Testosterone during Pregnancy and Gender Role Behavior of Preschool Children: A Longitudinal, Population Study

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Levels of testosterone (T) and sex hormone-binding globulin (SHBG) were measured in blood samples from pregnant women and related to gender role behavior in 342 male and 337 female offspring at the age of 3.5 years. Gender role behavior was assessed using the Pre-School Activities Inventory, a standardized measure on which a parent indicates the child’s involvement with sex-typical toys, games, and activities. Levels of T, but not SHBG, related linearly to gender role behavior in preschool girls. Neither hormone related to gender role behavior in boys. Other factors, including the presence of older brothers or sisters in the home, parental adherence to traditional sex roles, the presence of a male partner in the home, and maternal education, did not relate to gender role behavior in this sample and did not account for the relation observed between T and behavior. Although other, unmeasured factors may explain the relation, the results suggest that normal variability in T levels prenatally may contribute to the development of individual differences in the gender role behavior of preschool girls.

INTRODUCTION

Sex differences in childhood gender role behavior, including toy, playmate, and activity preferences, develop as a consequence of numerous influences, involving social, cognitive, and biological processes. Boys and girls are socialized differently as to the types of toys and activities that are appropriate. For instance, parents, teachers, and peers provide more positive reinforcement for sex-congruent play than for play that is not sex-congruent (Fagot 1977, 1978; Langlois & Downs, 1980; Lytton & Romney, 1991; Snow, Jacklin, & Maccoby, 1983). In addition, children develop a cognitive understanding of gender and of sex-appropriate toys and activities, and come to value these. This can be seen in their more positive feelings about themselves and other children when they play in gender-appropriate versus gender-inappropriate ways (Bussey & Bandura, 1992), in their imitation of same-sex over other-sex models (Huston, 1983; Perry & Bussey, 1979), and in their preferences for objects labeled as being for children of their own sex (Masters, Ford, Arend, Grotevant, & Clark, 1979). With regard to biological influences, gonadal hormones may play a role in the development of gender-typical behavior. Most notably, girls exposed to high levels of androgens prenatally because of the genetic disorder congenital adrenal hyperplasia (CAH), show increased preferences for masculine-typical toys and activities (Berenbaum & Hines, 1992; Dittmann et al., 1990; Ehrhardt & Baker, 1974; Ehrhardt, Epstein, & Money, 1968; Slijper, 1984). This relationship between prenatal androgen and childhood gender role behavior is the best-established link between the early hormone environment and human psychosexual development.

The hypothesis that androgen could contribute to the development of human gender role behavior derives largely from evidence that androgens influence neural and behavioral sexual differentiation in other mammals (Collaer & Hines, 1995; Goy & McEwen, 1980). Beginning early in gestation the testes produce high levels of androgens, particularly testosterone (T), which then enters the brain, where it (or hormones produced from it) stimulates receptors, resulting in enhanced masculine-typical development and impaired feminine-typical development (Goy & McEwen, 1980). The neural characteristics influenced by testicular hormones include the size of cells, cell death and survival, dendritic growth, and synapse formation (e.g., Arnold & Gorski, 1984; Matsumoto, 1991). These hormonal influences have been documented in a wide range of mammals, including rodents as well as non-human primates. Typically, they occur during critical periods of early development that correspond to times when T levels are higher in males than in females. In the human, these times appear to be weeks 8 through 24 of gestation, and the first 6 months of infancy (Smail, Reyes, Winter, & Faiman, 1981).

Because hormones influence basic processes of brain development, they also exert permanent influences on behavior. For example, female rats treated with a single injection of T on the day of birth show...
reduced female-typical sexual behavior and increased male-typical sexual behavior in adulthood (Goy & McEwen, 1980). Similar hormonal treatments also influence nonreproductive behaviors that show sex differences (i.e., that differ, on average, for male and female animals; Collaer & Hines, 1995; Goy & McEwen, 1980). An example is rough-and-tumble play, which is more common in juvenile males than females. In both rats and rhesus monkeys, genetic female animals treated with T during critical periods of prenatal or early postnatal life show increased levels of this male-typical play behavior as juveniles (Goy, Bercovitch, & McBrair, 1988; Meaney & Stewart, 1981).

