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Brief report

The association between perinatal testosterone concentration and early vocabulary development: A prospective cohort study

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ABSTRACT

Prenatal exposure to testosterone is known to affect fetal brain maturation and later neurocognitive function. However, research on the effects of prenatal testosterone exposure has been limited by indirect measures of testosterone and small unrepresentative samples. This study investigated whether bioavailable testosterone (BioT) concentrations in umbilical cord blood are associated with expressive vocabulary development, in a large birth cohort. Cord blood samples were taken immediately after delivery and expressive vocabulary was measured at two years of age using the language development survey (LDS). BioT concentration significantly predicted vocabulary size in males ($n = 197$), such that higher concentrations were associated with lower LDS scores, indicating smaller vocabulary. This relationship between BioT concentrations and vocabulary at aged 2 years was not observed in girls ($n = 176$). Higher circulating prenatal testosterone concentrations at birth may be associated with reduced vocabulary in early childhood among boys.

1. Introduction

A large body of literature has revealed consistent sex differences in early expressive vocabulary development, with boys developing language at a slower rate than girls (Berglund et al., 2005; Feldman et al., 2000; Zubrick et al., 2007). It has been hypothesised that exposure to higher levels of prenatal testosterone may underlie these sex differences (Whitehouse, 2010). However, existing studies have been limited by measures of prenatal testosterone available. Published studies have mainly relied on proxy measures of prenatal testosterone exposure such as the 2D:4D digit ratio (Manning et al., 1998), or extrapolated from populations with excessive prenatal testosterone exposure, such as girls with Congenital Adrenal Hyperplasia (Nass and Baker, 1991). These studies have provided preliminary evidence consistent with an association between higher exposure to prenatal testosterone and lower verbal ability (Helleday et al., 1994; Kelso et al., 2000; Luxen and Buunk, 2005; Resnick et al., 1986). However, the relationship between the 2D:4D digit ratio and prenatal testosterone concentrations is inconsistent (Hickey et al., 2010). Furthermore, it is difficult to extrapolate from clinical populations, such as Congenital Adrenal Hyperplasia, to variations in language development within the general population.

Few studies have examined the relationship between prenatal testosterone exposure and language development more directly. Using testosterone levels derived from amniotic fluid to estimate prenatal testosterone exposure, Finegan et al. (1992) found a significant inverse association between amniotic testosterone concentrations and language comprehension in girls, but not boys, aged 4 years (28 girls, 30 boys). No relationship was observed between fetal testosterone levels and expressive language in either sex.

The Cambridge Fetal Testosterone Project has examined the association between fetal testosterone level (measured from amniotic fluid at 18 weeks pregnancy) and various aspects of childhood language development. Initially, with the sexes analysed together, a significant inverse relationship was found between testosterone concentration and expressive vocabulary size when the children were 18 and 24 months old ($n = 87$; Lutchmaya et al., 2002). However, there was no significant relationship when males and females were investigated separately.

When this same cohort was 4 years of age, parents of 58 children (35 male, 23 female) completed the Children's Communication Checklist (Bishop, 1998). No sex differences were observed for the subscales assessing speech, syntax or pragmatic language, and

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therefore the association between amniotic testosterone and language capability was not further investigated (Knickmeyer et al., 2005). Furthermore, when the children from the Cambridge cohort ($n = 74$; 43 male, 31 female) were between 6 and 10 years of age, no sex differences were found on the Verbal IQ scores of the Wechsler Abbreviated Scale of Intelligence, and these scores did not correlate with testosterone concentrations (Auyeung et al., 2009).

However, amniotic fluid testosterone concentrations are only weakly associated with circulating fetal testosterone levels (Rodeck et al., 1985). In addition, amniocentesis is performed only in high risk pregnancies, so amniotic fluid samples do not allow for representative sampling of pregnancies. Furthermore, most studies investigating the relationship between amniotic testosterone and language development have measured total testosterone levels. Most circulating testosterone is bound to sex hormone-binding globulin (SHBG) and only the free (unbound) fraction is biologically active (Dunn et al., 1981). The relationship between language development and free versus bound testosterone is still unclear.

A more recent study by Whitehouse et al. (2012) used umbilical cord serum to measure bioavailable testosterone (BioT). This methodology allows for collection of larger and more representative samples and previous studies have found relationships between umbilical cord testosterone and postnatal behaviour (e.g. timidity; Jacklin et al., 1983, 1988). Whitehouse et al. (2012) found that higher levels of testosterone were associated with increased likelihood of clinically significant language delay for males in early childhood. Conversely, in females, higher concentrations of testosterone were associated with reduced frequency of language delay, indicating a possible protective effect. However, it was not possible from this study to determine whether early testosterone exposure was related to normal variation in language development. Furthermore, the association with the most obvious marker of early language ability, expressive vocabulary, remains unclear.

