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Effects of prenatal alcohol exposure on testosterone and pubertal development

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Abstract

Background—Animal models have demonstrated fetal alcohol-related disruptions in neuroendocrine function in the hypothalamic-pituitary-gonadal (HPG) axis and downstream effects on pubertal development and sexual behavior in males and females, but little is known about these effects in humans. This study examined whether prenatal alcohol exposure is associated with alterations in testosterone during adolescence and whether it affects timing of pubertal development.

Methods—The sample consisted of 265 African American adolescents from the Detroit Longitudinal Cohort Study for whom testosterone and/or pubertal development data were available. Subjects were offspring of women recruited at their first prenatal clinic visit to over-represent moderate-to-heavy alcohol use, including a 5% random sample of low-level drinkers/abstainers. Mothers were interviewed at every prenatal visit about their alcohol consumption using a timeline follow-back approach and about their smoking and drug use and sociodemographic factors. At age 14 years, adolescents provided salivary samples, which were analyzed for testosterone (pg/mL), self-reported Tanner stages for pubertal development, and age at menarche (females).

Results—Prenatal alcohol exposure was related to elevated testosterone concentrations for males and females but not to changes in Tanner stages or age at menarche, after controlling for confounders. In regression models stratified by alcohol exposure, the expected relation between testosterone and pubic hair development was seen among males with light-to-no prenatal alcohol

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exposure but not among those with moderate-to-heavy prenatal alcohol exposure. This interaction between testosterone and prenatal alcohol exposure was confirmed in multivariable models including an alcohol exposure group X testosterone interaction term and potential confounders.

Conclusions—This study was the first to show a relation between prenatal alcohol exposure and increased testosterone during adolescence and evidence of decreased testosterone responsiveness in tissues related to pubertal development. Further studies examining androgen receptor expression and other hormonal and cellular factors affecting pubertal development may reveal important mechanisms underlying these teratogenic effects of alcohol exposure.

Keywords

Fetal alcohol spectrum disorder; prenatal alcohol exposure; puberty; testosterone; Tanner stages

INTRODUCTION

Fetal alcohol spectrum disorders (FASD), the most common preventable cause of developmental delay, are characterized by a range of teratogenic effects of prenatal alcohol exposure, including cognitive impairment, structural neurologic deficits, growth restriction, and birth defects reported for every organ system. Animal models have demonstrated fetal alcohol-related disruptions in endocrine function and neuroendocrine regulation in the hypothalamic-pituitary-gonadal (HPG) axis as well as downstream effects on pubertal development and sexual behavior. In the hypothalamus, prenatal alcohol exposure has been associated with alterations in cellular morphology of gonadotropin-releasing hormone (GnRH) neurons in females (McGivern and Yellon, 1992) and alterations in GnRH regulation in both males and females (Lan, Hellemans, et al., 2009; Weinberg et al., 2008). Prenatal alcohol exposure also appears to disrupt pituitary function and responsiveness. In males, prenatal alcohol exposure has been shown to blunt normal luteinizing hormone (LH) patterns in infancy and to decrease levels of LH in later life (Handa et al., 1985; Udani et al., 1985; Wilson and Handa, 1997). In females, prenatal alcohol exposure has led to decreased basal LH levels, marked changes in developmental and adult patterns of LH and follicle stimulating hormone (FSH) levels, decreased LH expression in response to direct stimulation, and increases in prolactin, a pituitary hormone that inhibits GnRH production.

Marked reductions in the normal developmental prenatal and early postnatal testosterone surges have also been demonstrated (McGivern et al., 1988; 1993). However, little is known about how alcohol-related disruptions in the developing HPG axis affect basal hormone levels in adulthood. Using a late-gestation/early-postnatal alcohol exposure model, Udani et al. (1985) found alcohol-related decreases in testosterone levels through the period following puberty, but not later in life. Effects of prenatal alcohol exposure on gonad development and function in animals have also been seen, including decreased testicular size (Udani et al., 1985), alterations in testicular morphology (McGivern et al., 1988), testicular insensitivity to luteinizing hormone (McGivern et al., 1988), and inhibition of testicular steroidogenesis (Kelce et al., 1990). Potential effects of prenatal alcohol exposure on ovarian function are less well studied, but Weinberg and colleagues have shown menstrual-phase-specific increases in basal and post-stress levels of estradiol in rats with prenatal alcohol exposure (Lan, Yamashita, et al., 2009).

