Original Research Article

Maternal Cortisol Disproportionately Impacts Fetal Growth in Male Offspring: Evidence from the Philippines

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Objectives: Lower birth weight (BW) reoccurs across generations, but the intermediate mechanisms remain poorly understood. One potential pathway involves cortisol, which may be elevated in women born small and in turn could lead to fetal growth restriction in offspring. To test this possibility, we evaluated whether BW predicts hypothalamic-pituitary-adrenal (HPA) function in the nonpregnant state in a cohort of young Filipino women, and whether differences in HPA function predict offspring BW.

Methods: Multiple regression relating maternal BW, adult salivary cortisol profiles and recalled offspring BW ($N = 488$) among participants of the Cebu Longitudinal Health and Nutrition Survey.

Results: Maternal BW related inversely to evening cortisol in adulthood ($P < 0.04$). Maternal BW and evening cortisol were both stronger predictors of male than of female BW (maternal BW: $P < 0.0001$ for males; $P = 0.07$ for females; bedtime cortisol: $P = 0.003$ for males; $P = 0.3$ for females). Waking and 30-min postwaking cortisol did not predict offspring BW. Controlling for evening cortisol did not diminish the relationship between maternal and offspring BW in males or females.

Conclusions: Being born small predicted higher evening cortisol in adulthood among these young mothers. Lower maternal BW and elevated evening cortisol independently predicted giving birth to lower BW offspring, with effects greatest and only significant among males. We speculate that sex differences in sensitivity to maternal stress hormones could help explain the stronger relationships between BW and cardiovascular disease (CVD) risk factors reported among the males in this and other populations. Am. J. Hum. Biol. 24:1–4, 2012.   © 2011 Wiley Periodicals, Inc.

It is well recognized that birth weight (BW) tends to be similar in mother and offspring independent of genetic heritability (Ounsted and Ounsted, 1968). Although the underlying mechanisms remain poorly understood, modifications to maternal hypothalamic-pituitary-adrenal (HPA)-axis function is one plausible contributing factor (Drake and Walker, 2004). The hormone cortisol is the primary product of the HPA-axis in humans and helps coordinate physiologic responses to various forms of environmental challenge. Although the fetus is largely shielded from maternal cortisol through the action of placental enzymes, this buffering is incomplete, thus allowing some maternal cortisol to reach the fetus (Seckl, 2004). Elevated maternal cortisol is associated with reduced offspring birth size (Pike, 2005). Strikingly, individuals born small often have altered HPA-axis function in adulthood (Entringer et al., 2009; Reynolds et al., 2005).

Because the gestational environment shapes adult HPA-axis function, and adult HPA-axis function influences the gestational environment experienced by the next generation, variation in HPA-axis set points established in early development could help explain the tendency for lower BW to be perpetuated across generations (Drake and Walker, 2004; Kuzawa and Sweet 2009). To test this model, we evaluated the relationship between maternal and offspring BW in a large multigenerational cohort from Cebu City, Philippines, and explored the role of maternal salivary cortisol in this relationship. To evaluate the role of stable, trait-level variation in female HPA-axis function, we limited analyses to parous women, whose saliva was obtained outside of pregnancy, allowing us to avoid effects of the temporary and marked regulatory changes in the HPA-axis during pregnancy (Wadhwa, 2005). Because past work in this sample identified sex differences in later life outcomes related to early growth rate (Kuzawa and Adair, 2003; Kuzawa et al., 2010), we also evaluated whether these relationships differed by sex of offspring.

METHODS

Data were obtained from the Cebu Longitudinal Health and Nutritional Study (CLHNS), which enrolled 3,327 pregnant women who gave birth to 3,080 singleton, live-born offspring from 1983–1984 in Cebu City, Philippines (Adair et al., 2011). The present study included 488 singleton live-born offspring born to the 308 female members of the original birth cohort who were parous by the time of questionnaire and sample collection and had all variables available (Table 1). Reproductive histories recorded birth date, sex, and recalled BW and length of gestation for each offspring among study women in 2005, 2007, and 2009.