The developmental influences of T on the mammalian brain and behavior are dose dependent, with effects being more dramatic in animals exposed to more hormone (Goy & McEwen, 1980; Mullins & Levine, 1968). In addition, normal variability in hormones prenatally appears to influence the development of individual differences in sex-typical behavior within a given sex. Female rodents are behaviorally masculinized by proximity to males in utero (Clark & Galef, 1998; Meisel & Ward, 1981). Those located adjacent to male littermates show more masculine-typical and less feminine-typical behavior than those located adjacent to other females. This is thought to occur because they are exposed to blood containing T from male littermates.

Information regarding similar hormonal influences on human development is limited. As noted above, girls exposed to high levels of androgen prenatally because of CAH show more masculine-typical behavior. The altered behaviors include increased preferences for boys’ toys, boys’ activities, and boys as playmates (Berenbaum & Hines, 1992; Dittmann et al., 1990; Ehrhardt & Baker, 1974; Ehrhardt et al., 1968; Slijper, 1984). However, the relevance of these findings to normal sexual differentiation has been questioned, in part because the prenatal androgen elevation caused by CAH masculinizes the external genitalia. Typically, the genitalia are surgically feminized early in life and hormonal treatment regulates postnatal androgen production. Nevertheless, it has been suggested that the masculine appearance of the genitalia at birth could alter parental perceptions or self-perceptions of femininity and thus influence sex-typical behavior in girls (Fausto-Sterling, 1992; Quadagno, Briscoe, & Quadagno, 1977). A second concern is that the experience of illness itself could alter behavior in girls with CAH, because the disorder and its treatment typically involve surgery during infancy, and, in most cases, additional hospitalization for salt-losing crises associated with the adrenal hormone abnormality (Slijper, 1984). In addition, even if behavioral alterations in girls with CAH are caused by prenatal exposure to androgen, this would not necessarily imply that prenatal variability in T within the normal range influences the development of gender role behavior in children without CAH.

Attempts to determine whether normal variability in hormones contributes to human behavioral sex differences have produced inconsistent results. Androgen in umbilical cord blood at birth has been found to relate inversely to spatial ability in girls at the age of 6 years (Jacklin, Wilcox, & Maccoby, 1988). Similarly, T and progesterone have been found to relate negatively, and estradiol positively, to timidity in boys at the age of 6 to 18 months (Jacklin, Maccoby, & Doering, 1983). Assuming that spatial ability is something at which males excel, and timidity is more common in females, some of these results are consistent with predictions based on animal models (e.g., the negative relationship between T and timidity), whereas others are not (e.g., the negative relationship between androgen and spatial ability). Another study also found a negative relationship in girls between T in amniotic fluid and performance at the age of 4 years on block building and on tasks that assess counting and number facts (Finegan, Niccols, & Sitarenios, 1995). These results again contradict predictions, if it is assumed that males perform better on measures involving numbers and spatial stimuli. A study of these same children at the age of 7 years produced results more consistent with predictions. At this age, girls with higher levels of T in amniotic fluid performed a mental rotations procedure (on which boys generally excel) faster than did girls with lower levels, although their accuracy was not improved (Grimshaw, Sitarenios, & Finegan, 1995). A final study related maternal hormones during pregnancy to sex-typical behavior in adult female offspring (Udry, Morris, & Kovenock, 1995). Exposure to T during the second trimester of pregnancy, as reflected in levels of sex hormone-binding globulin (SHBG, a hormone that limits the ability of T to act) in combination with T levels in adulthood, predicted masculine-typical ratings on a broad measure of gender role behavior.