Alcohol-related disruptions in the developing HPG axis may play a role in the prenatal alcohol-related effects that have been seen on downstream pubertal development and sexual behavior. In males, alcohol-related decreases in masculinization have been manifested in decreases in anogenital length, smaller testicular and accessory sexual organ weights, and decreased sexual motivation (Udani et al., 1985). Fetal alcohol-related pubertal delays have been observed in female rats, as well as a shortened window of reproductive competence (Esquifino et al., 1986; McGivern and Yellon, 1992; McGivern et al., 1995; Udani et al., 1985; Weinberg et al., 2008). Reduced sexual dimorphism in nonsexual behaviors has also been reported in rats with prenatal alcohol exposure when compared to pair-fed controls (McGivern et al., 1984).

Despite this animal literature demonstrating effects of prenatal alcohol exposure on the HPG axis and pubertal development, very little is known about these potential effects in humans. In a case series of 61 adolescents and adults with FAS or fetal alcohol effects, males had a slight delay in onset of puberty that the authors deemed clinically insignificant (Streissguth et al., 1991). Day et al. (2002) found that the effects of alcohol on 14-year growth indices were independent of the timing of puberty assessed on the Pubertal Development Scale (Petersen, 1988) but did not report whether alcohol was associated with pubertal delays. Given the importance of HPG function for somatic growth, cognitive development, and other endocrine systems, such as the hypothalamic-pituitary-adrenal (HPA) axis (Hellemans et al., 2010; Jacobson et al., 1999; Oberlander et al., 2010), better understanding of potential effects of fetal alcohol exposure on the HPG axis in humans is needed. As part of the Detroit Longitudinal Cohort Study, a large study of children with a broad range of prenatal alcohol exposure levels followed from birth through age 19 years, the current study examined whether prenatal alcohol exposure is associated with altered levels of testosterone during adolescence and whether it affects timing of pubertal development

METHODS

Sample

The sample consisted of 265 African American adolescents for whom testosterone concentration and/or pubertal development data were available, who participated in the 480-subject Detroit Longitudinal Cohort Study (Carter et al., 2013; Jacobson et al., 2002; 2004). Women presenting for their initial visit to the prenatal clinic of a large inner-city maternity hospital were interviewed regarding alcohol consumption both currently and at conception between 1986 and 1989. Only African American women were included since the hospital served primarily (92%) women from this ethnic/racial group. Women who reported alcohol consumption at conception of at least 0.5 oz absolute alcohol (AA), the equivalent of 1 standard drink/day, and a random sample of 5% of lower-level drinkers and abstainers were invited to participate. To reduce the risk that prenatal alcohol and cocaine effects might not be separable statistically, an additional 48 women with both high cocaine (≥ 2 days/week) and low alcohol use (< 0.5 AA/day) were also included in the sample. Infants were excluded for birth weight < 1500 g, gestational age (GA) at delivery < 32 weeks, major chromosomal anomalies or neural tube defects, or multiple gestation pregnancy. Children were assessed at 6.5 and 13 months postpartum and at 7.5, 14, and 19 years. Informed consent was obtained

from the mother at recruitment and at each visit. Children gave oral assent at 7.5 and 14 years and written consent at 19 years. Approval for human research was obtained from the Wayne State University Human Investigation Committee.

Maternal alcohol, smoking, and drug use

The mother was interviewed at each prenatal clinic visit (mean = 5.3 visits) regarding her drinking during the previous 2 weeks using a timeline follow-back interview (Jacobson et al., 2002). Volume consumed was recorded for each type of alcohol beverage and converted to oz of absolute alcohol (AA) using weights proposed by Bowman et al. (1975; liquor—0.4, beer—0.04, wine—0.2). Average oz AA/day across pregnancy was calculated. Due to wide variability in the dosage and degree of purity of illicit drugs, data were summarized in terms of the average number of days/month for cocaine, opiates (e.g., heroin, methadone, codeine), and marijuana. Details regarding assessment of illicit drug use are provided in Jacobson et al. (1993).

Control variables

Data were obtained on demographic background variables, including number of prior pregnancies, maternal age at delivery, years of education, and marital status. The Hollingshead (Hollingshead, 1975) Scale for Socioeconomic Status was administered at 6.5 months and 14 years. Stressful life events were assessed in separate maternal and child interviews at 14 years (Holmes and Rahe, 1967; Yumoto et al., 2008). Weight, length/height, and head circumference were measured by a trained research assistant, who was blind with respect to prenatal alcohol, smoking, and drug exposure, at each postnatal visit using methods described in Carter et al.(2013). Z-scores were calculated for weight-for-age and length- or height-for-age as well as weight-for-length at birth and body mass index (BMI) at ages 14 and 19 using 2000 Centers for Disease Control (CDC) norms (Kuczmarski et al., 2002).