TABLE 1. *Characteristics of mothers and offspring*

Variable	All (N = 308) Mean (SD)	Range	Low evening cortisol (N = 154) Mean (SD)	Range	High evening cortisol (N = 154) Mean (SD)	Range	P^a
Maternal characteristics							
Age (years)	21.5 (0.3)	(20.8, 22.3)	21.5 (0.31)	(20.8, 22.3)	21.5 (0.3)	(20.8, 22.3)	0.35
Birth weight (g)	2,984 (407)	(1,814, 4,100)	3,010 (376)	(1,956, 4,082)	2,958	(1,814, 4,100)	0.26
Weight (kg)	47.2 (7.9)	(30, 85)	48.8 (8.3)	(34, 85)	45.6 (7.0)	(30, 64)	0.0002
Height (cm)	150.6 (5.4)	(136.5, 167.1)	151.2 (5.4)	(139.8, 167.1)	149.9 (5.3)	(136.5, 162.8)	0.04
BMI (kg/m^2)	20.7 (3.0)	(14.6, 33.5)	21.3 (3.1)	(15.7, 33.5)	20.2 (2.8)	(14.6, 29.8)	0.0009
Underweight (%)[b]	23.1%		16.9%		29.2%		0.010
Overweight (%)[c]	8.1%		10.4%		5.8%		0.14
Obese (%)[d]	0.01%		1.9%		0%		0.15
Cortisol							
Waking (nmol/l)	7.3 (3.8)	(0.4, 23.2)	6.7 (3.2)	(0.4, 18.7)	7.9 (4.2)	(0.9, 23.2)	0.003
30-min post waking (nmol/l)	8.7 (4.5)	(0.4, 34.0)	8.0 (3.9)	(0.4, 23.6)	9.5 (4.9)	(0.6, 34.0)	0.01
Evening (nmol/l)	1.9 (2.2)	(0.1, 17.1)	0.6 (0.3)	(0.1, 1.1)	3.1 (2.5)	(1.1, 17.1)	0.08
Individual Pregnancies	(N = 488)		(N = 244)		(N = 244)		
Offspring birth weight (g)	3,032 (558)	(1,500, 4,500)	3,086 (552)	(1,591, 4,500)	2,979 (560)	(1,500, 4,455)	0.03
Offspring male (%)	53%		55%		50%		0.23
Born early (<9 months gestation)	6%		5%		7%		0.24
Primiparity (%)	56%		57%		55%		0.78

[a]Significance level for difference between women with high and low cortisol from two-sided *t*-tests for continuous variables and χ^2 for categorical variables.
[b]BMI ≤18.5.
[c]BMI ≥ 25 and <30.
[d]BMI ≥ 30.

Standard anthropometric techniques were used to measure standing height and weight in women. For each birth, maternal prepregnancy BMI was calculated using height and weight measured during the survey preceding that birth by at least 9 months. We defined underweight as a BMI ≤ 18.5; when women were younger than 18 years of age (N = 33), we defined underweight status using BMI cut-offs recommended by the Childhood Obesity Working Group of the International Obesity Taskforce using the zbmicat command in Stata (Cole et al., 2000). The mothers' BW was measured by hospital nurses or birth attendants trained in the use of Salter scales, and by survey interviewers during follow-ups. The mother's gestational age at delivery was estimated from the date of last menstrual period (see Adair et al., 2011). This research was conducted under conditions of informed consent, and with approval of the Institutional Review Boards of the University of North Carolina (Chapel Hill), Northwestern University (Evanston, Illinois), and the Office of Population Studies Foundation (Cebu, Philippines).

Cortisol measurement

Saliva samples were obtained at waking, 30 minutes after waking and immediately prior to bed (for protocol see Gettler et al., 2011). Cortisol was assayed in duplicate by a laboratory in Trier, Germany, using a time-resolved immunoassay with fluorometric detection (DELFIA). The intra assay and interassay coefficients of variation (CVs) were between 4.0 and 6.7% and 7.1–9.0%, respectively. Samples with CVs over 12% were rerun.

Statistical analysis

All analyses were conducted using Stata 10.1 (College Station, TX). Cortisol was log transformed and adjusted for time of saliva collection, usual wake time, exercise, and illness symptoms prior to saliva collection, with residuals used in models. Multiple regression was used to determine the relationship between maternal BW, cortisol and offspring BW with maternal BMI, age, recalled offspring age at parturition, primiparity, and mothers gestational age considered as covariates. In Stata, the regress command was used with the cluster option to account for nonindependence of BWs among multiparous women. While a total of 11 regressions were run, which could increase the risk of Type I error, we set alpha at 0.05 because many of the tests involved models built sequentially to test pathways and thus were not independent.

RESULTS

Maternal BW was a significant inverse predictor of bedtime cortisol measured in young adulthood (β = −50.5 ± 21.5 log-nmol/g, $P < 0.02$, $R^2 = 0.07$) but not of other cortisol measures (both $P > 0.12$). Maternal BW adjusted for gestational age was positively associated with recalled BW in male and female offspring, with effects stronger in males (males: β = 0.4 ± 0.09 g offspring BW/g mother's BW; $P < 0.001$, $R^2 = 0.16$; females: β = 0.2 ± 0.1 g/g, $P = 0.08$, $R^2 = 0.07$).

We next evaluated whether maternal HPA-axis function in young adulthood predicted offspring BW. Maternal bedtime cortisol was a significant, inverse predictor of offspring BW in males but not females (males: β = −90.6 ± 45.5 g/log-nmol/l; $P = 0.05$, $R^2 = 0.10$; females: β = −46.0 ± 43.8 g/log-nmol/l, $P = 0.3$, $R^2 = 0.04$; Fig. 1a). As a result, absolute sex differences in BW were reduced in half among individuals with above-median maternal bedtime cortisol compared with individuals born to mothers with below-median bedtime cortisol (Fig. 1b). Waking cortisol was a modest inverse but nonsignificant predictor of offspring BW in males and females (both $P > 0.1$), while 30-min postwaking cortisol was not associated with offspring BW (both $P > 0.6$).

