensitivity to timing and high variability. The PM slope was calculated as the least squares regression line from Time 3 to Time 5.

Linear regression models were implemented to examine associations between maternal BMI or obesity status and each cortisol index entered in a separate model (cortisol measurements at Time 1, Time 2, Time 3, Time 4, and Time 5; CAR; diurnal slope; and PM slope), adjusting for maternal age, race, education level, and gestational age at saliva collection.

TABLE 1. DESCRIPTIVE CHARACTERISTICS

Categorical Covariates	All Participants (n = 261) n (%)	Repeated Wheeze Status		P*
		No (n = 239) n (%)	Yes (n = 22) n (%)	
Boys	128 (49.0%)	114 (47.7%)	14 (63.6%)	0.18
Maternal prepregnancy obesity (BMI ≥ 30 kg/m ²)	86 (33.5%)	74 (31.5%)	12 (54.5%)	0.03
Maternal race				0.60
Hispanic	148 (56.7%)	134 (56.0%)	14 (63.6%)	
Black	63 (24.1%)	57 (23.8%)	6 (27.3%)	
White/other	50 (19.1%)	48 (20.2%)	2 (9.1%)	
Maternal education ≤12 yr	159 (61.0%)	144 (60.3%)	15 (68.2%)	0.48
Prenatal smoking†	46 (17.6%)	43 (18.0%)	3 (13.6%)	0.77
Maternal atopy‡	94 (36.0%)	85 (35.6%)	9 (40.9%)	0.65
Continuous Covariates	Mean ± SD	Mean ± SD	Mean ± SD	P§
Maternal prepregnancy BMI, kg/m ²	28.5 ± 6.4	28.3 ± 6.3	30.6 ± 6.5	0.12
Maternal enrollment age, yr	27.0 ± 5.9	27.1 ± 5.9	25.9 ± 5.4	0.36
Weeks of pregnancy at saliva sampling	29.0 ± 4.9	29.0 ± 4.8	29.4 ± 5.7	0.72
Birthweight (z value for gestational age)	−0.26 ± 1.1	−0.27 ± 1.1	−0.14 ± 1.4	0.61
Cortisol Measures	Mean ± SD	Mean ± SD	Mean ± SD	P*
Time 1 (awakening, µg/dl)	2.64 ± 0.45	2.63 ± 0.46	2.75 ± 0.37	0.25
Time 2 (0.5–1.5 h after awakening, µg/dl)	2.70 ± 0.49	2.70 ± 0.50	2.66 ± 0.41	0.68
Time 3 (4–6.5 h after awakening, µg/dl)	2.23 ± 0.45	2.23 ± 0.46	2.20 ± 0.41	0.72
Time 4 (7.5–11.5 h after awakening, µg/dl)	1.80 ± 0.51	1.78 ± 0.50	1.95 ± 0.57	0.17
Time 5 (before bedtime, µg/dl)	1.58 ± 0.61	1.55 ± 0.60	1.83 ± 0.58	0.05
CAR (difference between Time 2 and Time 1, µg/dl)	0.05 ± 0.46	0.07 ± 0.46	−0.10 ± 0.43	0.12
Diurnal slope (over entire day)	−0.07 ± 0.05	−0.07 ± 0.05	−0.06 ± 0.05	0.19
PM slope (from Time 3–5)	−0.06 ± 0.06	−0.06 ± 0.06	−0.03 ± 0.06	0.05

Definition of abbreviations: BMI = body mass index; CAR = cortisol morning rise.

*Chi-square tests comparing wheeze and nonwheeze groups for categorical variables.

†Mothers reported smoking at baseline and in the third trimester; women were classified as prenatal smokers if smoking at either visit.

‡Self-reported clinician-diagnosed asthma, eczema, and/or hay fever.

§Logistic regressions comparing wheeze and nonwheeze groups for continuous variables.