Testosterone concentration assays

5-mL saliva samples were obtained for testosterone analysis from a subset of 114 adolescents (63 males, 51 females) from the larger cohort of children seen at the 14-year visit. The samples were collected in the morning upon the participant's arrival at the laboratory, at least 1 hour had elapsed since the participant's last meal. Samples were frozen at −70°C and subsequently sent in batches on dry ice via overnight delivery to Salimetrics laboratories (State College, PA), where they were assayed for testosterone using an enzyme immunoassay specifically designed for use with saliva according to the manufacturer's recommended protocol without modification (Granger et al., 1999; 2004). The assay's test volume is 50 μL with a range of sensitivity from 1.5 to 360 pg/mL, and the average inter- and intra-assay coefficients of variation were less than 15% and 5% respectively. All saliva samples were assayed in duplicate, and the average of the duplicates was used in all analyses.

Pubertal development

Tanner stages for breast and pubic hair development for females and for genital and pubic hair development for males were assessed at the 14-year visit for 178 adolescents, after their primary caregivers were shown the Tanner stage drawings (Marshall and Tanner, 1969; 1970) and gave us permission to show them to their children. The adolescent was shown the drawings for each Tanner stage and asked to indicate which drawing best represented his/her current development. Females were also asked to provide their age at first menses.

Statistical analyses

All variables were checked for normality of distribution. AA/day during pregnancy was positively skewed (skew > 3.0) and normalized by means of log(X + 1) transformation. For both cocaine and marijuana use during pregnancy, frequency of use was equally distributed throughout the reported range among users, and women were categorized to create five similarly-sized groups based on days/month of use (0; 0-0.99; 1-2; > 2–4; > 4). For maternal smoking, which had a normal distribution with a few extreme outliers (> 3 SD from the cohort mean), the seven outliers were transformed by recoding to one point greater than the highest observed value (Winer, 1971).

Using SPSS (IBM, Armonk, NY), the relation of prenatal alcohol exposure and the control variables to the following outcomes was examined separately for males and females using Pearson *r*: child salivary testosterone concentration, Tanner stages (pubic hair, genital development (males), breast development (females), sum of the Tanner stage subtypes), and age at first menses (females). Any outcome that was related to prenatal alcohol exposure at $p < .10$ was then examined in a multiple regression analysis in relation to prenatal alcohol and any control variables related to the given outcome at $p < .10$.

RESULTS

Maternal characteristics, alcohol consumption, and drug use

At recruitment, 62.6% of the women were high school graduates (Table 1). Thirty-seven percent were nulliparous, and 47.9% reported 1-2 prior pregnancies. Among drinkers, women reported drinking an average of 5.2 standard drinks/occasion at conception and 4.2 drinks/occasion across pregnancy. Alcohol consumers also reported drinking on an average of 12.2 days/month at conception and 4.5 days across pregnancy. Smoking was common (62.5%) with 33.9% of smokers averaging ≥ 1 pack/day. Marijuana use was common (30.6%), with almost 25% reporting monthly use and almost 20% reporting weekly use. Of the 91 women (34.3%) reporting any cocaine use, 48 (52.8%) reported heavy use (≥ 2 days/week), and of these, only three reported drinking one or more drinks/day on average. Twenty-five women (9.4%) reported opiate use, with 16 using opiates at least once monthly.

Child characteristics

Based on assessments performed at 7.5 years, only three children met criteria for a diagnosis of FAS (Jacobson et al., 2004). At age 14, 17.6% met CDC definition for obesity (BMI ≥ 95th %ile), and an additional 13.1% were overweight (BMI between 85th and 95th %iles).

Primary caregivers reported higher perceived stress in their children's lives at 14 years than the adolescents reported themselves ($t(224) = 4.86$, $p < .001$).

