Psychoneuroendocrinology 68 (2016) 111–116

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Early postnatal testosterone predicts sex-related differences in early expressive vocabulary

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ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form 29 February 2016
Accepted 2 March 2016

Keywords:
Testosterone
Postnatal development
Sex
Gender
Vocabulary
Language development

ABSTRACT

During the first few years of life, girls typically have a larger expressive vocabulary than boys. This sex difference is important since a small vocabulary may predict subsequent language difficulties, which are more prevalent in boys than girls. The masculinizing effects of early androgen exposure on neurobehavioral development are well-documented in nonhuman mammals. The present study conducted the first test of whether early postnatal testosterone concentrations influence sex differences in expressive vocabulary in toddlers. It was found that testosterone measured in saliva samples collected at 1–3 months of age, i.e., during the period called mini-puberty, negatively predicted parent-report expressive vocabulary size at 18–30 months of age in boys and in girls. Testosterone concentrations during mini-puberty also accounted for additional variance in expressive vocabulary after other predictors such as sex, child's age at vocabulary assessment, and paternal education, were taken into account. Furthermore, testosterone concentrations during mini-puberty mediated the sex difference in expressive vocabulary. These results suggest that testosterone during the early postnatal period contributes to early language development and neurobehavioral sexual differentiation in humans.

1. Introduction

Certain aspects of language development differ between the sexes. Females are typically better at speech production than males (Hyde and Linn, 1988). In particular, during the first few years of life, girls on average speak more words and thus have a larger expressive vocabulary than boys (Berglund et al., 2005; Feldman et al., 2000; Fenson et al., 1994; Zubrick et al., 2007). Early expressive vocabulary development is particularly important, since a small vocabulary may indicate language delay and predict subsequent language difficulties, which are more prevalent in boys than girls (Hawa and Spanoudis, 2014; Rescorla, 2011).

Early androgen exposure may contribute to the sex difference in early expressive vocabulary development. Androgens, particularly the testicular hormone, testosterone, are elevated in male fetuses between about 8 and 24 weeks of gestation (Reyes et al., 1974). There is also an early postnatal surge of testosterone in male infants, called "mini-puberty", with testosterone peaking at about 1–3 months of age, and declining to baseline by about 6 months of age (Winter et al., 1976). During these periods, the adrenal glands produce some androgens in both sexes, but in males the gonads produce larger amounts of testosterone. In studies of non-human mammals, manipulations of testosterone prenatally or neonatally exert enduring influences on behavioral characteristics that differ for the two sexes (Arnold, 2009). Higher concentrations of testosterone produce more male-typical behavior whereas lower concentrations of testosterone produce more female-typical behavior (Arnold, 2009).

Two studies have investigated whether early testosterone exposure contributes to expressive vocabulary size in humans. The first study included 40 boys and 47 girls aged 18–24 months and used amniotic fluid testosterone to estimate prenatal testosterone exposure (Lutchmaya et al., 2001). The second study included 197 boys and 176 girls aged 2 years and used umbilical cord blood testosterone to estimate late prenatal testosterone exposure (Hollier et al., 2013). Both studies employed parent-report questionnaires and found the expected differences between boys and girls in testosterone and in vocabulary. The first study found no correlation between testosterone and vocabulary in either boys or girls, whereas the second study found the expected negative correlation in boys, but not in girls. Similarly, studies relating amniotic or umbilical cord blood testosterone to other aspects of language

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http://dx.doi.org/10.1016/j.psyneuen.2016.03.001