Methodological limitations could explain the lack of support in some studies for predicted relationships between normal variability in the prenatal hormone environment and subsequent behavior. Measures used in the studies that did not find predicted relationships did not focus on childhood gender role behaviors, and, in fact, the majority of measures did not show sex differences. For example, Jacklin et al. (1988) correlated androgen with a spatial factor that they obtained via a principal components analysis of scores on four cognitive measures. The spatial factor did not
show a sex difference in their study and has not been used in other studies. Similarly, Finegan et al. (1992) assessed block building, counting, and number facts using the Block Building, Counting, and Number Facts and Concepts tasks from the McCarthy Scales of Children’s Abilities, none of which showed a sex difference in their sample. Separate research suggests that the measures also do not show sex differences in other samples (Tierney, Smith, Axworthy, & Ratcliffe, 1984). Because animal models suggest that gonadal hormones influence only characteristics that show sex differences (Collaer & Hines, 1995), measures that do not show reliable sex differences are unlikely to be able to detect the predicted relationships. Another problem is that the studies depended on small samples, further limiting their power.

In the current study, we related maternal levels of T and SHBG during pregnancy to gender role behavior in male and female offspring from a longitudinal, population sample. We examined the hypothesis that higher levels of T (or lower levels of SHBG) predict more masculine-typical gender role behavior in girls. We also examined the hypothesis that higher levels of T (or lower levels of SHBG) predict more masculine-typical gender role behavior in boys. However, for three reasons, predictions for boys were made with less confidence than those made for girls. First, although numerous studies of female rodents suggest that normal variability in the early hormone environment predicts variability in sex-related behavior (Clark & Galef, 1998), only limited data are available for males. Second, studies in which developing male animals have been treated with androgen have produced inconsistent results; T sometimes increases masculine-typical characteristics, sometimes reduces them, and sometimes leaves them unchanged (Baum & Schretlen, 1975; Diamond, Llacuna, & Wong, 1973; Dohler et al., 1984). This contrasts with outcomes for female animals in which treatment with T consistently promotes the development of masculine-typical behavior (Goy & McEwen, 1980). Finally, prior studies of human beings exposed to unusual levels of T or other hormones prenatally, as well as prior studies of normal variability in hormones during human development, have produced more evidence linking hormones to behavior in females than in males (Collaer & Hines, 1995).

METHOD
The data analyzed for this study were collected as part of the Avon Longitudinal Study of Parents and Children (ALSPAC: formerly called the Avon Longitudinal Study of Pregnancy and Childhood), which is measuring biological, environmental, and social factors associated with pregnancy outcomes and child health. Full details of the study are given in Golding, Pembrey, Jones, & the ALSPAC Study Team (2001).

Participants
The ALSPAC cohort consists of all pregnant women who were resident within a geographically defined area of Avon, U.K. and had expected delivery dates between April 1, 1991 and December 21, 1992. A total of 13,998 pregnant women enrolled in ALSPAC, representing approximately 90% of all pregnancies occurring in the geographical area during the defined time period. The cohort of 13,998 pregnant women produced 14,138 children. Data from 679 of these children were analyzed for the present study.

Procedures
Gender role behavior. Gender role behavior was assessed using the Pre-School Activities Inventory (PSAI), a questionnaire measure on which parents indicate their child’s involvement in various sex-typed behaviors (Golombok & Rust, 1993a, 1993b). The PSAI includes 24 items measuring the child’s frequency of play with respect to a variety of sex-typical toys, games, and activities. Each item is scored on a 5-point scale ranging from “never” to “very often.” Higher scores on the inventory represent more masculine-typical behavior and lower scores represent more feminine-typical behavior. The mother or the child’s primary caretaker completed the inventory when the child was 3.5 years of age.

The PSAI is a psychometrically constructed screening instrument specifically designed to differentiate “masculine” and “feminine” boys and girls within a normal population sample of preschool children (i.e., to differentiate within, as well as between, the sexes). The measure has been standardized on more than 2,000 children in the United Kingdom, the Netherlands, and the United States (Golombok & Rust, 1993a). Mean scores on the inventory do not differ for children from these three countries and for the entire group are 61.66 (SD = 9.40) for boys and 38.72 (SD = 9.66) for girls. Test–retest reliability over a 1-year period was .62 for boys and .66 for girls, and split-half reliability was .66 for boys and .80 for girls. The inventory has been validated by comparing parental ratings to teacher ratings of children attending five different day-care centers. For boys, the correlation between parent and teacher ratings was .37 and for girls it was .48.