As expected, salivary testosterone concentrations at 14 years were higher for males than females ($t(110) = 6.62$, $p < .001$) and consistent with previously published norms for both males and females (Matchock et al., 2007). Self-reported Tanner stages were 4 or 5 in more than 90% of the males for pubic hair and more than 85% for genital development. Similarly, more than 90% of the females reported pubic hair Tanner stages of 4 or 5, and more than 70% reported breast development stages of 4 or 5. Tanner stage subtypes were moderately intercorrelated for both males ($r = .47$, $p < .001$) and females ($r = .49$, $p < .001$). All females reported that their first menstrual period had occurred prior to their 15th birthday with a median age at first menses of 12 years. For females, younger age at first menses predicted a higher sum of Tanner breast ($r = -.35$, $p < .001$and pubic hair stages ($r = -.27$, $p = .008$). Children with more advanced pubertal development at 14 years were more likely to be closer to their adult height, as indicated by inverse associations between sum of Tanner stages and change in height between ages 14 and 19 for both males ($r = -.25$, $p = .056$) and females ($r = -.32$, $p = .045$). BMI *Z*-score at age 14 was associated with higher summed Tanner stages for males ($r = .17$, $p = .036$) and females ($r = .42$, $p < .001$) and with earlier age at first menses for girls ($r = -.22$, $p = .032$).

Correlates of salivary testosterone concentration

Adolescent testosterone concentration was not related to maternal cigarette smoking, marijuana use, or cocaine use during pregnancy among males or females. By contrast, higher average daily maternal alcohol consumption was associated with higher adolescent salivary testosterone concentration, after controlling for potential confounders, for both males and females (Table 3). In both males and females, testosterone concentrations among the heaviest exposed children were still within the range seen for unexposed children. Using ANCOVA, bar graphs were created to examine mean salivary testosterone levels, adjusted for potential confounders, by prenatal alcohol exposure level (Fig. 1). For males, the effect on testosterone was mainly seen at exposure levels of 0.5 oz AA/day or higher. For females, a linear effect was seen at lower levels of exposure (AA/day >0.1 oz). From a study in which paired serum and saliva samples were assayed, Salimetrics Laboratories (2012) provides parameters to estimate serum testosterone from salivary measures. Based on these parameters, the salivary values in Figure 1 correspond to serum testosterone levels of 6.9 ng/mL, 6.8 ng/mL, and 8.3 ng/mLin the three groups of males, respectively, and 0.5 ng/mL, 0.6 ng/mL and 0.6 ng/mL for the three groups of females, respectively.

Correlates of pubertal development

Prenatal alcohol exposure was not related to Tanner stages in males or females or to age at first menses for females (Table 2). In light of the effect of alcohol exposure on testosterone concentrations in the males, we examined the degree to which this exposure might alter the normal relation between testosterone and pubertal development (Table 4). For these analyses prenatal alcohol exposure was dichotomized to compare moderate-to-heavy [AA/day across pregnancy ≥ 1.0 OR binge drinking (AA/occasion ≥ 2.0) at least monthly, $n = 19$] vs. light-to-no exposure (exposure < heavy-to-moderate, $n = 43$). In univariate analyses, testosterone

concentration was weakly associated with male pubic hair Tanner stage for the sample as a whole, $r = .26$, $p = .058$, although not to genital development Tanner stage, $r = .08$, $p > .20$. Multivariable models including a prenatal alcohol exposure group X testosterone interaction term and potential confounders revealed interaction effects between prenatal alcohol exposure and testosterone on Tanner stages. In regressions stratified by alcohol exposure (Table 5), testosterone was related to pubic hair development ($\beta = .36$, $p = .041$) among the males with light-to-no prenatal alcohol exposure but not among those with moderate-to-heavy prenatal alcohol exposure ($p = .495$). Similarly, the relation of testosterone to genital development fell just short of statistical significant for males with light-to-no prenatal alcohol exposure ($\beta = .29$, $p = .091$) but was not seen in those with moderate-to-heavy prenatal alcohol exposure ($p = .215$).

DISCUSSION

In this prospective, longitudinal cohort study, prenatal alcohol exposure was related to increased salivary testosterone concentrations in both males and females at age 14 years. These effects were independent of other maternal drug use or smoking during pregnancy and persisted after adjusting for potential confounders. Although relatively small numbers of subjects at the highest levels of exposure in this study make the identification of true exposure thresholds for the effects seen difficult, effects on testosterone for males appeared at moderate-to-heavy levels of prenatal alcohol exposure (Fig. 1). In females, we saw effects at lower levels of prenatal exposure (0.1 oz AA/day) than are generally found in the FASD literature, although we did observe effects at these low levels in this cohort in one recent study examining the association of prenatal alcohol to gray matter volume measured using MRI in young adulthood (Eckstrand et al., 2012).

