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ORIGINAL RESEARCH

# Genetic and Environmental Contributions to Covariation Between DHEA and Testosterone in Adolescent Twins

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**Abstract** Although several studies have shown that pubertal tempo and timing are shaped by genetic and environmental factors, few studies consider to what extent endocrine triggers of puberty are shaped by genetic and environmental factors. Doing so moves the field from examining correlated developmentally-sensitive biomarkers toward understanding what drives those associations. Two puberty related hormones, dehydroepiandrosterone and testosterone, were assayed from salivary samples in 118 MZ (62 % female), 111 same sex DZ (46 % female) and 103 opposite-sex DZ twin pairs, aged 12–16 years ($M = 13.1$, $SD = 1.3$). Pubertal status was assessed with a composite of mother- and self-reports. We used biometric models to estimate the genetic and environmental influences on the variance and covariance in testosterone and DHEA, with and without controlling for their association with puberty, and to test for sex differences. In males, the variance in testosterone and pubertal status was due to shared and non-shared environmental factors; variation in DHEA was due to genetic and non-shared environmental factors. In females, variance in testosterone was due to genetic and non-shared environmental factors; genetic,

shared, and non-shared environmental factors contributed equally to variation in DHEA. In males, the testosterone-DHEA covariance was primarily due to shared environmental factors that overlapped with puberty as well as shared and non-shared environmental covariation specific to testosterone and DHEA. In females, the testosterone-DHEA covariance was due to genetic factors overlapping with pubertal status, and shared and non-shared environmental covariation specific to testosterone and DHEA.

## Introduction

Despite decades of research on the hormonal mechanisms that underlie pubertal development (Bordini and Rosenfield 2011), the extent to which peripheral sex hormones like testosterone and dehydroepiandrosterone (DHEA) capture the full suite of biological changes of pubertal maturation (and vice versa), and through what mechanism, is not fully resolved. The physical and hormonal manifestations of puberty are not synonymous (Dorn et al. 2006; Huang et al. 2009; Matchock et al. 2007; Shirtcliff et al. 2009), and a number of studies have demonstrated unique relationships between hormones and behavior even after controlling for pubertal maturation (Granger et al. 2003; Marceau et al. 2012; Slap et al. 1994). Thus, while the majority of genetically-informative research on pubertal development has focused on morphological changes, it is equally important to understand the genetic and environmental etiology of its hormonal underpinnings, as well as potential shared genetic and environmental influences on these indices. Pubertal development is also characterized by

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significant sex differences in many respects, from hormone concentrations to physical indices to the timing of on-set (Bordini and Rosenfield 2011). Despite these sex differences, there is also overlap between the sexes (e.g., gonads originate from the same fetal tissues; DHEA and testosterone are present in both sexes) that supports the argument that males and females differ partly by the strength of the underlying mechanisms. Therefore, we examine the same hormonal biomarkers in both males and females, but explicitly examine sex differences in their genetic architecture.

## Testosterone, DHEA, and puberty

Examining testosterone and DHEA directly is important because these hormones inform development beyond measures of puberty alone. According to the organizational-activational hypothesis, the same hormones that organize the body, brain, and behavior will later activate those circuits (Schulz et al. 2009; Shirtcliff 2005). Even after sex hormones have guided sexual differentiation of the developing organism in utero (i.e. organizational effects), sex hormone effects persist largely to influence later maturational events like the timing and tempo of puberty (Brooks-Gunn 1988; Ellis et al. 2011; van Weissenbruch and Delemarre-van de Waal 2006). These activational effects begin once those same sex hormones are released in more frequent pulses and start to guide pubertal maturation. While testosterone is classically recognized as an organizing–activating hormone (Goel and Bale 2008; Meaney and McEwen 1986), DHEA likewise shows organizational and activational properties (Compagnone and Mellon 1998; Parker 1991). Because DHEA is a prohormone for testosterone, it may overlap functionally with testosterone in its role as a sex hormone, but this understudied sex hormone may also exert unique effects across development (Yen and Laughlin 1998).

