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Short Communication

Maternal salivary cortisone to cortisol ratio in late pregnancy: An improved method for predicting offspring birth weight

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ABSTRACT

Overexposure to maternal cortisol in utero has been associated with lower birth weight of offspring. In order to regulate fetal exposure to this hormone, placental expression of 11-β-hydroxysteroid dehydrogenase-2, an enzyme that converts active cortisol to inactive cortisone, increases across pregnancy. Because of this increase in 11-βHSD2 activity, measuring maternal cortisol in isolation may not reflect actual fetal exposure to the hormone. Previous work by Hellgren et al. (2016) has shown that maternal serum cortisone:cortisol ratio was a better predictor of offspring birth weight than cortisol measured in isolation. This paper sought to replicate these results when examining maternal salivary cortisone:cortisol ratio. Data come from 55 pregnant women from Auckland, New Zealand. Cortisol and cortisone were measured in saliva samples collected at waking and prior to going to sleep on two consecutive weekdays between 34 and 36 weeks of gestation. We found that salivary cortisol and cortisone followed the expected diurnal rhythm and that cortisone was higher than cortisol at both times of day. Maternal bedtime cortisone:cortisol ratio was significantly and inversely related to offspring birth weight. However, waking and bedtime cortisol, as well as waking cortisone:cortisol ratio, were unrelated to birth weight. These results show that maternal salivary cortisone:cortisol ratio, like serum cortisone:cortisol ratio, is a more sensitive biomarker for predicting infant birth weight than cortisol measured in isolation. This ratio could be a valuable, minimally-invasive measurement for future studies interested in understanding the relationship between maternal HPA-axis function and offspring birth weight.

1. Introduction

Elevated maternal cortisol levels influence offspring phenotypic outcomes. Previous research has demonstrated that higher maternal cortisol affects hypothalamic-pituitary-adrenal axis function across generations (Constantinof et al., 2016), and is associated with reduced birth size (Field et al., 2006). The enzyme 11-β-hydroxysteroid dehydrogenase 2 (11-βHSD2) regulates fetal exposure to cortisol in the placenta by converting biologically active cortisol into inactive cortisone (Murphy et al., 1974).

The expression of 11-βHSD2, like maternal cortisol levels, increases during pregnancy (Meulenberg and Hofman, 1990). The enzyme 11-βHSD2 is expressed in several tissues, including the placenta and parotid (salivary) glands. Within the parotid gland, cortisone increases across pregnancy at a greater rate than cortisol levels (2–3 times increase versus 1–2.5 times, respectively), presumably due to increased 11-βHSD2 activity (Meulenberg and Hofman, 1990). During late pregnancy, 11-βHSD2 activity also increases in the placenta, where almost all maternal cortisol is converted into cortisone (Benediktsson et al., 1997). Due to this increase in 11-βHSD2 activity, examining maternal cortisol levels alone may not be the best index of fetal cortisol exposure. Instead, measuring cortisone:cortisol ratio (cortisone/cortisol), which takes into account 11-βHSD2 activity, may be a more sensitive biomarker (Ghaemmaghami et al., 2014). Past research examining both placental 11-βHSD2 activity and cord vein cortisone:cortisol ratio suggests that both measures are significant predictors of birth weight in preterm infants (Kajantie et al., 2003).

Hellgren et al. (2016) examined maternal serum cortisone:cortisol ratio in late pregnancy as a predictor of offspring birth weight. While they did not find a significant relationship between maternal serum cortisol and birth weight, they showed that serum cortisone:cortisol ratio had a weak but significant positive correlation with offspring birth weight in women with psychiatric morbidity. These findings suggest that serum cortisone:cortisol ratio is a better predictor of offspring birth weight than maternal

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cortisol measured in isolation. In light of this, we were interested in seeing if we could find a similar relationship in healthy women when evaluating maternal salivary cortisone:cortisol ratio in pregnancy. Specifically, we examined whether maternal salivary cortisone:cortisol ratio in late pregnancy was a better predictor of offspring birth weight than cortisol measured in isolation.

There are important differences between serum and salivary cortisol and cortisone that could impact the relationship between cortisone:cortisol ratio and birth weight in these different tissues. Firstly, serum cortisol reflects both bound and unbound cortisol, while salivary reflects only the bioactive, unbound form (Levine et al., 2007). Secondly, the relationship between cortisone and cortisol varies between blood and saliva samples. In pregnant women serum cortisol levels are much higher than cortisone, (Ghaemmaghami et al., 2014; Hellgren et al., 2016), while in saliva the opposite is true (Greaves and West, 1968; Meulenberg and Hofman, 1990). Therefore the predicted relationship between salivary cortisone:cortisol ratio and birth weight, if any, is unclear. That said, if our findings show that salivary cortisone:cortisol is associated with birth weight, similar to Hellgren et al. (2016), this would suggest that saliva could be collected as a less invasive index of fetal exposure to glucocorticoids in pregnancy in future studies.