The mechanisms underlying these effects are as yet unknown. Our finding that alcohol-related increases in testosterone were seen in both males and females suggests that these increases were likely due to teratogenic effects of alcohol in the hypothalamus, the pituitary, and/or the adrenal glands rather than direct effects on ovarian and testicular function. Alcohol-related disruptions in HPG and/or HPA development (e.g., Jacobson et al., 1999) may lead to reductions in the normal testosterone surge that occurs in the first year of life, as seen in animal models (McGivern et al., 1988; 1993), setting the stage for increases in testosterone in adolescence. However, little is known about how such alterations in the infant testosterone surge might impact basal hormone levels in adolescence and adulthood. Only one animal model of alcohol effects on HPG development examined post-pubertal basal testosterone and found no effect s (Udani et al., 1985). This study used an exposure model that would correspond to late gestation and postnatal exposure in humans. Further studies examining testosterone in infants with prenatal alcohol exposure are needed to examine potential alcohol-related disruptions in HPG development and their downstream effects on basal hormonal levels in adolescence and adulthood.

Alternatively, teratogenic effects on the hypothalamus and/or pituitary *in utero* may be latent until puberty, when HPG axis maturation leads to a rise in testosterone levels. Given the increased sensitivity to stress in alcohol-exposed animals, which can reduce testosterone secretion through LH inhibition by corticosterone, it is possible that the adolescent

testosterone reduction reflected a secondary effect of HPA axis alterations on the HPG axis. Disruption of HPA axis development has been demonstrated in FASD animal models (e.g., Weinberg et al., 2008; Lan et al., 2006) and human studies (Jacobson et al., 1999; Oberlander et al., 2010), as have interaction effects between stress and alcohol on HPA development (e.g., Schneider et al., 2004). In the current study, life stress was positively correlated with testosterone in both males and females and accounted for a small portion of the effects of alcohol exposure on testosterone. The increased testosterone levels seen could also be the result of increased secretion by the adrenal gland, resulting from alcohol-related disruptions in the HPA axis that become apparent in middle childhood with adrenal maturation. Such HPA disruptions may also affect homeostasis of other sex hormones, such as estrogen, and further studies are needed to evaluate potential effects of prenatal alcohol exposure on these hormones.

We did not find effects of prenatal alcohol exposure on timing of pubertal development, as indicated by Tanner stages or age at menarche. These findings are consistent with animal model findings in males but contrast with studies demonstrating alcohol-related pubertal delays in females. This discrepancy may be due to differences between rat and human species or experimental models. Alternatively, pubertal delays may be subtle or short-term and thus may not have been revealed by our cross-sectional assessment of Tanner stages in which a large majority of children in this cohort were already in stages four or five. We found that prenatal alcohol exposure disrupted the normal relation between testosterone and male Tanner stages seen in children with light-to-no exposure, suggesting that prenatal alcohol exposure blunted the effects of testosterone on pubertal development. These findings help explain why prenatal alcohol exposure did not appear to accelerate male pubertal development despite higher testosterone levels in children with alcohol exposure. These interaction effects between alcohol and testosterone may be due to other, unmeasured hormonal factors, or to decreased tissue sensitivity to testosterone. In elegant studies examining teratogenic effects of alcohol on the HPA and HPG axes in rat models, Weinberg et al. (2008) have demonstrated decreased responsiveness to testosterone in the hypothalamus, which may have been due to down-regulation of androgen receptor as evidenced by decreased androgen receptor mRNA. In the current study, prenatal alcohol exposure may have led to decreases in androgen receptors and, in turn, decreased testosterone responsiveness in the HPG axis and other tissues related to pubertal development. Similar decreases in androgen receptors have been shown to result from low testosterone in infancy, much like the alcohol-related blunting of the early postnatal testosterone surge seen in animal models. Bingham and Viau (2008) demonstrated decreases in adult androgen receptors in the stria terminalis and amygdala of rats gonadectomized in infancy. Androgen receptor levels were normalized by testosterone treatment during infancy but not by treatment during adulthood, indicating that low testosterone in infancy was responsible for the long-term decreases in androgen receptors. Thus, alcohol-related blunting of the normal testosterone surge of infancy, like that seen in McGivern and colleagues' study (1993), may be the underlying cause of the decreased testosterone responsiveness in the hypothalamus in Weinberg et al.'s study as well as the disruption of the normal relation between alcohol testosterone and pubertal development in our study. Furthermore, decreases in androgen receptors in the HPG and/or HPA axis may disrupt the

physiologic negative feedback loop between androgen levels and androgen production and thus be partly responsible for the increases in testosterone we saw in our cohort. Further studies are needed to test these hypotheses in a single model.