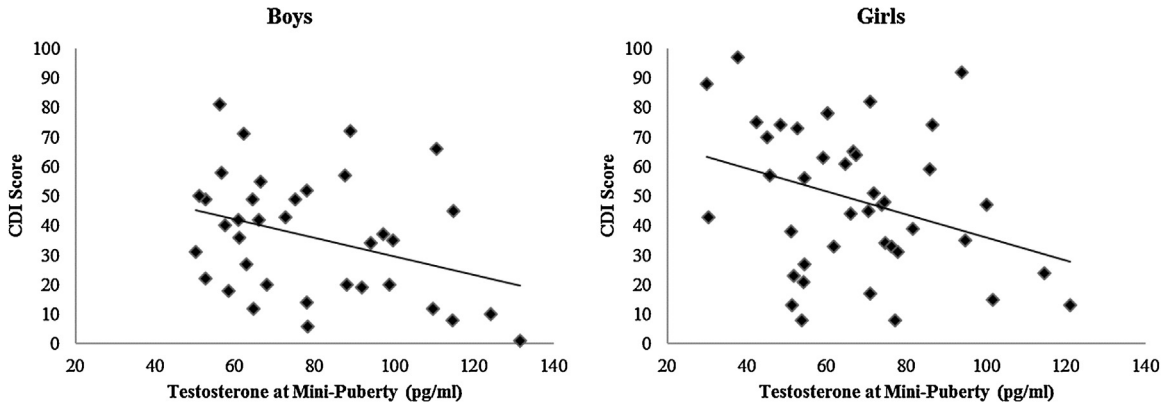

Fig. 1. Scatter plot showing the relationship between scores on the toddler short form for vocabulary production from the MacArthur Communicative Development Inventory (CDI) and concentrations of testosterone at mini-puberty in boys (left) and girls (right).

Table 1
Descriptive and Inferential Statistics for Differences Between Boys and Girls.

	Boys (B)			Girls (G)			All			B vs. G		
	n	M	SD	n	M	SD	n	M	SD	t	p	d[a]
Testosterone at mini-puberty (pg/ml)	36	79.09	22.75	42	67.37	20.79	78	72.78	22.36	2.38	0.020	0.55
CDI scores	37	36.97	20.83	42	48.62	24.54	79	43.18	23.48	−2.26	0.027	−0.51
Birth weight (kg)	37	3.42	0.53	42	3.46	0.42	79	3.44	0.47	−0.37	>0.250	−0.08
Child's age at saliva sampling (weeks)	37	7.64	1.72	42	8.14	2.27	79	7.90	2.03	−1.09	>0.250	−0.24
Child's age at CDI assessment (months)	37	22.59	3.54	42	22.19	3.23	79	22.38	3.64	0.53	>0.250	0.12
Maternal age (years)	37	34.48	3.28	41	34.05	4.58	78	34.25	3.99	0.47	>0.250	0.11
Paternal age (years)	36	36.03	3.79	41	36.78	6.10	77	36.43	5.13	−0.64	>0.250	−0.15
Maternal education	37	4.68	0.47	42	4.62	0.54	79	4.65	0.51	0.49	>0.250	0.12
Paternal education	37	4.46	0.65	42	4.69	0.52	79	4.58	0.59	−1.76	0.083	−0.39
Number of siblings	37	0.59	0.90	42	0.57	0.77	79	0.58	0.83	0.12	>0.250	0.02

Note. CDI = the toddler short form for vocabulary production from the MacArthur Communicative Development Inventory; Maternal and paternal education were rated on a 5-point scale from 1 (*primary education only*) to 5 (*postgraduate degree*).

[a] Positive *d*s indicate higher values in boys than girls.

development have produced mixed findings (e.g., Finegan et al., 1992; Grimshaw et al., 1995; Lust et al., 2010; Whitehouse et al., 2012, 2014). These largely negative findings could reflect insufficiently sensitive measures of testosterone exposure (Hines et al., 2015; Hollier et al., 2014). The negative findings in some studies could also reflect the use of language outcome measures that showed no sex differences (e.g., Finegan et al., 1992; Grimshaw et al., 1995; Lust et al., 2010).

No studies have yet related the early postnatal testosterone surge, mini-puberty, to later expressive vocabulary. However, testosterone exposure during mini-puberty appears to be important for normal development of the male genitalia and reproductive function (Boas et al., 2006; Kuiri-Hänninen et al., 2011; Main et al., 2005). Testosterone during mini-puberty also has been found to predict subsequent sex-typical play behavior (Lamminmaki et al., 2012; Pasterski et al., 2015). In addition, brain plasticity remains high and the brain continues to develop rapidly throughout the early postnatal period (de Graaf-Peters and Hadders-Algra, 2006). Language-related neural development can be expected to involve both prenatal and early postnatal influences. Thus, testosterone during mini-puberty, as well as prenatally, may relate to later expressive vocabulary.