We used PSAI scores to select six groups of chil-
dren for hormone assays: boys (n = 128) and girls (n = 113) with extremely masculine PSAI scores, boys (n = 112) and girls (n = 118) with extremely feminine PSAI scores, and boys (n = 102) and girls (n = 106) randomly selected from among the remaining children. Mean (and SD) PSAI scores for masculine, medium, and feminine boys were 79.92 (6.16), 62.15 (6.53), and 44.07 (4.64), respectively; and for masculine, medium, and feminine girls were 56.70 (4.70), 35.73 (7.25) and 17.54 (4.40), respectively. Scores of all feminine boys and all masculine girls were more than 1.5 SDs from mean scores for their own gender, and were within .5 SDs of mean scores for the other gender. Scores of all masculine boys and all feminine girls were more than 2 SDs from mean scores for their own gender.

Hormone measures. Maternal blood samples were obtained by venipuncture during routine prenatal medical care. The target period for obtaining samples was gestational weeks 16 to 20. However, women generally had samples taken when convenient, based on the timing of their medical appointments. As a result, the time of sampling ranged from Week 5 to Week 36 of gestation, with a mean of Week 16 and an SD of 8 weeks. Fifty-five percent of the women had blood sampled between Weeks 8 and 24 of gestation, the time of dramatic T elevation in the human male fetus. Twenty-four percent had their samples taken between Weeks 5 and 7 of gestation and 21% between Weeks 25 and 36 of gestation. Time of day when blood was sampled was not controlled. Once obtained, blood was spun to form plasma aliquots of .5 ml. Initial storage was at −20°C and long-term storage was at −70°C.

Assays of T and SHBG were conducted by the Lewis Laboratories, Southmead Hospital, U.K. Testosterone was measured using an automated chemiluminescence system (ACS) from Cheron Diagnostics (The ACS:180 assay, a competitive immunoassay using direct chemiluminescent technology). The assay shows high specificity for T and the assay range is .35 to 52.0 nmol/L. The intra-assay and inter-assay coefficients of variation are 11.3% and 13.8% at 1.7 nmol/L and 4.9% and 7.7% at 43.8 nmol/L, respectively. Sex hormone-binding globulin was measured using the DELFIA SHBG time-resolved fluorimunoassay (Wallac). The detection limit of this assay is approximately .5 nmol/L. The intra-assay and inter-assay coefficients of variation are 1.4% and 8.2% at 19.7 nmol/L and 1.8% and 10.1% at 130 nmol/L, respectively.

Background factors. The ALSPAC dataset includes approximately 10,000 variables for each pregnancy, mostly assessed by questionnaires mailed to the enrolled women. We identified background variables with theoretical links to gender development, to determine whether these factors might explain any observed relationships between hormones and gender role behavior in preschool-age children. These variables were analyzed for control purposes; that is, we wanted to determine if any relationship we might observe between hormones and gender role behavior remained after accounting for background factors that could relate to gender development. Variables of interest included maternal education, the presence of older brothers or sisters in the home, the presence of a male partner living with the mother in the home, and parental adherence to traditional sex roles.

The mother’s highest educational attainment was categorized into five levels with 1 being the lowest level and 5 the highest. The presence of older brothers or sisters in the home, and the presence of a male partner in the home formed three dichotomous variables: (1) older brother(s) present in the home or not, (2) older sister(s) present in the home or not, and (3) a male partner lives with the mother in the home or not. Parental adherence to traditional sex roles was assessed for mothers who had partners living in the home. They completed a questionnaire containing items indicating the frequency with which each partner carried out seven sex-typed household duties.

Data were analyzed using correlation coefficients and analyses of covariance (ANCOVAs). Because the data were distributed trimodally within girls and boys, pooled within-group correlation coefficients were used.

RESULTS

Initial Analyses

Prior to data analyses, variables were examined for the existence of extreme scores and for normality, using SPSS 7.5. To identify outliers, data values were recoded to standard (z) scores. Five cases had extreme z scores (in excess of 3.29) for one or more of the hormone variables and these values were not included in the data analyses. In the remaining data, the distributions of values for the two hormone measures were skewed. Therefore, a logarithmic transformation was carried out on T values and a square-root transformation was carried out on SHBG values.