Salivary testosterone concentrations and predicted serum concentrations for unexposed children in this study were consistent with previously published norms for adolescent males and females (Konforte et al., 2013; Matchock, 2007; Ross et al., 1986). Furthermore, values stratified by Tanner stage were consistent with those seen in the CALIPER study (Konforte et al., 2013), a multi-ethnic cohort study of 1,234 children designed to establish pediatric reference norms. Thus, our data further validate the use of salivary testosterone and the use of the Salimetrics testosterone enzyme immunoassay in studies examining testosterone.

This study had limitations common to other longitudinal studies of development. Although the mothers in this study were recruited from the same inner city community, numerous unmeasured environmental, dietary, and genetic influences on the HPG axis and pubertal development may exist. We did, however, examine potential confounding effects of suspected factors, such as socioeconomic status, life stress as reported by both mother and child, and other prenatal exposures. Noise surrounding true estimates of true alcohol exposure to the fetus may obscure some effects, but such noise is likely minimal given the validity of maternal reports using our timeline follow-back interviewing methods demonstrated across multiple outcomes in this cohort (Jacobson et al., 2002) and in relation to meconium assays in the Cape Town Longitudinal Cohort (Bearer et al., 2003). Tanner stages were self-reported and thus vulnerable to bias among subjects. However, the Tanner stages in this cohort were consistent with those expected for age (Herman-Giddens et al., 2012; Susman, 2010). Furthermore, moderate intercorrelation between Tanner subtypes and between female Tanner stages and age at menarche as well as associations between Tanner stage and closeness to adult height all support validation of the Tanner data collected in this study. Although testosterone levels have been shown to differ between ethnic/racial groups for males (Ellis and Nyborg, 1992), such differences are unlikely to have contributed to our findings since all subjects in this study were African American.

This study is the first to report a relation between prenatal alcohol exposure and increased basal testosterone during adolescence in humans, extending current knowledge of alcohol-related disruptions in the HPG and HPA axes. Future studies in humans are needed to determine when in development these effects begin, examine whether they persist or change later in life, and evaluate underlying mechanisms. Our findings of interaction effects between alcohol and testosterone suggest decreased testosterone responsiveness in tissues related to pubertal development as have been seen in the hypothalamus. Further studies examining expression of androgen receptors and other hormonal and cellular factors affecting pubertal development may reveal important mechanisms underlying these teratogenic effects of alcohol exposure.

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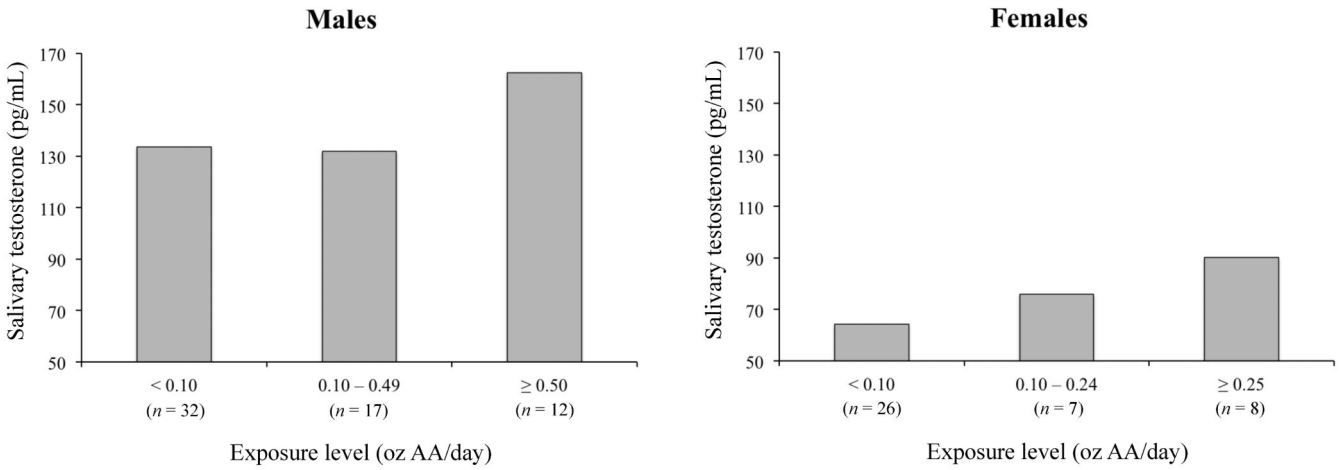

Figure 1.