2. Methods

2.1. Sample

Data come from pregnant women (34–36 weeks gestation) living in Auckland, New Zealand. Detailed recruitment and sample information is described in Thayer and Kuzawa (2014). All participants were interviewed by the same study author in their homes. Inclusion criteria for the study included having no pre-existing health conditions and being pregnant with a singleton. With regards to pre-existing health conditions, women were asked if they had ever been told by a general practitioner that they had: Type 1 diabetes, Type 2 diabetes, Gestational diabetes, High blood pressure, Heart disease, High Cholesterol, Asthma, or Bipolar disorder. They were also asked if they had any other serious health complications. Having any of these conditions excluded women from the study. This study was conducted under conditions of written consent and approved by the Northwestern Institutional Review Board (IRB # STU00049286) and the New Zealand Upper South B Health and Disability Ethics Committee (Ethics ref: URB/11/09/036).

2.2. Maternal and offspring data

Maternal age was collected via self-report. Socioeconomic status was assessed with the NZ Individual Deprivation Index (NZiDep) questionnaire, which creates a continuous score of material deprivation ranging from zero (least deprived) to five (most deprived)(Salmond et al., 2006). Infant sex, gestational age at birth, and birth weight were retrieved from infant health and wellness records.

2.3. Saliva collection

Saliva collection is a minimally invasive method for assessing cortisol that reflects the unbound, bioactive fraction of the hormone (Kirschbaum and Hellhammer, 1989). Following recruitment women were provided four 2.0 mL saliva tubes and instructions for saliva collection. Women passively drooled into each tube at waking (waking sample) and prior to going to sleep (bedtime sample) on two consecutive weekdays between 34 and 36 weeks gestation. Participants were instructed to not eat, drink or brush their teeth in the 30 min prior to collecting samples, and they recorded start and stop times for saliva collection, mood and, for morning samples, sleep quality the previous night. Participants stored samples in their freezer until they were retrieved by the interviewer during an interview the following week. Following retrieval by the researcher, samples were stored at −80 °C at a laboratory until analysis. While 64 women were enrolled, sufficient saliva for cortisol measurement in both morning and evening samples was collected from 55 women.

2.4. Laboratory analyses

Cortisol and cortisone samples were analyzed using triple quadrupole mass spectrometry at the Liggins Institute in Auckland, New Zealand. Samples were run in duplicate, with all samples from each participant run in the same assay. The internal standards were cortisol-d2 for cortisol; corticosterone-d8 for cortisone. Both steroids were extracted using 1 mL of ethyl acetate (Merck KGaA Darnstadt, Germany) (Rumball et al., 2008). After removal of the organic supernatant, samples were dried by vacuum concentration (Savant SC250EXP, Thermo Scientific, Asheville, NC, USA), resuspended in 60 μL of mobile phase 72% methanol (Merck) and 28% water, and transferred to HPLC injector vials. Twelve μL were injected onto an HPLC mass spectrometer system consisting of an Accela MS pump and autosampler followed by an Ion Max APCI source on a Finnigan TSQ Quantum Ultra AM triple quadrapole mass spectrometer all controlled by Finnigan Xcaliber software (Thermo Electron Corporation, San Jose, CA, USA)(Rumball et al., 2008). The mobile phase was isocratic, flowing at 250 μL min^{-1} through a Luna HST 2.6 μm C18(2) 100 × 3.0 mm column at 40 °C (Phenomenex, Auckland, New Zealand). Cortisol and cortisone retention times were 6.1 min and 5.7 min, respectively, ionisation was in positive mode, and Q2 had 1.2 mTorr of argon. The mass transitions followed were 363.2–122.2 at 28 V for cortisol and 361.1–163.0 at 28 V for cortisone. Intra-assay coefficients of variation ranged from 3.4% to 12.1%.

2.5. Statistical analysis

Descriptive statistics were used to evaluate cortisol and cortisone values at waking and at bedtime. Cortisone:cortisol ratio was initially calculated as (cortisone/cortisol) (Hellgren et al., 2016). Multivariate regression was used to assess the relationship between cortisol and cortisone:cortisol ratio, respectively, with birth weight. Analyses were run separately for morning and evening values. Consistent with Hellgren et al. (2016), all analyses controlled for maternal height, parity, smoking, gestational age, offspring sex, and time of day of sample collection.

3. Results

3.1. Summary of maternal and offspring characteristics

The average age of participants was 30.8 years of age (SD = 4.8 years). Fifty-three percent of the sample had a NZiDep score of 0, indicating no material deprivation, while 18% had a score of 2 or above, indicating moderate to high material deprivation. The average gestational age of infants at birth was 39.2 weeks (SD = 1.4 weeks) and average weight was 3486.4 g (SD = 530.9), both of which are considered healthy. Fifty-eight percent of the sample offspring were male.

3.2. Summary of relationship between cortisol, cortisone, and cortisone:cortisol ratio

There was an expected diurnal rhythm in both cortisol and cortisone, with morning values for each hormone being higher than