Because the time during gestation when blood samples were obtained varied from one woman to another, correlations were calculated between the gestational day of blood sampling and hormone measures. In both boys and girls, hormone measurements correlated significantly with the time of sampling, with both T and SHBG increasing as the pregnancy progressed, $r(333) = .19, p < .001$, and $r(321) = .15, p < .01$, for the
correlation of T with gestational age in boys and girls, respectively; and \( r(332) = .59, p < .001 \), and \( r(324) = .52, p < .001 \), for the correlation of SHBG with gestational age in boys and girls, respectively. We also examined the relationship of maternal age to T. Maternal age correlated negatively with T in both boys, \( r(331) = -.18, p < .01 \), and girls, \( r(321) = -.16, p < .01 \), but not with SHBG in either sex, \( r(330) = -.02, ns \) for boys, and \( r(324) = -.10, ns \) for girls. Because of the significant correlations, gestational age at the time of blood sampling was included as a covariate in subsequent analyses involving SHBG; and gestational age at the time of blood sampling, as well as maternal age, were included as covariates in subsequent analyses involving T.

Maternal Hormones and Gender Role Behavior

Table 1 shows maternal hormone measures during pregnancy for masculine, medium, and feminine boys and for masculine, medium, and feminine boys. For T, the two-way (sex: male, female \( \times \) group: masculine, medium, feminine) ANCOVA indicated no significant main effects of group or sex, \( F(2, 640) = 2.95, p = .053 \) for group; \( F(1, 640) = .000, p = .99 \) for sex; but a significant interaction between group and sex, \( F(2, 640) = 3.42, p = .03 \). For SHBG, there were no significant main or interaction effects, \( F(2, 659) = 1.03, p = .38 \) for group; \( F(1, 659) = .65, p = .42 \) for sex; and \( F(2, 659) = .06, p = .95 \) for the interaction.

The absence of main effects of sex for either hormone measure indicates that levels of T and SHBG do not differ in the maternal circulation for pregnancies producing male versus female offspring.

The interaction between sex and group for T levels during pregnancy is consistent with the expectation that hormone-behavior relationships might differ for girls and boys. This interaction was explored further by conducting separate one-way ANCOVAs by sex.

For girls, ANCOVA indicated a significant relationship between maternal T and group, \( F(2, 310) = 5.80, p = .003 \). A trend analysis also indicated a significant linear trend, \( F(1, 328) = 7.39, p < .001 \), such that as maternal T levels increased, so did masculine-typical gender role behavior. The size of the difference in T levels between the feminine girls and masculine girls was .35 SDs, and maternal T during pregnancy accounted for approximately 2% of the variance in the gender role behavior of preschool girls.

Because a prior report found that SHBG related to gender role behavior in women (Udry et al., 1995), we also conducted a one-way ANCOVA on SHBG in girls. There was no main effect for SHBG, \( F(2, 325) = .29, p = .75 \). Similar ANCOVAs on data for boys indicated no relationship between T or SHBG and gender role behavior, \( F(2, 339) = .501, p = .60 \) for T; \( F(2, 338) = 1.42, p = .23 \) for SHBG.

Do Background Variables Account for the Relation Between T and Gender Role Behavior?

We next explored the possibility that the correlation between T and gender role behavior in preschool girls could be accounted for by background variables.

### Table 2  Correlations of Background Factors with Testosterone and with Childhood Gender Role Behavior in Preschool Girls

<table>
<thead>
<tr>
<th>Background Factor</th>
<th>Testosterone N</th>
<th>r</th>
<th>Gender Role Behavior N</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal education</td>
<td>309</td>
<td>.011</td>
<td>312</td>
<td>.052</td>
</tr>
<tr>
<td>Older brother(s) in the home</td>
<td>311</td>
<td>-.091</td>
<td>314</td>
<td>.067</td>
</tr>
<tr>
<td>Older sister(s) in the home</td>
<td>311</td>
<td>.018</td>
<td>314</td>
<td>-.059</td>
</tr>
<tr>
<td>Mother has a male partner living in the home</td>
<td>321</td>
<td>.002</td>
<td>326</td>
<td>-.090</td>
</tr>
<tr>
<td>Parental adherence to traditional sex roles</td>
<td>331</td>
<td>-.012</td>
<td>336</td>
<td>-.064</td>
</tr>
</tbody>
</table>