Puberty is made up of two overlapping processes that are differentially associated with testosterone and DHEA. Adrenarche, an early stage of sexual maturation, begins between the ages of 6 and 8 years with the activation of the hypothalamic–pituitary–adrenal (HPA) axis and is characterized by an increase in body hair, body odor, and skin changes. HPA axis activation triggers the release of DHEA, DHEA-sulfate (DHEA-S) and androstenedione from the adrenal glands. Concentrations of DHEA rise gradually and exert effects on the developing body, somewhat earlier for females than males, and continue to rise well after puberty is complete (Saenger and Dimartino-Nardi 2001). DHEA also tends to be found in higher concentrations in females than in males and has been more closely related to physical pubertal development in females compared to males (Dorn et al. 2006; Shirtcliff et al. 2009). About half of androgen

exposure in females comes from peripheral conversion from DHEA (Granger et al. 1999).

In contrast to adrenarche, and through dissociable neurobiological mechanisms (Saenger and Dimartino-Nardi 2001), gonadarche is related to the reactivation of the gonadotropin-releasing hormone (GnRH) pulse generator by the hypothalamic–pituitary–gonadal (HPG) axis and is marked by testicular (in males) and breast (in females) development. During pubertal development in males, testosterone switches from being adrenal in origin to being gonadal in origin, but remains largely of adrenal origin for females. By mid-puberty testosterone levels are much higher and are rising more quickly for males than females. Thus, while the masculinizing (e.g., deepening voice, hair growth) effects of testosterone are not sex-specific, they are more readily observed in males due to greater concentrations beginning in mid-puberty (Granger et al. 2004).

Although not the primary drivers of pubertal development for girls, examining DHEA and testosterone in both sexes is necessary to gain more complete insights into adolescent development as both hormones are responsible for many activational effects throughout the body and brain. Additionally, both hormones correlate well with pubertal status changes in both sexes (Akamine et al. 1980; Dorn et al. 2006; Shirtcliff et al. 2009). The observation that development of secondary sexual characteristics typically co-occur at parallel developmental rates as youth advance across pubertal stages suggests that there may be shared mechanisms driving the suite of physical and hormonal changes in the body (Kauffman et al. 2009). Conversely, the hormonal processes that culminate in puberty begin at different ages, originate largely from different organs, and can be dissociated from one another in certain medical conditions (Sklar et al. 1980), suggestive of divergent underlying neuroendocrine mechanisms for adrenal versus gonadal activation that happen to coalesce during puberty. Examining covariance between DHEA and testosterone would thus help elucidate why and through what mechanism these hormones share developmental pathways.

Beyond advancing understanding of the hormonal components of puberty, examining DHEA and testosterone covariance is useful as an emerging literature is pointing out that the combined influence or "coupling" of multiple hormones (including DHEA and testosterone) is shaped by early life experience (Ruttle et al. 2013), diurnal effects, and stressor exposure (Marceau et al. 2013, 2014) and impacts mental problems beyond the contribution of either hormone alone (Johnson et al. 2014). Extending this work by examining DHEA and testosterone covariation in a genetically-informed manner will help elucidate why covariation is observed across multiple studies, and to what extent pubertal development is driving the apparent relationship between DHEA and testosterone.

Genetic and environmental influences on testosterone, DHEA, and puberty

Genetically informative designs exploit the natural variation in genetic relatedness among types of family members (i.e. parent-offspring, siblings, cousins) to estimate the genetic and environmental influences on biological or behavioral outcomes (Neale and Cardon 1992). For example, monozygotic (MZ) twin pairs are genetically identical while dizygotic (DZ) twin pairs share on average half their genes. By comparing the trait similarity between pairs of MZ and DZ twins we can estimate the proportion of trait variance that is accounted for by genetic factors, referred to as the heritability, and two types of environmental factors: shared (i.e. those factors experienced by both twins and which tend to make twins similar) and non-shared (i.e. those factors that are specific to one twin and tend to make twins dissimilar). In a similar fashion, we can calculate the proportion of covariance between two traits that are due to both traits being influenced by the same set of genetic, shared, or non-shared environmental factors.