Next, each cortisol metric was fit individually in a logistic regression model to predict the binary repeated wheeze outcome. These models were run unadjusted and adjusted for race, education level, maternal atopy, smoking during pregnancy, child sex, obesity (BMI ≥ 30 kg/m²), and birthweight adjusted for gestational age (39). Odds ratios (ORs) of wheezing for an interquartile range (difference between the 75th percentile and 25th percentile) increase in the cortisol predictor were calculated. Separate logistic models were run to examine the association between maternal obesity and repeated wheeze adjusting for covariates other than cortisol. We next ran logistic models considering cortisol measures and obesity status together to see if there were independent effects of obesity and cortisol on repeated wheeze or if the addition of cortisol indices attenuated associations between maternal anthropometric measures and child wheeze (i.e., was cortisol disruption in the pathway).

We also explored patterns of cortisol production over the course of the day among women with and without children with repeated wheeze using linear mixed models (LMMs). The models specified the cortisol measures as the response and wheeze as the primary predictor, and contained a random subject-specific intercept to account for within individual correlation in repeated cortisol measures. Models included gestational age at time of saliva collection (centered at the mean). Splines were added to capture the morning rise and the rate of decline during the day. Using visual inspection of the data and biologic knowledge of diurnal cortisol rhythms, a knot was placed at 30 minutes and at 4 hours after awakening. To examine the association between child wheezing status and maternal cortisol trajectory, wheezing status was added along with interaction terms formed by multiplying this wheezing indicator by all time variables (the main effect of time and each spline term) in the model. We next stratified by maternal obesity to see if the association between maternal cortisol profiles and children's repeated wheeze differed based on mother's obesity status. We also used logistic regression to examine the interaction between maternal cortisol metrics (each entered in a separate model) and obesity on the association with repeated wheeze. Most analyses were done using SAS version 9.0 (SAS Institute, Cary, NC). LMMs were implemented in R statistical package (version 2.13.0, Vienna, Austria).

RESULTS

Mothers were primarily ethnic minority (57% Hispanic, 24% African American), low socioeconomic status (61% having ≤12 years of education), and nonsmokers (82%); 34% of mothers were obese and 8.4% of children had repeated wheeze (Table 1). Mean maternal cortisol measures are shown in Table 1. On average, cortisol followed a typical diurnal rhythm with higher levels in the morning that peak approximately 45 minutes after awakening, dropping rapidly and then declining more slowly over the remainder of the day (30, 31).

Maternal BMI and Obesity and Prenatal Cortisol

Associations among BMI, maternal obese status, and cortisol parameters are summarized in Table 2. Increased BMI was associated with lower cortisol levels at Times 1, 2, and 3; obesity status was similarly associated with lower levels at Times 1 and 3. Higher BMI was also associated with flatter diurnal ($P = 0.07$) and PM slope ($P = 0.05$) measures (i.e., slower decline in cortisol over the day), albeit the former did not quite reach statistical significance.

Maternal Prenatal Cortisol and Children's Wheeze

Table 3 shows associations between maternal cortisol metrics and children's repeated wheeze levels. Results are shown for unadjusted and fully adjusted models. Higher maternal cortisol levels measured at Time 5 (bedtime) were most strongly associated with children's repeated wheeze status (OR, 2.21; 95% confidence interval [CI], 1.20–4.11; corresponding to the effect of an interquartile range increase in log-transformed cortisol), adjusting for covariates. In addition, PM slope was associated with repeated wheeze in the adjusted model, indicating that