Mean salivary testosterone concentrations (adjusted for age, socioeconomic status, and adolescent self-reported life stress for the males; and for age and maternal report of adolescent life stress for the females) by prenatal alcohol exposure group.

Table 1

Sample characteristics

	N	Mean or *n*	(SD) (%)	Range
Maternal characteristics				
Parity (number of prior pregnancies)	265	1.3	(1.5)	0.0 – 10.0
Gestational age at recruitment (weeks)	265	23.4	(8.0)	6.0 – 37.0
Age at delivery (years)	265	26.9	(6.0)	14.1 – 43.9
Prepregnancy weight (kg)	265	65.1	(19.1)	41.8 – 159.1
Years of school completed	265	11.8	(1.6)	7.0 – 18.0
Marital status (married)	265	30	(11.3)	
Socioeconomic status[1]				
Infancy	224	20.0	(8.1)	11.0 – 56.0
14-year visit	265	29.7	(10.0)	8.0 – 66.0
Perceived life stress at 14-year visit[2]	236	31.1	(22.7)	0.0 – 154.0
Number of stressful events[2]	236	6.9	(4.5)	0.0 – 26.0
Maternal prenatal alcohol, smoking, and drug use (users only[3])				
Average oz absolute alcohol (AA)/day				
At conception	213	1.2	(2.0)	0.01 – 24.8
During pregnancy	202	0.4	(0.8)	0.01 – 6.5
Average oz AA/drinking day				
At conception	213	2.6	(3.4)	0.1 – 25.6
During pregnancy	202	2.1	(2.4)	0.2 – 24.8
Average number drinking days/month				
At conception	213	12.2	(8.4)	4.4 – 30.5
During pregnancy	202	4.5	(5.1)	0.2 – 30.5
Cigarettes/day	168	14.3	(10.3)	1.0 – 41.0
Marijuana use (days/month)	78	3.4	(3.9)	0.1 – 19.2
Cocaine use (days/month)	91	3.9	(3.7)	0.1 – 17.0
Opiate use (days/month)	25	2.5	(2.5)	0.1 – 9.0
Child characteristics				
Age at 14-year visit (years)	265	14.4	(0.6)	13.3 – 16.4
Weight-for-age *Z*-score	261	0.9	(1.1)	−3.8 – 3.7
Length-for-age *Z*-score	261	0.2	(1.1)	−3.8 – 3.8
Body mass index (BMI)	261	24.2	(6.1)	15.4 – 46.9
BMI *Z*-score	261	0.9	(1.1)	−2.4 – 3.0
Number of stressful events[2]	236	3.6	(2.4)	0.0 – 14.0
Perceived life stress (caregiver report)[2]	226	18.5	(10.0)	2.0 – 66.0
Perceived life stress (child report)[2]	226	14.0	(10.8)	0.0 – 61.0
Testosterone concentration: males (pg/mL)	62	141.1	(66.6)	14.1 – 303.1

	N	Mean or *n*	(SD) (%)	Range
Testosterone concentration: females (pg/mL)	50	69.9	(47.0)	4.8 – 212.0
Pubic hair Tanner stage: males	149	4.3	(0.6)	2–5
Genital development Tanner stage: males	150	4.1	(0.7)	1 – 5
Pubic hair Tanner stage: females	102	4.5	(0.7)	2 – 5
Breast development Tanner stage: females	104	3.9	(0.8)	2 – 5
Age at first menses: females (yr)	100	11.9	(1.2)	8 – 14

[1] Hollingshead Four Factor Index of Social Status (Hollingshead, 1975).

[2] Matemal reports of self and on behalf of child using Life Events Scale (Holmes and Rahe, 1967).

[3] Data available for all 265 mothers.