Note: Data are pooled within-groups correlation coefficients. No correlations were significant.
First, we examined correlations of the potentially relevant background variables (maternal education, the presence of older brothers or sisters in the home, a male partner living with the mother in the home, and parental adherence to traditional sex roles) with PSAI scores and testosterone. None was significant (see Table 2). In addition, we conducted an analysis in which these background variables were entered as covariates, along with the covariates, maternal age and gestational age, included in the earlier analyses. The correlation between maternal T and gender role behavior remained significant (see Table 3). Thus, these background variables cannot account for the observed relation between T and PSAI scores in preschool age girls.

**DISCUSSION**

The main finding of the present study was that T during pregnancy shows a positive, linear relationship to gender role behavior in female offspring at the age of 3.5 years. No relationship was seen between T and gender role behavior in male offspring at the same age.

A review of the sources of T in male and female fetuses and of the relationship between maternal T and fetal T is relevant to interpretation of the results. In the male fetus, the gonads differentiate into testes early in gestation and by Week 8 are producing appreciable quantities of T and other androgens (Wilson, George, & Griffin, 1981). As a consequence, the gonads are the main source of endogenous androgen in the male fetus. These testicular hormones cause the external genital structures, which are originally identical in male and female fetuses, to develop into penis and scrotum (Wilson et al., 1981). In contrast, in the female fetus, the gonads differentiate into ovaries, which produce very little androgen, or other hormones, prenatally (Reyes, Winter, & Faiman, 1973). In the absence of high levels of androgen, the external genital structures in the female develop into clitoris and labia. In both sexes, adrenal androgens are produced prenatally (Reyes et al., 1973). In the male, amounts are negligible compared with androgen production by the testes. However, because the fetal ovaries produce little or no hormone, the adrenal gland is the main source of endogenous androgen in the female fetus.

Hormones can also come from the maternal system to the fetus. Although the placenta provides some protection from maternal androgen, this protective system is not perfect. As a consequence, female offspring of women with medical conditions causing elevated androgen during pregnancy can be born with ambiguous (i.e., somewhat masculinized) genitalia (Barbieri, 1999). This is because maternal androgen has passed into the female fetus and had the same effect as did androgen produced by the testes of the male fetus. Similar evidence comes from female infants whose mothers took androgenic hormones during pregnancy for medical conditions. These infants also can be born with masculine-appearing external genitalia, because the hormones taken by the mother can pass into the fetal system (Ehrhardt & Money, 1967; Wilkins, 1960). In contrast, hormones do not appear to pass in appreciable quantities from the fetus to the mother. Androgen levels in pregnant women carrying fetuses who have CAH are not elevated, despite the high levels of androgen in the fetus. In addition, the great majority of studies that have compared levels of T or other androgens in pregnant women carrying male versus female fetuses have found no differences (Bammam, Coulam, & Jiang, 1980; Dawood & Saxena, 1977; Demisch, Grant, & Black, 1968; Forest, Ances, Tapper, & Migeon, 1971; Glass & Klein, 1981; Mizuno, Lobotsky, Lloyd, Kobayashi, & Murasawa, 1968; Nagamani, McDonough, Ellegood, & Mahesh, 1979; Reyes et al., 1973; Rivarola, Forest, & Migeon, 1968; but cf. Klinga, Bek, & Runnebaum, 1978; Meulen-berg & Hofman, 1991). We also did not find T to be elevated in mothers carrying male fetuses compared with those carrying female fetuses.