TABLE 2. ASSOCIATIONS BETWEEN MATERNAL ANTHROPOMORPHIC MEASURES AND PRENATAL LOG CORTISOL METRICS*

Body Size Measurement	Cortisol Measurement	Difference in Log Cortisol Associated with IQR Increase in BMI [†]	SE	P Value
BMI	Time 1 (awakening)	−0.12	0.04	0.01
	Time 2 (0.5–1.5 h after awakening)	−0.11	0.05	0.02
	Time 3 (3–6.5 h after awakening)	−0.11	0.04	0.01
	Time 4 (7.5–11.5 h after awakening)	0.05	0.05	0.39
	Time 5 (before bedtime)	−0.01	0.06	0.80
	CAR (morning rise)	0.01	0.05	0.77
	Diurnal slope (over entire day)	0.01	0.00	0.07
	PM slope (from Time 3–5)	0.01	0.01	0.05
Obesity	Time 1 (awakening)	−0.19	0.07	0.01
	Time 2 (0.5–1.5 h after awakening)	−0.16	0.07	0.04
	Time 3 (3–6.5 h after awakening)	−0.21	0.07	0.002
	Time 4 (7.5–11.5 h after awakening)	−0.01	0.08	0.87
	Time 5 (before bedtime)	−0.15	0.09	0.10
	CAR (morning rise)	0.03	0.07	0.66
	Diurnal slope (over entire day)	0.005	0.01	0.50
	PM slope (from Time 3–5)	0.01	0.01	0.44

Definition of abbreviations: BMI = body mass index; CAR = cortisol morning rise; IQR = interquartile range.

*Each model adjusted for maternal age, race, education level, and gestational age at saliva collection.

[†] For BMI, it corresponds to an IQR increase in BMI (IQR = 8.7 kg/m²; 23.7–32.4 kg/m²). For obesity, it corresponds to obesity (BMI ≥ 30 kg/m²) versus nonobesity (BMI < 30 kg/m²).

mothers with a flatter PM cortisol slope (slower decrease over the afternoon and evening) were more likely to have children with repeated wheeze. Other cortisol metrics were not associated with repeated wheeze. This table also shows remarkable consistency in effect estimates and confidence bounds comparing the unadjusted and adjusted models suggesting that results are robust to control for a number of covariates without concern for over fitting the models.

Maternal Obesity and Children's Wheeze

Maternal prepregnancy obesity (BMI ≥ 30 kg/m²) was associated with increased odds of having a child with repeated wheeze in the unadjusted model (OR, 2.61; 95% CI, 1.08–6.32); this association fell just below the 95% CI after adjusting for all covariates other than cortisol measures (OR, 2.56; 95% CI, 0.99–6.60) (Table 4; Model 0). We next added the obesity indicator and cortisol indices in the same models, adjusting for other covariates (Models 1–8). If altered cortisol production, indexed by any of the metrics, was on the causal path between maternal obesity and child wheeze, we would expect the effect estimate for obesity to be reduced, assuming that there are no

unmeasured confounders of the cortisol-wheeze associations. Notably, the effect estimate for obesity remained significant and the magnitude of effect was remarkably constant when adjusting for the summary indicators and was actually stronger when bedtime (Time 5) cortisol was included. Moreover, analyses also showed independent effects for a flatter PM cortisol slope and higher bedtime cortisol levels in association with repeated wheeze.

Interactions between Cortisol and Obesity

Stratified analyses using LMMs demonstrated that the relationship between disrupted maternal cortisol production and children's repeated wheeze was most evident in obese pregnant women. Figure 1 shows that for nonobese mothers, the cortisol trajectories were similar throughout the day, regardless of the children's wheeze status (−0.050 change and −0.061 change for children with and without wheeze, respectively [*P* for slope change = 0.51]). In the obese strata, children born to mothers with a flatter PM slope were more likely to have repeated wheeze (−0.017 change and −0.061 change for children with and without wheeze, respectively [*P* for slope difference = 0.009]).

TABLE 3. ODDS RATIO (95% CI) OF REPEATED WHEEZE IN CHILDREN IN RELATION TO MATERNAL PRENATAL CORTISOL METRICS*

Predictor	Unadjusted		Adjusted [†]	
	OR	95% CI	OR	95% CI
Cortisol measures [‡]				
Time 1 (awakening)	1.74	(0.78–2.67)	1.71	(0.82–3.51)
Time 2 (0.5–1.5 h after awakening)	0.79	(0.48–1.31)	0.83	(0.47–1.48)
Time 3 (4–6.5 h after awakening)	0.86	(0.52–1.43)	0.94	(0.52–1.67)
Time 4 (7.5–11.5 h after awakening)	1.41	(0.76–2.62)	1.58	(0.81–3.10)
Time 5 (before bedtime)	1.71	(1.00–2.95)	2.21	(1.20–4.11)
CAR (difference between Time 2 and Time 1)	0.73	(0.55–0.99)	0.71	(0.50–1.00)
Diurnal slope (over entire day)	1.56	(0.81–2.99)	1.72	(0.83–3.57)
PM slope (from Time 3–5)	1.56	(0.87–2.81)	1.65	(1.00–2.74)

Definition of abbreviations: CAR = cortisol morning rise; CI = confidence interval; OR = odds ratio.

