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Full-term deliveries without antecedent labor reveal sex differences in umbilical cord glucocorticoid concentrations

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ABSTRACT

Background: Previous studies have shown that pregnant women have higher salivary cortisol levels when the fetus is female. These findings suggest a basis for the sex differences observed in many offspring outcomes after exposure to in utero stress, but it is not known if fetal adrenal glucocorticoid synthesis differs by sex.
Methods: Arterial and venous umbilical cord blood samples were collected immediately after scheduled cesarean delivery (n = 52, 25 female). Cortisol and corticosterone concentrations were quantified by liquid chromatography coupled to tandem mass spectrometry.
Results: Sex differences were observed for fetal arterial and venous cortisol and venous corticosterone, with higher levels present when the fetus was female. However, sex differences were not observed for fetal synthesis of cortisol, suggesting that the fetus does not control the differences observed in cord blood glucocorticoids.
Conclusions: The presence of sex differences in umbilical cord glucocorticoid concentrations in the absence of sex differences in glucocorticoid synthesis by the fetal adrenal gland suggests that these differences have a maternal or placental origin. Thus, the in utero glucocorticoids in circulation are sex-specific and may have developmental importance for sex differences in psychiatric and neurodevelopment disorders that display sex biases.

Sex differences within the intrauterine milieu have profound and enduring implications for fetal and child development (Clifton, 2010; Sandman et al., 2013). For example, in the presence of maternal asthma, female fetuses reduce their growth whereas males do not (Murphy et al., 2003). However, if the mother experiences an acute exacerbation of asthma, males have a greatly increased incidence of intrauterine growth retardation, preterm delivery and stillbirth (Clark et al., 2007; Murphy et al., 2005). In addition, sex differences in neurodevelopmental and behavioral outcomes are observed following intrauterine exposure to stress and stress hormones (Sandman et al., 2013), suggesting that sex differences within the intrauterine environment may be linked to sex differences in neurodevelopmental and psychiatric disorders (Davis and Pfaff, 2014). Given that glucocorticoid receptors are highly expressed in the fetal brain (Sanchez et al., 2000), affecting the expression of more than one-thousand genes (Salaria et al., 2006), sex differences in prenatal cortisol exposure have the potential to shape many aspects of brain development.

Maternal adaptation to pregnancy can alter the developmental environment of the fetus in response to fetal sex. For example, women pregnant with a female fetus had flatter daytime salivary cortisol slopes compared to women pregnant with a male (Giesbrecht et al., 2015), and mean salivary cortisol levels were also higher in women pregnant with a female during the last weeks of pregnancy (DiPietro et al., 2011). It has, however, been challenging

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to determine whether the two sexes differentially alter their own developmental environments.

The intrauterine glucocorticoid milieu is complex, receiving contributions from the placenta (which is steroidogenic) and from both maternal and fetal circulation. In addition to producing steroid hormones, the placenta also expresses high levels of the enzyme 11ß hydroxy-steroid dehydrogenase (11ßHSD) that converts both cortisol and corticosterone to cortisone and 11-dehydrocorticosterone, respectively (White et al., 1997). This conversion of glucocorticoids from biologically active to inactive forms is thought to limit fetal exposure to maternal glucocorticoids transferred into placental circulation (Benediktsson et al., 1997). By approximately mid gestation the fetal adrenals begin synthesizing glucocorticoids (Ishimoto and Jaffe, 2011) and this synthesis can be observed in the increase in umbilical glucocorticoid concentrations from venous (circulation toward the fetus from the placenta) to arterial (circulation from the fetus toward the placenta) circulation (Wynne-Edwards et al., 2013). Here we focus on umbilical circulation to address questions about fetal exposure and glucocorticoid synthesis.

Elective Caesarian sections offer an extraordinary window into full-term fetal stress steroid exposure and synthesis. The mother presents at the delivery site without going into labor, and without acute clinical indications of distress in the mother or fetus that require emergency intervention. In a previous sample of 10 elective Caesarian sections for which mixed cord blood (predominantly venous blood) was collected, there was no evidence of a sex difference in cortisol concentration (Clifton et al., 2007).