Although no research has related testosterone during mini-puberty to expressive vocabulary, three studies have examined other language outcomes in relation to early postnatal testosterone concentrations. The first study related testosterone in infant blood samples collected at age 1 month to lateralization of language function measured by electroencephalography (EEG) at the same age in 18 boys and 18 girls (Friederici et al., 2008). It was found that EEG responses differed by sex and that testosterone related to EEG responses in boys and in girls. Nevertheless, because the study was contemporaneous, findings could reflect transient influences of androgen, instead of the enduring influences that testosterone can exert on behavior. The second study related testosterone in infant saliva samples collected at age 3–4 months to durations of vocalizations and to the number of words expressed during laboratory play sessions at age 18–24 months in 47 boys and 37 girls (Saenz and Alexander, 2013). The third study related testosterone in infant blood samples collected at age 5 months to scores on measures of language comprehension completed at age 3–5 years in 9 girls and 11 boys (Schaadt et al., 2015). Correlational findings from both of these longitudinal studies were mostly non-significant, perhaps partly due to the use of outcome measures that showed no sex differences. Also, one of the longitudinal studies reported no sex difference in testosterone, which could reflect the collection of saliva samples at 3–4 months postnatal, after the peak of the postnatal surge (Saenz and Alexander, 2013). In addition to limitations in measures of testosterone and sex-related language development, these prior studies did not test whether testosterone may mediate sex differences in early language development.

The present study investigated whether salivary testosterone at 1–3 months of age predicts expressive vocabulary size at 18–30 months of age. Saliva sampling was employed since it affords a non-invasive approach for assessing testosterone concentrations. Expressive vocabulary during toddlerhood was assessed, because it shows a reliable sex difference and because low expressive vocabulary during toddlerhood may predict subsequent language difficulties. It was expected that boys would have higher salivary testosterone concentrations, and a smaller expressive vocabulary, than girls. We also tested the hypotheses that testosterone would:

(1) correlate negatively with expressive vocabulary in boys and in girls and (2) mediate the sex difference in expressive vocabulary.

2. Method

Participants were recruited from a larger longitudinal study investigating the influences of testosterone during mini-puberty on child development. Because the pattern of transient gonadal activation during the early postnatal period differs between full term (37 or more weeks of gestation) and preterm infants (Kuiri-Hänninen et al., 2011), only full term infants were recruited. In the larger study, parents of 118 healthy, full term infants were recruited in Cambridgeshire, England, by distributing flyers in the local community and by advertising in local papers and on social media. Saliva samples were taken from these infants for testosterone assays when they were 1–3 months old. When the children were 18–30 months old, all of the parent participants were invited to complete an online questionnaire assessing the children's expressive vocabulary size. Parents (77 mothers, 2 fathers) of 37 boys and 42 girls (mean age = 22.38 months; *SD* = 3.37 months; range = 18.00–30.32 months) completed the questionnaire. In the current sample, most of the children are of Caucasian descent (87.3%); the rest are of mixed descent (12.7%). English is the first language for all of the children. There were no significant differences in sex, ethnicity, predictor, or control variables between the current sample and the larger sample, except that mothers and fathers of the current child sample were significantly older at their child's birth than those in the larger sample. Parents gave informed consent for themselves and their children to participate in the study. The study was approved by the Psychology Research Ethics Committee at Cambridge University.

2.1. Outcome variable

The toddler short form for vocabulary production from the MacArthur Communicative Development Inventory (CDI; Fenson et al., 2000) is a parent-report measure designed to assess expressive vocabulary production in toddlers aged 16–30 months. Parents indicate whether or not their child says the words on the checklist. The short form consists of 100 vocabulary words selected from the 680 words on the long form (Fenson et al., 1994). The long form has excellent test-retest reliability ($r = 0.95$) over a period of around 1–3 months and correlates highly ($r = 0.78$–0.79) with tester-administered measures of vocabulary (Fenson et al., 1994; Ring and Fenson, 2000). The correlation between scores on the long and the short forms is $r = 0.98$ (Fenson et al., 2000). Higher CDI scores indicate a larger expressive vocabulary. The CDI was the only measure of language development that was included in the study.