Finally, we tested whether the higher evening cortisol among lower BW women might mediate the relationship between maternal BW and offspring BW. The relationship between maternal and offspring BW was essentially unchanged after adjusting for bedtime cortisol (males:
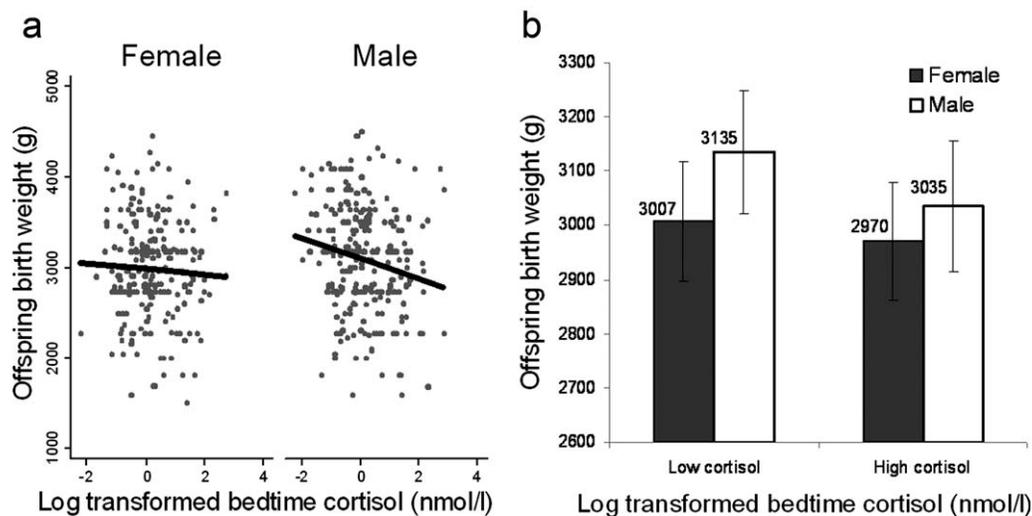
Fig. 1. (a) Linear trends relating maternal evening cortisol to gestational timing-adjusted offspring birth weight plotted separately for male and female offspring (see results for slopes); (b) The relationship between offspring birth weight and above- and below-median maternal bedtime cortisol, illustrating the reduction in sexual size dimorphism at higher levels of maternal cortisol.

maternal BW: $\beta = 0.4 \pm 0.1$ g/log-nmol/l, $P < 0.001$, bedtime cortisol: $\beta = -76.7 \pm 47.4$ g/log-nmol/l, $P = 0.1$, adjusted $R^2 = 0.18$; females: maternal BW $\beta = 0.2 \pm 0.1$ g/log-nmol/l, $P = 0.07$, bedtime cortisol: $\beta = -35.1 \pm 43.3$ g/log-nmol/l, $P = 0.4$, adjusted $R^2 = 0.08$).

DISCUSSION

We hypothesized that the gestational environment of the mother, as reflected in her own BW, would predict her adult HPA-axis function, and that this in turn would predict BW of her offspring. Consistent with this expectation, women born small did have significantly higher evening cortisol. Women who were born small had lower BW offspring, with disproportionate effects on males. Similarly, women with higher bedtime cortisol tended to give birth to smaller offspring, but this effect was stronger, and only significant, in males. Adjusting for bedtime cortisol had essentially no effect on the relationship between maternal and offspring BW, suggesting that a woman's own BW and HPA-axis function in adulthood are independent predictors of offspring BW.

The evidence we report for disproportionate effects of maternal cortisol on fetal growth in male offspring is a novel finding and could shed light on prior findings of the developmental predictors of male function and health. Notably, we previously reported that BW was a stronger predictor of blood pressure and cholesterol levels measured in this cohort during adolescence (Adair et al., 2001; Kuzawa and Adair, 2003), and that early postnatal weight gain disproportionately predicted adult size, muscle, and life history characteristics in males (Kuzawa et al., 2010). Among species with male-biased sexual size dimorphism (SSD), such as humans, SSD tends to be reduced in the context of marginal nutrition or environmental quality (Badyaev, 2002). It has been speculated that the faster growth rate of male fetuses, and the corresponding increased energy requirements, could make males more sensitive to maternal stressors (Kuzawa and Adair, 2003).

In summary, being born small and having elevated evening cortisol in young adulthood independently predict giving birth to smaller newborns in this population, with both effects strongest among male offspring. Although awaiting replication in other populations and with more intensive cortisol sampling protocols, these findings do not support a strong role of basal HPA-axis function as a pathway linking BW across generations. However, the evidence reported for increased male sensitivity to maternal stress hormones *in utero* could help explain our previous reports that relationships between BW and adult CVD risk factors are stronger among the males than in the females in this population. We hypothesize that these sex differences in prenatal sensitivity to maternal cortisol reflect past selection for traits related to SSD in humans.

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