*Each row is a separate model.

[†] Adjusted for maternal age, race, education, prenatal smoking, maternal atopy, weeks of pregnancy at saliva sampling, child's sex, birthweight adjusted for gestational age, and maternal prepregnancy BMI.

[‡] Corresponds to an interquartile range increase in each cortisol metric (log scaled). The 25th to 75th percentile for each cortisol metric was 2.41 to 2.93 for Time 1, 2.41 to 3.00 for Time 2, 1.98 to 2.51 for Time 3, 1.43 to 2.15 for Time 4, 1.22 to 1.97 for Time 5, −0.22 to 0.32 for CAR, −0.11 to −0.03 for diurnal slope over entire day, and −0.10 to −0.03 for afternoon slope.

TABLE 4. ODDS RATIO (95% CI) OF REPEATED WHEEZE IN MODELS INCLUDING MATERNAL PREPREGNANCY OBESITY AND CORTISOL METRICS*

Model†	Predictor	OR	95% CI
Model 0	Obesity	2.56	(0.99–6.60)
Model 1	Time 1 cortisol (awakening)	1.84	(0.91–3.74)
	Obesity	3.44	(1.19–10.0)
Model 2	Time 2 (0.5–1.5 h after awakening)	1.11	(0.61–2.04)
	Obesity	2.85	(1.01–8.04)
Model 3	Time 3 (4–6.5 h after awakening)	0.96	(0.54–1.72)
	Obesity	2.76	(0.96–7.97)
Model 4	Time 4 (7.5–11.5 h after awakening)	1.51	(0.74–3.09)
	Obesity	2.96	(1.02–8.57)
Model 5	Time 5 cortisol (bedtime)	2.20	(1.19–4.09)
	Obesity	3.43	(1.26–9.35)
Model 6	CAR (morning rise)	0.71	(0.51–1.01)
	Obesity	2.69	(1.02–7.06)
Model 7	Diurnal slope (over entire day)	1.70	(0.81–3.53)
	Obesity	2.71	(1.03–7.12)
Model 8	PM slope (Time 3–5)	1.81	(1.03–3.16)
	Obesity	2.65	(1.01–6.95)

Definition of abbreviations: CAR = cortisol morning rise; CI = confidence interval; OR = odds ratio.

*Corresponds to an IQR increase in each cortisol metric (log scaled). The 25th to 75th percentile for each cortisol metric was 2.41 to 2.93 for Time 1, 2.41 to 3.00 for Time 2, 1.98 to 2.51 for Time 3, 1.43 to 2.15 for Time 4, 1.22 to 1.97 for Time 5, –0.22 to 0.32 for CAR, –0.11 to –0.03 for diurnal slope over entire day, and –0.10 to –0.03 for afternoon slope.

†Each model also adjusted for maternal age, race, education, prenatal smoking, maternal atopy, weeks gestation at saliva sampling, child's sex, and birthweight adjusted for gestational age.

Because the outcome is log cortisol, we can also interpret these coefficients in terms of percent change (e.g., –0.017 vs. –0.061 coefficients for time on the log cortisol scale correspond to a –1.7% and –5.9% change in cortisol per hour, respectively).

In logistic regression models examining the interaction between individual maternal cortisol measures and prepregnancy obesity on the association with repeated wheeze, the interaction was significant between Time 5 (bedtime) cortisol and obesity ($P = 0.02$). Interactions between obesity and a flattened cortisol slope over the day (diurnal slope) (P for interaction = 0.13) and afternoon (PM slope, Times 3–5) (P for interaction = 0.13) were suggestive albeit did not reach statistical significance at the $P = 0.05$ level.