2.2. Predictor variable

Saliva was collected from the infant using a small-sized inert polymer swab at the age of 4–14 weeks (mean age = 7.90 weeks; *SD* = 2.03 weeks; range = 4.14–14.43 weeks). Testosterone exhibits a diurnal rhythm. It is highest in the morning and lowest at around midnight (Ankarberg and Norjavaara, 1999; Diver et al., 2003). Hence, in accordance with previous research (Caramaschi et al., 2012), saliva samples were collected in a specific time frame, between 8:30 am and 12 noon. A correlational analysis based on a subset of the sample ($n = 60$) suggests that the relationship between the time of day and testosterone concentrations within the 3.5 h time window was negligible ($r = 0.03$). To reduce the possibility that the infant's saliva contained substances that could interfere with the immunoassay (such as breastmilk), mothers were instructed not to feed their child for at least an hour before the scheduled sample. Following collection, samples were stored in a freezer set to −25 °C before being sent to Salimetrics (Cambridgeshire, England) for testosterone assays. Concentrations of testosterone in saliva were measured in duplicate using enzyme immunoassays (assay sensitivity <1 pg/ml, intra-assay coefficient of variation = 5%, inter-assay coefficient of variation = 10%). One male infant produced insufficient saliva for the hormonal assay. Therefore, data on testosterone concentrations were available for 36 boys and 42 girls.

2.3. Control variables

Birth weight, maternal and paternal age and education, number of siblings, child's age at saliva sampling, and child's age at CDI assessment were assessed for control purposes. Information on these variables was obtained from parental reports. Maternal and paternal education were rated on a 5-point scale from 1 (*primary education only*) to 5 (*postgraduate degree*).

3. Results

Most variables were normally distributed (skewness statistics < 1.0), but child's age at saliva sampling, number of siblings in boys, and paternal education in girls were slightly skewed. Log transformations were carried out so that values for these variables became normally distributed. Because analyses based on raw values and analyses based on log transformed values yielded highly comparable results, in terms of both effect sizes and significance levels, descriptive statistics and statistical analyses based on raw values of these variables are presented here. There were no univariate outliers ($z < 3.29$) for any variables. As indicated by Mahalanobis distance, there were no bivariate or multivariate outliers. All tests were two-tailed, with α set at 0.05.

As expected, boys had significantly higher concentrations of testosterone during mini-puberty and significantly lower CDI scores at age 18–30 months than girls (see Table 1 for descriptive statistics, independent samples *t*-test results, and Cohen's *d* statistics). None of the control variables differed significantly for girls versus boys. In addition, there was a significant negative correlation between concentrations of testosterone during mini-puberty and later CDI scores in boys, in girls, and in the entire sample (see Table 2 for Pearson's *r* statistics and Fig. 1 for a scatter plot depicting these results).

To examine the unique contribution of mini-puberty to expressive vocabulary, concentrations of testosterone during mini-puberty were included in the second block in a hierarchical regression model predicting CDI scores. Any other variables that had a significant bivariate association with CDI scores were included in the first block of the model (see Table 3 for regression analysis statistics). For within-sex regression analyses, child's age at CDI assessment was entered in the first block. The inclusion of concentrations of testosterone during mini-puberty accounted for a significant additional 12% and 9% of the variance in CDI scores in boys and in girls, respectively. For regression analyses in the entire sample, sex, child's age at CDI assessment, and paternal education were entered in the first block. After including concentrations of testosterone during mini-puberty, the regression model accounted for a significant additional 10% of the variance in CDI scores in the entire sample.

A bias-corrected bootstrapping analysis with 10,000 resamples (Hayes, 2013) was conducted to examine the significance of the indirect effect of sex on CDI scores through the mediator, concentrations of testosterone during mini-puberty, in the entire sample. Because child's age at CDI assessment and paternal education significantly correlated with CDI scores, their effects on CDI scores

Table 2
Correlations of Scores on the Toddler Short Form for Vocabulary Production from the MacArthur Communicative Development Inventory (CDI) with Predictor and Control Variables.