Thus, it seems unlikely that the relationship between maternal T during pregnancy and gender role behavior in female offspring occurs because fetuses with high T pass these elevated levels on to their mother. However, it is possible that T from the maternal system passes into the developing female fetus and promotes masculinize-typical development. In addition, levels of T are determined, in part, genetically, with heritability estimates ranging from 40% to 60% (Harris, Vernon, & Boomsma, 1998; Sluyter et al.,

### Table 3 Summary of Covariance Analysis Including Background Factors and Testosterone as Predictors of Gender Role Behavior in Preschool Girls

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age when blood was sampled</td>
<td>1</td>
<td>7.175</td>
<td>.008</td>
</tr>
<tr>
<td>Maternal age</td>
<td>1</td>
<td>9.030</td>
<td>.003</td>
</tr>
<tr>
<td>Older brother(s) in the home</td>
<td>1</td>
<td>.260</td>
<td>.611</td>
</tr>
<tr>
<td>Older sister(s) in the home</td>
<td>1</td>
<td>.814</td>
<td>.368</td>
</tr>
<tr>
<td>Maternal education</td>
<td>1</td>
<td>.889</td>
<td>.346</td>
</tr>
<tr>
<td>Parental adherence to traditional sex roles</td>
<td>1</td>
<td>.076</td>
<td>.783</td>
</tr>
<tr>
<td>Mother has male partner living in the home</td>
<td>1</td>
<td>1.300</td>
<td>.255</td>
</tr>
<tr>
<td>Groupa</td>
<td>2</td>
<td>4.250</td>
<td>.015</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>267</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Dependent variable = Testosterone (transformed).

A Group refers to masculine, medium, or feminine girls.
There is some evidence that this genetic connection is clearer in females than in males (Harris et al., 1998). Thus, a second possibility is that mothers with relatively high T have daughters with relatively high T, because of a genetic predisposition that is passed from mother to daughter. In this case, the female fetus’s own T could masculinize her development. We were unable to distinguish between these possibilities; however, either would be consistent with findings in other mammals in which T influences sexual differentiation of brain and behavior during early critical periods of development. In addition, both interpretations are consistent with findings from girls exposed to androgen prenatally because of CAH. Girls with CAH also show enhanced masculine-typical gender role behavior, including childhood toy, game, and activity preferences similar to those measured by the PSAI (Berenbaum & Hines, 1992; Dittmann et al., 1990; Ehrhardt & Baker, 1974; Ehrhardt et al., 1968; Slijper, 1984). Girls with CAH also show more masculine-typical scores on the PSAI itself (Fane, Brook, & Hines, 2001). Thus, the present study’s results extend prior findings by suggesting that normal variability in T levels prenatally is one factor that contributes to individual variability in gender role behavior in young girls.

A prior report found that lower maternal SHBG during pregnancy (thought to provide a measure of available T), combined with higher T levels in adult female offspring, predicted more masculine-typical gender role behavior in women (Udry et al., 1995). The present findings for preschool girls are consistent with this prior report to the extent that we also found a relationship between prenatal T and postnatal gender role behavior. However, it is not clear why the relationship in the prior study was to prenatal SHBG (but not T itself), whereas in this study we found no relationship of SHBG on its own to gender role behavior. It was not necessary to consider T levels in offspring to predict behavior in the present study for more obvious reasons. In contrast to the prior study, subjects in this study were preschool children and thus were in a period of life when gonadal hormones are at low levels. Also, the childhood behaviors assessed in this study, unlike some adult behaviors, do not require activation by postpubertal hormone elevation. Hence, in the current study, the relationship between prenatal hormones and postnatal behavior could be seen without accounting for hormone levels in offspring at the time of the behavioral assessments.

Although we observed a relationship between prenatal T and postnatal gender role behavior in girls, we did not see a similar relationship in boys. Amniotic fluid levels of T have also been found to relate to speed of mental rotations in girls, with findings less clear for boys (Grimsaw et al., 1995). Studies of individuals exposed to abnormal hormone environments prenatally also present a clearer picture for girls than for boys. Findings of more masculine-typical play behavior in girls with CAH were mentioned above. Similar outcomes have been reported for girls exposed to androgenic progestins because their mothers were prescribed these hormones during pregnancy (Ehrhardt & Money, 1967). In addition, females with CAH show more masculine-typical behavior in areas in addition to play, including, for example, sexual orientation (for review, see Collaer & Hines, 1995; Hines, 2002; Zucker, 1999). In contrast, boys with CAH have generally not been found to differ from other boys in sex-typical behavior (Collaer & Hines, 1995). When differences are found, their direction is inconsistent. Two reports (Hines & Kaufman, 1994; Slijper, 1984) suggest some evidence of less masculine-typical childhood behavior, and one (Ehrhardt & Baker, 1974) suggests some evidence of more masculine-typical childhood behavior. Studies of males exposed to exogenous hormones prenatally also have produced inconsistent results. There are occasional reports that estrogen, progesterone, or the two in combination impair some masculine-typical behaviors (Kester, Green, Finch, & Williams, 1980; Yalom, Green, & Fisk, 1975) or that they enhance them (Kester et al., 1980; Wilcox, Maxey, & Herbst, 1992). However, as the authors themselves sometimes note, the studies assessed a large number of variables, raising the possibility that the few significant findings resulted from chance.

