



# Prenatal Testosterone Exposure (2D:4D ratio) is Associated with Lower Stress-Induced Cortisol Response in Female Infant Rhesus Monkeys

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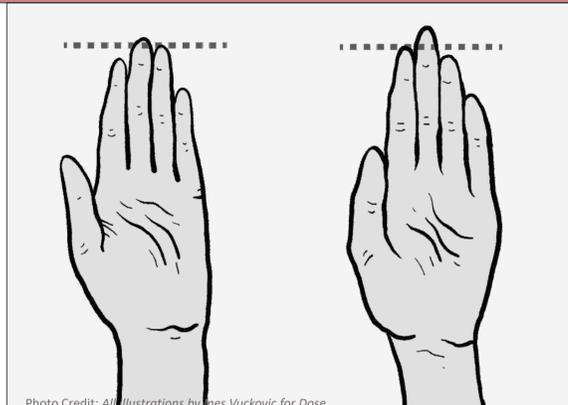


## Abstract

The second-to-fourth-digit ratio (2D:4D ratio) is widely accepted as a biomarker for the degree of prenatal androgen exposure, which is the primary factor responsible for masculinizing a developing fetus. Some studies suggest that females prenatally exposed androgens (such as testosterone) are less likely to suffer from anxiety and anxiety disorders later in life. Plasma cortisol is used as a measure of stress and anxiety. Using 2D:4D ratio as a proxy for the degree of prenatal androgen exposure, we hypothesized that a more masculinized 2D:4D ratio would be associated with lower levels of stress-induced circulating cortisol in female (but not male) rhesus monkeys (*Macaca mulatta*). Two blood samples were obtained during a two-day experimental mother-infant social separation stressor from rhesus monkeys infants ( $N = 268$ : 180 females, 88 males) when they were three-to-four months of age. Samples were assayed for concentrations of plasma cortisol and the values were averaged for analysis. Subjects' second and fourth digits were measured at varying ages ( $M = 6.70$  years of age) and a 2D:4D digit ratio was calculated for each subject. Results from a subset of the sample ( $n = 70$ ) showed a positive correlation between early and later life 2D:4D digit ratio, indicating stability across life. Female monkeys with a more masculinized 2D:4D ratio pattern, exhibited lower plasma cortisol when they were three-to-four months of age ( $p = .01$ ). These findings suggest that in females, higher levels of prenatal androgen exposure may be related to an attenuated stress-induced hypothalamic-pituitary-adrenal (HPA) axis response, as measured by plasma cortisol levels. To the extent that these findings generalize to humans, results suggest that prenatal androgen exposure, as measured by 2D:4D ratio, modulates the HPA axis response to stress, reducing risk for anxiety later in life.

## Introduction

- Prenatal androgen exposure (mostly testosterone) is thought to be the main organizational influence that masculinizes the body and brain during development<sup>1</sup>.
- The second-to-fourth-finger (2D:4D) ratio is widely accepted as a biomarker for the degree of prenatal androgen exposure<sup>2</sup>, allowing a retrospective assessment of prenatal androgen exposure.
- Studies show sex differences in the activity of the hypothalamic-pituitary-adrenal (HPA) axis<sup>3,4,5</sup>.
- Studies in humans show that individual differences in the 2D:4D ratio pattern are stable throughout life<sup>6</sup>, allowing digit ratio measurements performed early (or later) in life to assess prenatal androgen exposure.
- Rhesus monkeys are ideal subjects for studying prenatal androgen exposure, because of their similarity to humans at the genetic<sup>7</sup> and CNS<sup>8</sup> level.
- We hypothesize that infant female rhesus monkeys with a more masculinized 2D:4D ratio (suggesting a higher degree of prenatal masculinization) would exhibit lower stress-induced plasma cortisol levels.



Rhesus monkeys (*Macaca mulatta*)

Photo Credit: Kathy West, © CNPRC, UC Davis

## Methods

### Subjects

- $N = 268$  (180 females, 88 males) rhesus monkeys studied at the California National Primate Research Center.
- They were housed in semi-naturalistic settings, in social conditions that approximate the natural rhesus social composition.

### Independent Variable: Mean 2D:4D Ratio

- Fingers were measured between 1-17 years later ( $M = 6.70$ ) using a digital caliper.
- Second and fourth digits were both measured at least twice, until two measurements were within  $\pm 1.5$  mm.
- Subjects' 2D:4D ratios were determined by dividing the length of the pointer finger (2D) by the length of the ring finger (4D).
- Because the right and left hands were positively correlated, for sake of analyses, measurements were averaged.
- Preliminary analyses showed cohort-year differences. Thus, mean 2D:4D ratio was standardized within measurement year and individual z-scores were used as the independent variable.