DISCUSSION

These analyses corroborate studies showing an association between increased maternal BMI or obesity and childhood wheeze

(11) or asthma (12–14), and make three novel contributions to the literature. To our knowledge, these are the first population-based data examining associations between prenatal maternal HPA axis functioning and repeated childhood wheeze. Independent effects of maternal obese status and indicators of prenatal cortisol disruption on children's wheeze shown in models including only main effects (no interaction term) suggest that the pathways linking maternal obesity and *in utero* HPA axis disruption to child wheeze operate, at least in part, through different mechanisms. Stratified analyses and models including interaction terms suggested that prenatal cortisol associations with child wheeze occur only among obese mothers.

Mechanisms linking prenatal stress to early childhood wheeze and asthma remain poorly elucidated. These findings suggest a role for maternal cortisol disruption during pregnancy in programming childhood wheeze. The HPA axis is a major component of the stress response. Disturbed regulation of maternal stress systems in pregnancy, in particular the HPA axis, may

Figure 1. Maternal cortisol trajectories comparing children with and without repeated wheeze, stratified by maternal obesity status (*left*, nonobese mothers; *right*, obese mothers), linear mixed model analysis. Linear mixed models with a random intercept were fit to assess associations between children's repeated wheeze and maternal cortisol trajectories, assuming that repeated cortisol measurements within individuals are correlated. All models were adjusted for gestational age at time of saliva sampling. Children's repeated wheeze status was added to the model and interacted with each of the slope terms in the model. The P values for comparisons between children with and without repeated wheeze for obese mothers: morning slope (over first 4 h) $P = 0.14$, afternoon slope (from 4 h to bedtime) $P < 0.01$. For nonobese mothers: morning slope $P = 0.97$, afternoon slope $P = 0.67$. Dashed and solid lines represent mean log cortisol values and the shading and dotted lines outline the 95% confidence bounds.

modulate offspring immune function starting *in utero* (40). Maternal glucocorticoid effects on the placenta and more directly on the fetus play a role in immunomodulation and fetal lung development (5, 6, 41). Stress-induced alterations in maternal cortisol may influence fetal immune development, possibly through influence of stress hormones on cytokine production (42), with selective suppression of Th1-mediated cellular immunity and enhanced Th2-mediated humoral immunity (42, 43). HPA axis disruption may also contribute to enhanced placental oxidative stress (44) and programming of autonomic imbalance in offspring (45, 46), which are linked to early wheeze and immunomodulation (6). Notably, although altered glucocorticoid production in the mother may influence fetal growth (8) and low birthweight is linked to early wheeze, adjusting for birthweight did not change relationships between cortisol and wheeze in these analyses.

Although there are inconsistencies with regard to the physiologic changes that are associated with various disease outcomes, a blunted HPA axis (characterized by flattening of the diurnal slope) has been specifically associated with increased autoimmune and inflammatory disease susceptibility (47, 48). Similar to Beijers and coworkers (10), we found that profiles including increased cortisol levels late in the day and a flattened slope were associated with childhood respiratory outcomes. We extend these findings because our study more specifically considers repeated wheeze, which may be a precedent of asthma and reduced lung function (49) as opposed to a more nonspecific composite of respiratory symptoms and outcomes that may have differing underlying mechanisms.

Obesity was related to cortisol as seen in studies of nonpregnant women. Increased BMI was associated with decreased AM cortisol and a flatter slope, consistent with Kumari and coworkers (18). Larsson and coworkers (17) found an association between increased waist/hip ratio and a flatter diurnal slope. Notably, peripheral body fat distribution has been associated with lower nocturnal cortisol, whereas visceral adiposity (increased waist/hip ratio) has been associated with higher nocturnal cortisol (50), and visceral adiposity is more likely related to chronic stress. Central or visceral adiposity also seems to be most associated with asthma (22). We do not have information on fat distribution in these women and thus were unable to examine this further.