A larger sample of 265 deliveries (Wynne-Edwards et al., 2013), of which 53 were elective Caesarian deliveries, determined both cortisol and corticosterone concentrations for the umbilical vein and artery. Our previous study demonstrated that the full-term fetus synthesizes both cortisol and corticosterone, and, specifically, that fetal corticosterone synthesis, rather than cortisol synthesis, was associated with fetal distress (Wynne-Edwards et al., 2013). However, sex of the fetus was excluded as an analytic covariate in those analyses before focusing on the subset of participants who underwent elective Caesarian section without antecedent labor, and therefore without elevation in maternal or fetal glucocorticoids consequent to labor. The current analysis re-visited the data from that study to test the hypothesis that fetal sex would influence fetal exposure and glucocorticoid synthesis. Maternal-fetal communication operates bi-directionally via both cortisol and corticosterone, and under conditions of stress the mother preferentially secretes cortisol (Cawson et al., 1974) whereas the fetus preferentially secretes corticosterone (Wynne-Edwards et al., 2013). Accordingly, we set out to determine if concentrations of cortisol or corticosterone in cord blood are sexually dimorphic and if fetal synthesis differs for males and females. We reasoned that sex differences in umbilical corticosterone would suggest fetal control whereas sex difference in cortisol would suggest a role for the maternoplacental unit.

1. Methods

Full methods are available in Wynne-Edwards et al. (2013). Within a sample of 265 healthy, full-term (>37 completed weeks), singleton births, the subset of 53 women with elective Caesarian section without antecedent labor were included in this analysis. Race/ethnicity and socioeconomic status were not assessed, however the catchment area for the participating hospital was predominately white, middle class and suburban, and likely underrepresents a broad diversity of races/ethnicities and socioeconomic levels. Written consent was obtained from participants upon arrival at the labor and delivery unit, and covered only the maternal birth record. Chart data for the mother and child were not examined. The study was approved by the University of Calgary Conjoint Health Research Ethics Board.

Table 1
Sample characteristics.

	Females (n = 25)		Males (n = 27)		p
	Mean	SE	Mean	SE	
Maternal age (years)	33.48	0.69	32.63	0.86	0.44
Parity	1.04	0.12	1.22	0.20	0.45
APGAR at 1 min	8.52	0.22	8.33	0.24	0.57
Birthweight (g)	3307	91	3596	114	**.05**

Note: *p* values refer to *t*-test comparisons between males and females.

At the research site, standard postpartum protocol routinely samples umbilical arterial and venous blood separately for pH and acid-base status. After that routine sampling, but prior to delivery of the placenta, one non-heparinized, 'red top', vial of venous and another of arterial whole blood were collected by needle aspiration (16 gauge) for this study. Samples were immediately refrigerated, and allowed to stand for at least one hour (range 1–12 h, median < 4 h), before being centrifuged (4000 G for 5 min). Serum was separated and stored at −20 °C until delivery to the research laboratory for hormone analysis.

Detailed methods for simultaneous quantitation of cortisol and corticosterone concentrations by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) have been previously reported (Koren et al., 2012; Wynne-Edwards et al., 2013). Briefly, cortisol and corticosterone concentrations were determined as area ratios relative to a bio-identical deuterated internal standard. Intra- and inter-assay coefficients of variation were 4.5% and 2.9% for cortisol, and 5.8% and 3.6% for corticosterone. No samples fell outside of the linear range of the calibration curve (Wynne-Edwards et al., 2013).

Sex differences were assessed through three parameters: 1) the concentration of corticosteroid (ng/ml) in venous and arterial circulation; 2) the increase (ng/mL) in concentration between venous and arterial circulation (i.e., increase in concentration due to fetal synthesis); and 3) the proportional change across the fetus that considers the fetal synthesis as a proportion of the arriving concentration ((arterial-venous)/venous)). Comparisons between the sexes used a *t*-test with degrees of freedom adjusted for unequal variances. All statistical tests used JMP 12.1.0 (www.jmp.com) and applied a critical alpha of 0.05. Effect sizes were estimated with Cohen's *d*. Effects greater than $d = 0.41$ are, by convention, considered clinically significant (Ferguson, 2009).

2. Results

One female fetus had an arterial cortisol value of 253 ng/ml, which was an outlier relative to the sample median of 35 ng/ml (range 8–125 ng/ml; SD 30.5). Recent antenatal glucocorticoid administration to the mother was suspected. That female fetus was excluded, resulting in a final sample size of 25 females and 27 males.

As expected, common antecedent risk factors for this sample were previous Caesarian section (n = 38, 73%, 19 F, 19 M) and maternal age over 35 (n = 22, 42%, 10 F, 12 M). Both factors contribute to physician decisions to schedule an elective section. Other antecedent risk factors (e.g., gestational diabetes or hypertension) were too rare to be analyzed within this cohort. Also as expected, there were sex differences in birthweight, with females smaller than males (Table 1, $p = 0.05$).