	Boys			Girls			All		
	n	r	p	n	r	p	n	r	p
Testosterone at mini-puberty	36	−0.35	0.037	42	−0.33	0.033	78	−0.38	<0.001
Birth weight	37	−0.14	>0.250	42	0.05	>0.250	79	−0.03	>0.250
Child's age at saliva sampling	37	−0.16	>0.250	42	0.00	>0.250	79	−0.05	>0.250
Child's age at CDI assessment	37	0.46	0.005	42	0.53	<0.001	79	0.46	<0.001
Maternal age	37	−0.19	>0.250	41	−0.03	>0.250	78	−0.10	>0.250
Paternal age	36	0.18	>0.250	41	−0.11	>0.250	77	0.00	>0.250
Maternal education	37	−0.22	0.197	42	0.19	0.218	79	0.02	>0.250
Paternal education	37	0.17	>0.250	42	0.26	0.096	79	0.25	0.025
Number of siblings	37	0.08	>0.250	42	0.01	>0.250	79	0.04	>0.250

Table 3
Hierarchical Multiple Regressions Predicting Scores on the Toddler Short Form for Vocabulary Production from the MacArthur Communicative Development Inventory (CDI).

	Boys ($n = 36$)			Girls ($n = 42$)			All ($n = 78$)		
	B	SE	p	B	SE	p	B	SE	p
Block 1:									
Child's age at CDI assessment	2.52	0.91	0.009	3.99	1.02	<0.001	3.10	0.70	<0.001
Sex	–	–	–	–	–	–	12.30	4.69	0.011
Paternal Education	–	–	–	–	–	–	4.18	4.04	>0.250
	$R^2 = 0.19$, $F = 7.72$, $p = 0.009$			$R^2 = 0.28$, $F = 15.23$, $p < 0.001$			$R^2 = 0.30$, $F = 10.31$, $p < 0.001$		
Block 2:									
Child's age at CDI assessment	2.51	0.85	0.006	3.87	0.97	<0.001	3.04	0.66	<0.001
Sex	–	–	–	–	–	–	8.35	4.54	0.070
Paternal Education	–	–	–	–	–	–	4.06	3.78	>0.250
Testosterone at mini-puberty	−0.31	0.13	0.023	−0.36	0.15	0.022	−0.34	0.10	<0.001
	$\Delta R^2 = 0.12$, $F = 5.72$, $p = 0.023$			$\Delta R^2 = 0.09$, $F = 5.60$, $p = 0.022$			$\Delta R^2 = 0.10$, $F = 11.53$, $p < 0.001$		
	$R^2 = 0.31$, $F = 7.26$, $p = 0.002$			$R^2 = 0.37$, $F = 11.39$, $p < 0.001$			$R^2 = 0.40$, $F = 11.71$, $p < 0.001$		

– Variables not entered in the block.

were controlled in this analysis. The indirect effect was entirely above zero, $B = 3.96$, $SE = 2.29$, 95% CI = [0.69, 10.00], suggesting that testosterone during mini-puberty was a significant mediator between sex and CDI scores, when other predictor variables were controlled.

4. Discussion

The present study is the first to demonstrate that testosterone during the early postnatal period of mini-puberty predicts the sex-related differences that have been observed in early expressive vocabulary development. Differences were found between boys and girls in salivary testosterone at 1–3 months of age and in expressive vocabulary size at 18–30 months of age. A negative relationship between testosterone during mini-puberty and expressive vocabulary was found in boys, in girls, and in the entire sample. Results also showed that testosterone accounted for significant additional variance in expressive vocabulary, when other predictors, such as child's age at vocabulary assessment and paternal education were controlled, suggesting that the effects of testosterone are independent from those of other predictors. Furthermore, the mediation analysis suggested that the sex difference in expressive vocabulary can be partly attributed to the sex difference in testosterone during mini-puberty.

Consistent with predictions based on non-human mammal research (Arnold, 2009), the current findings suggest that early androgen exposure contributes to subsequent behaviors that differ by sex. However, the current findings differ from prior findings that have reported little to no effect of mini-puberty on other aspects of subsequent language development in young children (Saenz and Alexander, 2013; Schaadt et al., 2015). The current findings also differ from prior findings that show no sex differences in salivary testosterone measured during the early postnatal surge (Auyeung et al., 2012; Saenz and Alexander, 2013). The measurement of testosterone during the peak of mini-puberty at 1–3 months of age, rather than at later ages, and the examination of expressive vocabulary, an outcome that shows a substantial and consistent sex difference during early development, may account for the present study's ability to detect the sex difference in salivary testosterone and to detect the relationship between testosterone during mini-puberty and later expressive vocabulary.