Interrelationships observed in these analyses between maternal obesity, prenatal cortisol, and wheeze raised, perhaps, the most interesting questions to be considered in ongoing work. Whereas it has been proposed that the metabolic consequences of obesity may contribute to HPA axis disruption and this may be a pathway linking obesity to chronic disease (24, 51), these analyses did not support this scenario. Rather, obesity and indices of cortisol disruption were independently associated with wheeze. Our data supported an interaction between maternal cortisol production *in utero* and maternal obesity in relation to early childhood wheeze (i.e., women who were obese and had the more adverse cortisol profiles with elevated levels later in the day were most likely to have children with repeated wheeze). What underlies the observed interaction is not clear. Both states may independently enhance a proinflammatory immune milieu or an oxidative state that influences the programming of the child's propensity to develop airway inflammation and/or hyperresponsiveness. Both maternal obesity and HPA axis disruption may contribute to placental oxidative stress (19, 44) and programming of autonomic imbalance (45, 46, 52, 53) in offspring, which in turn influence early wheeze and immunomodulation (6). Because they may operate through overlapping pathways, this may contribute to synergistic effects. Obesity is also associated with metabolic changes that result in intracellular

amplification of active glucocorticoid metabolism resulting in local glucocorticoid excess, a process that may further potentiate immunomodulatory effects of systemic cortisol (54). Thus, when both conditions are present, one may potentiate the effects of the other.

Strengths of this study include the prospective design, the reasonably large lower socioeconomic status, ethnically mixed cohort, and consideration of important confounders. This is the first study to examine effects of HPA axis disruption in pregnant women on wheeze assessing cortisol rhythms through repeated measures over multiple days. We acknowledge that use of maternal-reported wheeze may be a limitation albeit it has been shown to be a reasonable surrogate for those who may be more likely to develop asthma or have compromised lung function (55). Nonetheless, it is important to examine relationships among maternal HPA axis disruption, prepregnancy obesity, and more definitive outcomes as these children grow (i.e., immunophenotypes, physician-diagnosed asthma, lung function) and see if relationships hold. For example, it is interesting to know whether children of mothers with a blunted prenatal cortisol response are more likely to develop asthma. It is also important to elucidate how intervening processes may affect these relationships, including how maternal-fetal endocrine indicators of stress (e.g., prenatal cortisol), prenatal and postnatal maternal psychological functioning, and postnatal caregiving behaviors impact the infant's developing stress response systems (i.e., HPA axis, autonomic functioning, immune function) and ultimately examine whether the associations between maternal cortisol disruption *in utero* and child wheeze and asthma is, in part, mediated through effects on child stress physiology. The number of children with repeated wheeze was relatively small in this sample; however, effect estimates were consistent comparing adjusted and unadjusted models even when controlling for a number of covariates suggesting robust findings not subject to over fitting the models. As in any observational study and given the somewhat wide confidence bounds around significant associations, these findings warrant replication in future studies with increased sample size. The sample size may have also impacted our power to fully examine interactions. Although self-reported height and weight may be subject to nondifferential misclassification, internal validation demonstrated good agreement between self-reported and measured values. Moreover, observed differences between reported and measured values add variability to the analysis that would be expected to diminish the chance of finding an association. Nonetheless, direct measurement of height and weight will enhance precision.

These data support a role for maternal HPA axis disruption *in utero* in programming childhood wheeze. Cortisol disruption did not seem to operate in the path between maternal obesity and child wheeze. Perhaps most intriguing, obesity and HPA axis disruption acted synergistically to influence early childhood wheeze. Obesity (56) and stress correlates (57) including measureable cortisol disruption in pregnancy (36, 58) are more prevalent in lower income women of childbearing age. Thus, continued exploration of the links between stress, maternal obesity, and HPA axis disruption in pregnancy in relation to childhood wheeze and asthma may be particularly relevant for urban, ethnic minority populations of lower socioeconomic status who are particularly burdened by these interrelated adverse health conditions.

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