The aim of the current study is to investigate the relationship between umbilical cord testosterone and variations of typical expressive vocabulary development within the same cohort investigated by Whitehouse et al. (2012). It is predicted that higher levels of umbilical cord testosterone will be associated with lower expressive vocabulary development in males and improved expressive vocabulary development in females.

2. Methods

2.1. Participants

Participants were part of the Western Australian Pregnancy Cohort (Raine) study (www.rainestudy.org.au). Between May 1989 and November 1991, 2900 pregnant women were recruited from King Edward Memorial Hospital or nearby private practices. Eight-hundred and sixty-one children had umbilical cord serum collect at birth, and a further 426 (227 male; 199 female) of these children also had the language measure collected at 2 years. Inclusion criteria for the current study required the mothers to have English as a first language and the presence of no known developmental disorders among the children that could otherwise account for delayed language development (e.g., autism and Down syndrome). The final sample size for the current study was 373 (197 males; 176 females).

2.2. Language development survey

The language development survey (LDS; Rescorla, 1989) contains 310 words arranged alphabetically in 14 semantic categories (e.g. animals, foods, vehicles). Parents checked the words their child used spontaneously, not including those the child merely comprehended or imitated. Words pronounced using "baby talk" (i.e. "baba" for bottle) were included. Total number of words spoken was used as the outcome measure.

Parent completed questionnaires have yielded reliable results for early developmental assessments (Johnson et al., 2008). In particular, the LDS has been shown to correlate highly with standardised measures of language development (Rescorla and Alley, 2001).

2.3. Testosterone levels

Ten randomly selected cord blood samples confirmed the absence of detectable maternal contamination (Whitehouse et al., 2012). Total testosterone was measured by liquid chromatography-tandem mass spectrometry after solvent extraction as described by Keelan et al. (2012). Limit of quantitation was 0.025 ng/ml (0.08 nmol/l). Inter-batch imprecision was 6–11% ($n = 24$); recovery from cord serum was 93–98%. Sex hormone binding globulin was measured by ELISA using a commercial kit (IBL International, Hamburg, Germany). BioT, representing the fraction of total testosterone either free (unsequestered by SHBG) or bound to serum albumin, was calculated by summing the concentrations of free testosterone and albumin-bound testosterone (Keelan et al., 2012). Albumin levels were adjusted using published reference values to account for the decrease in serum albumin concentrations with gestational age (Zlotkin and Casselman, 1987).

2.4. Covariates

A range of sociodemographic, antenatal and obstetric variables argued by Whitehouse (2010) to potentially influence neurocognitive outcomes were prospectively collected and investigated (Table 1).

2.5. Statistical analysis

An independent samples t-test was conducted to compare scores on the LDS and BioT for males and females. In addition, bivariate and point-biserial correlations between possible covariates and the LDS were examined. Hierarchical multiple regression analyses were used to investigate the relationship between LDS and BioT concentrations separately for males and females, with control for covariates.

3. Results

Table 1 presents the characteristics of male and female participants. As assessed by the LDS, boys had significantly smaller expressive vocabularies than girls. Additionally, boys were heavier at birth and had significantly higher BioT concentrations than girls. There were no other sex differences observed. Covariates found to significantly correlate with LDS scores (at $p < .05$) were maternal education, child's age at language assessment, parity and gestational age, these variables were included in the regression analysis.

Of central importance, BioT concentrations showed a significant negative relationship with LDS scores in males ($r = -.19$, $p < .05$), but no relationship was found in females ($r = .08$, *ns*). Similarly, a significant negative correlation was found between total testosterone concentrations and LDS scores in males ($r = -.17$, $p < .05$), but not females ($r = .05$, *ns*). For each of the testosterone measures, there was a significant differences between the correlations for males and females ($z = 2.61$, $p < .05$ for BioT; $z = 2.12$, $p < .05$ for total testosterone). Due to the observation of comparable relationships between LDS scores and both total testosterone and BioT, and the very high correlation between the two testosterone measures ($r = .91$, $p < .001$), the regression analysis focused on BioT only.

To further examine the relationship between BioT testosterone and language development in males, a hierarchical multiple regression analysis was conducted in which BioT was added to a model where the covariates noted above were entered first in predicting vocabulary scores. The inclusion of BioT significantly improved the model (see Table 2), with higher levels of BioT associated with lower scores on the LDS.

4. Discussion

Consistent with extensive published literature (Berglund et al., 2005; Feldman et al., 2000; Zubrick et al., 2007), we found that on average boys had smaller expressive vocabulary than girls at two years of age. Also, males had significantly higher umbilical cord blood testosterone levels than girls. The major finding of the current study was that higher levels of cord blood testosterone predicted lower vocabulary size in boys aged 2 years, which supports previous findings from amniotic testosterone studies looking at early language development (Finegan et al., 1992; Lutchmaya