Maternal corticosterone exposure in the mouse programs dysregulation of plasma corticosterone levels and adrenal size in offspring in an age and sex specific manner

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Maternal exposure to synthetic glucocorticoids during pregnancy is known to reduce nephron number and program offspring to develop hypertension in a sex specific manner. While synthetic glucocorticoids are often administered to women at risk of preterm delivery, natural glucocorticoids are elevated endogenously in response to maternal stress. However, the effects of excess natural glucocorticoid exposure on fetal development are poorly characterized. We have previously demonstrated that maternal exposure to Corticosterone (Cort), the endogenous glucocorticoid in mice, reduces nephron number in both sexes but induced hypotension and impaired renal function in male offspring only at 12 months of life. This demonstrates that maternal Cort exposure programs different physiological outcomes than synthetic glucocorticoid exposure and highlights that a nephron deficit alone does not always lead to increased blood pressure. Recent finding by our laboratory have demonstrated that prenatal Cort exposure programs alterations in the expression of renal glucocorticoid receptors and glucocorticoid metabolizing enzymes in male but not female offspring. This suggests that programmed alterations to renal glucocorticoid sensitivity may play a role in the programming of hypotension in this model. This led us to investigate the regulation of adrenal development and function in offspring following prenatal Cort exposure.

Pregnant C57/BL/6 mice were surgically implanted with osmotic minipumps primed to release Corticosterone (33µg/kg/h for 60h beginning at E12.5). Untreated animals served as controls. Mice were allowed to litter down and were killed at 1 month, 6 months and 12 months of life for collection or plasma and adrenal glands. Steroids were extracted from the plasma using a dichloromethane extraction and plasma Cort levels determined using a radioimmunoassay. Left adrenal glands were snap frozen for molecular analysis. RNA was extracted, reverse transcribed and mRNA expression measured for cytochrome P450, family 11, subfamily A, polypeptide 1 (Cyp11a1), steroidogenic acute regulatory protein (Star) and melanocortin 2 receptor (Mc2r). The right adrenal glands were fixed in 4% PFA, serially sectioned, stained with periodic-acid Schiffs reagent and volume of adrenal zones determined using unbiased stereology.

Prenatal Cort exposure did not affect plasma Cort concentrations in offspring at 1 month of life. At this age, plasma Cort levels were similar in males and females but at 6 and 12 months, Cort concentrations were higher in females than males. Maternal Cort exposure increased plasma Cort levels in male (75%) but not female offspring at 6 months of life. Plasma Cort levels tended to be increased in male but not female offspring at 12 months of life however this did not reach statistical significance (P=0.1). Adrenal weight was not affected by maternal Cort exposure or offspring sex at one month of life. Adrenal glands were significantly heavier in females compared to males at both 6 and 12 months of life. At 6 months of life, adrenal weight was increased in male (44%) offspring while at 12 months, adrenal weight was reduced in males (25%) Adrenal volume was reduced in male (35%) but not female offspring at 1 month of life and this was associated with reductions in three of the zones of the adrenal - *Zona fasciculata*, *Zona reticularis* and adrenal medulla. Adrenal volumes were not significantly affected by prenatal treatment at either 6 or 12 months of life. Cyp11a1 mRNA levels were not affected by prenatal treatment at 1 month of life, but were reduced at 6 months of life (0.6 fold) and similar to untreated animals at 12 months of life. Mc2R expression was significantly increased by prenatal Cort exposure at 1 month of life (1.2 fold) but not affected at either 6 months or 12 months of life. Prenatal Cort did not affect expression of other genes examined at any age.

This study demonstrates that prenatal Cort exposure can program elevated glucocorticoid production in offspring while dysregulating adrenal growth and mRNA expression in an age and sex specific manner. Importantly, prenatal Cort exposure did not impair adrenal development in female offspring. Together with our previous findings, this study suggests that prenatal Cort exposure may be eliciting the programmed hypotensive phenotype by impairing the development of multiple organs and systems which collectively dysregulate blood pressure. These findings provide potential mechanistic insight into the sex specific programming of adult disease and suggest other mechanisms in addition to nephron deficit may precede programmed renal and cardiovascular disease.