One pair of studies assessed sex-typical play in both boys and girls exposed to the same exogenous hormone prenatally. These studies looked at children exposed to medroxyprogesterone acetate (MPA), a progestational hormone that interferes with the ability of androgen to act (Ehrhardt, Grisanti, & Meyer-Bahlburg, 1977; Meyer-Bahlburg, Grisanti, & Ehrhardt, 1977). As in the present study, girls, but not boys, appeared to be affected by variations in the prenatal hormone environment. Girls exposed to MPA (and thus, assumedly, to reduced androgen prenatally) showed more feminine-typical behavior than did control girls, whereas MPA exposed boys and unexposed boys did not differ.

There are at least two possible explanations for a more consistent relationship between prenatal hormonal variability and postnatal gender role behavior in girls than boys. First, boys have much higher levels of T prenatally than do girls (Smail et al., 1981). Therefore, normal variability may be insignificant in comparison to the high levels of T present in virtually all boys. In contrast, hormonal variability in girls exists
against a background of relatively low T levels. This could allow the variability to have a greater impact in girls than in boys. Second, differences in the socialization of gender role behavior in boys versus girls might be important. Compared with girls, boys are more strongly encouraged to behave in sex-typical ways and are more strongly discouraged from engaging in cross-gendered behavior (Fagot, 1978; Langlois & Downs, 1980). Boys also are more likely than are girls to preferentially imitate models of the same sex (Perry & Bussey, 1979). Thus, girls may be more likely than are boys to manifest hormone-related predispositions to gender role behaviors more characteristic of the other sex, because these predispositions are less likely to be counteracted by other influences.

Finally, the relationship we observed between T and gender role behavior in preschool girls did not appear to be caused by differences in other theoretically important factors that we were able to assess. Variables of interest available in the ALSPAC dataset included maternal education, the presence of older brothers or sisters in the home, whether a male partner lived with the mother in the home, and parental adherence to traditional sex roles. None of these variables related significantly to either maternal T during pregnancy or to PSAI scores in girls at age 3.5 years in the current sample, and when included in a covariance analysis, they did not alter the main finding. Thus, these particular factors did not account for the correlation observed between maternal T and gender role behavior in preschool girls. Nevertheless, the relationship cannot be assumed to reflect a hormonal cause. It is possible that other factors that we could not measure—such as the degree to which parents encourage sex-typed behavior in their children—relate to maternal T levels; and that this factor, or some other nonhormonal factors, are responsible for the correlation observed. In addition, it should be noted that the relationship observed between T and behavior was small, accounting for only about 2% of the variance in the gender role behavior of preschool girls, leaving ample scope for influences from other factors.

At the same time, it also is possible that the relationship of prenatal T to gender role behavior in preschool girls is larger than suggested by the results of the present study. Maternal T is far removed from the fetal brain and may be a pale reflection of the relationship between a fetus’s own prenatal T levels and postnatal behavior. In addition, a single blood sample is unlikely to provide the most accurate estimate of the early hormone environment. Additional research—for example, relating fetal hormone levels or hormone levels assessed by repeated sampling to postnatal behavior—could provide more accurate information on the nature and magnitude of relationships between the prenatal hormone environment and childhood behavior. Additional research is also needed to determine whether the relationship between T and behavior will persist as these children grow older, and to determine whether T also relates to other behaviors that show sex differences. The findings of the present study encourage such future research.

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