The observed effect of testosterone would appear to be enduring, rather than transient, because vocabulary was assessed many months after testosterone was measured, when mini-puberty was over and testosterone concentrations would have declined to baseline. In addition, neural plasticity is high during early postnatal development (de Graaf-Peters and Hadders-Algra, 2006), and studies in non-human mammals have found that testosterone during similar periods of early development alters brain structures, as well as behaviors, that differ by sex (Arnold, 2009). It is thus possible that testosterone at mini-puberty influences subsequent expressive vocabulary and perhaps other sex-related cognitive and behavioral characteristics by altering brain development.

On a broader scale, the current study highlights the potential of measuring salivary testosterone during the peak of mini-puberty as an effective tool to study the influences of early androgen exposure on human behavior. Previous research has found sex differences in testosterone in blood samples collected at the age of 5 months (Schaadt et al., 2015). On the other hand, prior studies have found no sex differences in salivary testosterone at 3–4 months of age (Auyeung et al., 2012; Saenz and Alexander, 2013). These findings suggest that blood sampling may provide a more sensitive measure of sex differences in testosterone for infants, because sex differences can be detected in blood samples, but not saliva samples, after the peak of mini-puberty. Despite its increased sensitivity which may compensate for late sample collection, blood sampling

is relatively invasive. Because saliva is relatively easy to obtain from infants, repeated samples can be taken in larger study populations, potentially increasing reliability of measurement.

Notably, the critical window during mini-puberty is more accessible than that during fetal development, because direct sampling from the developing individual is more feasible after they are born. One approach that has been used to estimate prenatal androgen exposure during typical development involves measuring testosterone in amniotic fluid obtained for clinical purposes, during a process called amniocentesis. Due to its invasive nature, however, amniocentesis is usually only performed in cases with increased risks of genetic anomalies associated with family medical history or increased maternal age, which limits the generalizability of the findings obtained using this approach. Moreover, although several research teams have reported sex differences in testosterone concentrations in amniotic fluid (e.g., Finegan et al., 1992; Lust et al., 2010; Lutchmaya et al., 2001; Rodeck et al., 1985), the one study that related amniotic fluid testosterone to fetal blood testosterone found no correlation between the two (Rodeck et al., 1985), suggesting that amniotic testosterone may not offer a reliable estimate of fetal androgen exposure. It also is not possible to increase reliability through repeated measurement, because the clinical procedure is almost always only conducted once. Another approach to estimating early testosterone exposure has been to measure testosterone in umbilical cord blood samples collected at birth. This approach, like others, has some limitations. For instance, androgen concentrations in umbilical cord blood can be influenced by labor and by the type of delivery (Hollier et al., 2014). In addition, although several research teams have reported sex differences in cord blood testosterone (e.g., Hollier et al., 2013; Jacklin et al., 1988; Simmons et al., 1994 Jacklin et al., 1988; Simmons et al., 1994), cord blood may reflect exposure only in late gestation (Hollier et al., 2014; Keelan et al., 2012), after the time of the prenatal surge, which appears to occur from about gestational week 8–24 (Reyes et al., 1974), and before the time of the postnatal surge, which appears to begin at about one month of age (Winter et al., 1976). Thus, saliva sampling during mini-puberty may afford an alternative, and relatively convenient, non-invasive approach for repeatedly assessing hormone exposures in representative samples during a critical period that may be important for human neurobehavioral development.

In addition to the challenges related to measuring testosterone concentrations reliably, it is important to consider whether outcome measures show sex differences when evaluating research results on the influences of early testosterone exposure. Prior studies have related testosterone measured in amniotic fluid to language comprehension and expression (Finegan et al., 1992), language lateralization (Grimshaw et al., 1995), and verbal IQ (Auyeung et al., 2009), and have related cord blood testosterone to reading ability (Jacklin et al., 1988) and receptive vocabulary (Farrant et al., 2013). All these studies have reported largely negative findings. However, none of the language outcome measures employed in these studies showed significant sex differences. The large body of experimental research in non-human mammals showing that testosterone before or shortly after birth influences neural and behavioral development, finds that these influences are limited to characteristics that differ by sex (Arnold, 2009). Based on non-human mammal research, the language outcome measures used in these prior studies of humans would not be expected to relate to early testosterone exposure. Thus, the largely negative results are not surprising. The remaining prior studies relating amniotic or cord blood testosterone to later language outcomes, either did not report any information on sex differences in the language outcome measures used (Lust et al., 2010), or included only girls (Whitehouse et al., 2014), thus preventing the assessment of sex differences.