Table 2

Correlates of adolescent testosterone concentration, Tanner stage subtypes, and age at menarche (Pearson *r*'s)

	Males			Females			
	Testosterone	**Pubic hair Tanner stage**	**Genital Tanner stage**	**Testosterone**	**Pubic hair Tanner stage**	**Breast Tanner stage**	**Age at menarche**
Maternal characteristics							
Parity	−.04	.02	.01	−.13	−.14	−.08	.21*
Age at delivery	.08	.01	−.19*	−.18	−.09	−.08	.13
Prepregnancy weight	−.03	.03	.12	.05	.14	.10	.06
Education (years school completed)	.19	−.02	−.21*	−.13	.21*	.31**	−.19†
Marital status	.08	−.08	.04	.18	−.04	−.05	−.06
Socioeconomic status							
Infancy	.02	.09	.05	.05	.14	.02	.07
14-year visit	.29*	.05	−.09	.09	.19	.22*	−.17†
Perceived life stress at 14-year visit	−.02	−.02	−.05	.17	−.06	−.03	−.02
Number of stressful events	.01	.01	−.02	.20	−.07	.00	−.01
Maternal alcohol and drug use							
Average daily alcohol consumption	.25*	−.03	−.12	.27†	−.09	−.09	.23
Average drinks per occasion	.11	.11	.12	.21	.04	−.07	.05
Frequency of drinking (days/week)	.28*	−.10	.23**	.13	−.15	−.05	.12
Cigarette smoking	.02	.07	−.08	−.11	.09	.01	.17
Marijuana use	−.19	−.13†	−.13	−.15	.00	−.17†	.08
Cocaine use	.16	.09	−.13	−.17	−.03	−.09	−.09
Child characteristics							
Age at 14-year visit	.38**	.14	.13	.37**	.10	.21*	.10
Child's perceived stress (caregiver report)	−.11	−.03	.12	.41**	.08	.13	−.06
Child's perceived stress (child report)	.24 †	−.09	−.06	.09	.00	.01	.02
Number of stressful events	.21	−.11	−.04	.14	.01	−.02	−.04

†$p \leq 0.10$.

* $p \leq 0.05$.

** $p \leq 0.01$.

*** $p \leq 0.001$.

Table 3

Relation of maternal alcohol and potential confounders to salivary testosterone concentrations

	Males		Females	
	r [1]	ß [1]	*r* [1]	ß [2]
Maternal average daily alcohol consumption	.25*	.26*	.41**	.29*
Age at time of measurement	.38**	.36**	.38**	.18
Child's perceived stress (caregiver report)	--	--	.41**	.35**
Child's perceived stress (child report)	.24 †	.14	--	--
Socioeconomic status, age 14	.29*	.22 †	--	--

† $p \leq 0.10$.

* $p \leq 0.05$.

** $p \leq 0.01$.

*** $p \leq 0.001$.

[1] Pearson correlations.

[2] Standardized regression coefficients from multivariable models adjusting for potential confounders with $p < 0.10$ in univariate analyses.

Table 4

Relation of testosterone concentration to Tanner stage among males and the impact of prenatal alcohol exposure

	Pubic hair Tanner stage				Genital development Tanner stage			
	r [1]	*p*	ß [2]	*p*	*r* [1]	*p*	ß [2]	*p*
Testosterone	.26	.058	.31	.042	.08	.561	.27	.064
Alcohol exposure group [3]			.18	.258			.24	.145
Interaction term			−.29	.085			−.49	.004

[1] Pearson correlations.

[2] Standardized regression coefficients from multivariable models including testosterone, alcohol exposure group, and alcohol exposure group × testosterone interaction term, and potential confounders (maternal marijuana use for pubic hair and maternal age at delivery and education for genital development).

[3] Heavy-to-moderate exposure [AA/day across pregnancy ≥1.0 OR binge drinking (AA/occasion ≥ 2.0) at least monthly, $n = 19$] vs. light-to-no exposure (exposure < heavy-to-moderate, $n = 43$).

Table 5

Relation of testosterone concentration to Tanner stage among males stratified by alcohol exposure group[1]

	Pubic hair Tanner stage				Genital development Tanner stage			
	Heavy-to-Moderate exposure		Light-to-no exposure		Heavy-to-Moderate exposure		Light-to-no exposure	
	r[2]	ß[3]	r[2]	ß[3]	r[2]	ß[4]	r[2]	ß[4]
Testosterone	−.21	−.18	.37*	.36*	−.32	−.32	.26†	.29†

† $p \leq 0.10$.

* $p \leq 0.05$.

** $p \leq 0.01$.

*** $p \leq 0.001$.

[1] Heavy-to-moderate exposure [AA/day across pregnancy ≥1.0 OR binge drinking (AA/occasion ≥ 2.0) at least monthly, $n = 19$] vs. light-to-no exposure (exposure < heavy-to-moderate, $n = 43$).

[2] Pearson correlations.

[3] Standardized regression coefficients adjusted for maternal marijuana use.

[4] Standardized regression coefficients adjusted for maternal age at delivery and education.