### Dependent Variable: Plasma Cortisol

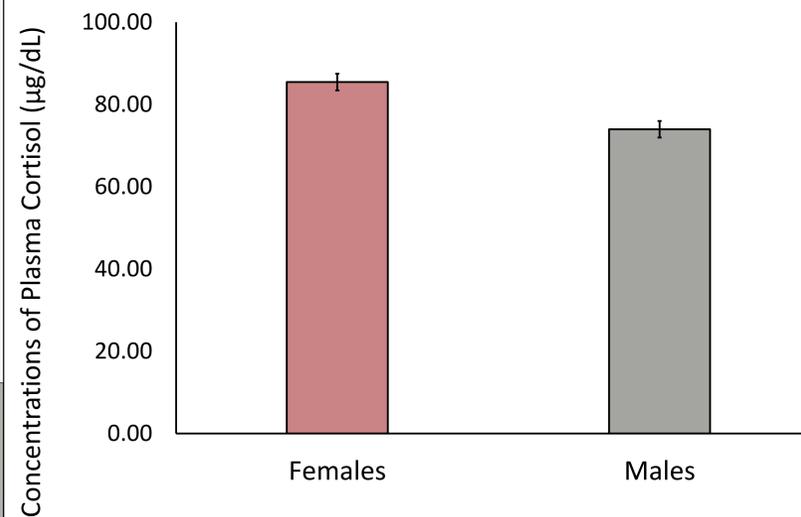
- Two blood samples were obtained via femoral venipuncture when subjects were 3-4 months of age during a social separation stressor, and they were assayed for plasma cortisol concentrations.
- As with finger ratio, there was cohort-year difference. Thus the values were z-scored by cohort year. The plasma cortisol response mean for each subject was used as the dependent variable.

### Analyses:

- A one-way between subject ANOVA was used to assess for sex differences in mean plasma cortisol concentrations.
- A bivariate correlation using a subset of subjects ( $n = 70$ ) that were measured at two different time points assessed whether individual differences in 2D:4D ratio are stable across time.
- Consistent with other studies<sup>9</sup>, preliminary analysis showed an effect of age at cortisol sampling; thus, age was statistically controlled in the analyses of finger ratio and plasma cortisol.
- Multiple regressions were performed using SPSS, version 25.

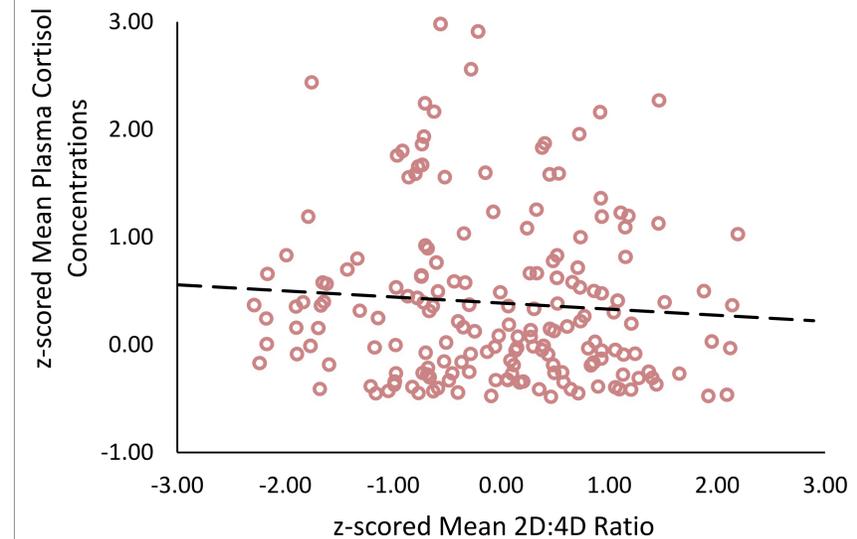
## Results

### Sex Difference in Mean Concentration of Plasma Cortisol at 3-4 Months of Age



There was a significant sex difference in mean plasma cortisol concentrations following a social separation stressor ( $F(1,265) = 13.41, p < .0001$ ).

### Association Between Mean 2D:4D Ratio and Mean Plasma Cortisol Concentrations in Female Monkeys



Result showed a significant correlation between plasma cortisol concentrations and 2D:4D ratio ( $\beta = -.182, p = .013$ ; Overall Model  $F(2, 177) = 6.453, p = 0.002$ ). There was no relationship between cortisol and 2D:4D ratio in males.

## Discussion

- Overall, the results showed that infant females exhibited higher stress-induced plasma cortisol levels when compared to same aged males.
- As androgens are the primary organizational factor masculinizing the brain, finding of a sex difference in stress-induced cortisol concentrations also supports the hypothesis of PAE-induced organizational effects.
- Results confirmed the hypothesis: infant female rhesus monkeys that exhibited a more masculinized 2D:4D ratio exhibited lower plasma cortisol concentrations during a social separation stressor.
- These findings suggest that in females, exposure to prenatal androgens has an organizational effect on the HPA-axis, leading to changes that may protect females from the effects of stress later in life.
- To the extent that these results generalize to humans, they suggest that females with higher levels of prenatal androgen exposure may be at lower risk for stress-related psychopathological outcomes.