Unlike most studies linking testosterone measured in amniotic fluid or in cord blood to language outcomes other than expressive vocabulary, Whitehouse et al. (2012) examined an outcome that showed a sex difference. This study related umbilical cord blood testosterone to language delay in early childhood, and found that language delay was more prevalent in boys than in girls. Also, as predicted, a positive relationship between cord blood testosterone and later language delay was found in boys. This result fits with predictions based on experimental research in other species and the finding of the current study showing that higher testosterone concentrations during mini-puberty predict smaller expressive vocabulary during toddlerhood. Both findings suggest that, in boys, relatively high testosterone concentrations during early life may contribute to increased risks of language-related problems. However, in the prior study (Whitehouse et al., 2012), contrary to prediction, a negative relationship between cord blood testosterone and language delay was found in girls, suggesting that testosterone exposure measured at birth may have protective effects on language development in girls (Whitehouse et al., 2012). Our finding that testosterone during mini-puberty may be a risk factor for language-related problems in both boys and girls contrasts with this prior finding, but is consistent with expectations based on animal research. The inconsistent findings in girls may be due in part to the above mentioned limitations related to using testosterone in cord blood as a measure of early androgen exposure. Also, the relationship between testosterone in umbilical cord blood and during mini-puberty is unknown. It is possible that contributions of androgen exposure measured at the time of birth and during mini-puberty to subsequent sex-related behaviors are sex-specific. Although this type of specificity based on the timing of exposure would not be predicted based on animal research, further research might usefully explore the possibility.

The relationship between testosterone prenatally and during mini-puberty also is unknown. If testosterone concentrations at these two time points correlate, our findings could reflect prenatal rather than early postnatal influences of testosterone. However, prior studies have found little to no associations between testosterone in amniotic fluid or umbilical cord blood and expressive vocabulary (Hollier et al., 2013; Lutchmaya et al., 2001 Lutchmaya et al., 2001), perhaps due to difficulty obtaining sufficiently sensitive and reliable measures of testosterone exposure. A recent study used aspects of genital development at birth and from birth to three months of age in boys to estimate prenatal and early postnatal testosterone exposure separately, and found that exposure at these two times each independently predicted subsequent sex-typical play behavior in the expected direction (Pasterski et al., 2015). Similar future research might usefully assess the independent contributions of prenatal and postnatal androgen exposure to expressive vocabulary and to other aspects of development that also differ by sex.

Children in the present study were aged 18–30 months when expressive vocabulary was assessed. Age was controlled statistically in the analyses, but additional research assessing expressive vocabulary at the same age in all children would also be of value. Another area for future work would involve using assays with better specificity than the immunoassay used in the present study to measure testosterone, as more specific assays might produce more reliable results.

In summary, higher concentrations of salivary testosterone during the peak of mini-puberty at age 1–3 months predicted smaller expressive vocabulary at age 18–30 months in boys and in girls. Also, the sex difference in expressive vocabulary was partly accounted for by the sex difference in salivary testosterone. These findings suggest that testosterone during mini-puberty may exert enduring influences on both within- and between-sex differences in expressive vocabulary. These findings also suggest that saliva

sampling at 1–3 months postnatal may provide a useful approach to studying the influences of early androgen exposure. Further research could relate salivary testosterone during mini-puberty to other cognitive and behavioral outcomes that show sex differences.

Conflicts of interest

The authors declare no conflict of interest.

Contributors

All authors contributed to the development of the study concept and research design. KTFK, WVB, and MC collected the data. KTFK performed the data analysis. KTFK drafted the manuscript, and WVB, MC, RMN, and MH provided critical revisions. All authors approved the final version of the manuscript.

Role of funding sources

This research was supported by the Economic and Social Research Council (ES/J500033/1; ES/I901957/1; ES/H016406/1) and the University of Cambridge. The grant agencies had no further role in the study design, data collection, analysis and interpretation of the data, in the writing of manuscript or the decision to submit the article for publication.

Acknowledgements

We thank all the families whose participation made this research possible.

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