Table 1 summarizes the behavior genetic studies of sex hormones and pubertal development during adolescence. Several studies indicate significant additive genetic and non-shared environmental influences on testosterone in adults (Kuijper et al. 2007; Meikle et al. 1986; Rice et al. 1993) and adolescents (Harden et al. 2014; Harris et al. 1998; Hoekstra et al. 2006; Koenis et al. 2013). In these adolescent samples, which span ages 9–19 years, genetic influences play a large role in individual variation in testosterone in males, with heritability estimates ranging from 55 to 70 % and non-shared environment accounting for the remaining variation (see Table 1). The results for females, however, are not nearly so uniform. Moreover, the evidence for sex differences in genetic and environmental influences on testosterone is mixed. While there is some evidence for greater heritability of testosterone in adolescent males compared to females (Harden et al. 2014; Harris et al. 1998; Hoekstra et al. 2006), at least two studies failed to find sex differences in the magnitude of genetic influences at earlier ages (Koenis et al. at age 9 years and Hoekstra et al. at age 12 years). Evidence for shared environmental influences on testosterone was found in only one study, and then only in females (Harden et al. 2014).

To our knowledge, no studies have examined genetic and environmental influences on DHEA in adolescence. Koenis et al. (2013) examined other sex hormones (luteinizing hormone, follicle stimulating hormone, and estradiol) in addition to testosterone in an adolescent twin sample. With the exception of age 12 estradiol, heritability estimates for these other sex hormones were lower for females than for males at ages 9 and 12 years. For females, shared environmental influences accounted for a majority

of the individual variation in estradiol at age 9 years, but were absent by age 12 years. Research on the genetic and environmental influences on the covariation between puberty related sex hormones is similarly limited. In one notable exception, most of the covariance between puberty hormones was attributable primarily to genetic factors, with some evidence for significant non-shared environmental influences (Koenis et al. 2013).

Behavioral genetic research on physical indices of pubertal development and timing tend to implicate moderate to strong genetic influences, moderate non-shared environmental influences, and very little shared environmental influences. Here, too, however, the evidence for sex differences is mixed (see Table 1). Dick et al. (2001) found evidence for substantial genetic influences on pubertal development in males at ages 12 and 14 years and females at 14 years of age, but lower heritability and stronger shared environmental influences for females at 12 years of age. Mustanski et al. (2004) reported genetic factors accounted for the majority of the variation in a latent measure of pubertal development, and the magnitude of these influences were the same for males and females, while most item specific residual variance could be explained approximately equally by genetic and non-shared environmental influences. Mustanski and colleagues also found evidence for common environmental influences specific to growth spurt and menarcheal status in females. Ge et al. (2007) found that additive genetic and nonshared environment influences each accounted for approximately half the variance in individual differences in pubertal *timing* for both males and females. A number of studies focused specifically on menarche have also demonstrated that genetic factors account for a majority of the individual differences in pubertal timing (Doughty and Rodgers 2000; Meyer et al. 1991; Treloar and Martin 1990; Van den Akker et al. 1987; van den Berg et al. 2006; van den Berg and Boomsma 2007).

Few genetically-informative studies include markers of both physical and hormonal pubertal development. This is surprising as shared genetic effects might point to common underlying genes which are frequently targeted in animal models designed to advance understanding of pubertal maturation (Terasawa 1994, 1995). Likewise, evidence for environmental effects would illustrate well the notion that sex hormones do more in the body and brain than advance puberty (Dorn et al. 2006), including being responsive to environmental stressors (Shirtcliff and Ruttle 2010). The few adolescent twin studies that included testosterone and pubertal status found modest correlations between the two ($r_s$ ranged 0.02–0.40), but this covariance could be explained almost entirely by genetic factors (Hoekstra et al. 2006; Koenis et al. 2013). Similarly, Harden et al. (2014) reported that the covariance between pubertal timing and

**Table 1** Summary of genetically-informative studies of testosterone and puberty in adolescent twins

| | Phenotype | Sample characteristics | Results |
|---|---|---|---|
| **Studies of pubertal development** | | | |
| Dick et al. (2001) | Pubertal Development Scale (PDS) | 926 twin pairs assessed at ages 12 and 14 years | *Males*<br>Age 12 years: $a^2 = .74$ and $c^2 = 0.13$<br>Age 14 years: $a^2 = 0.92$ and $c^2 = 0$<br>*Females*<br>Age 12 years: $a^2 = 0.40$ and $c^2 = 0.45$<br>Age 14 years:<br>$a^2 = 0.68$ and $c^2 = 0.02$ |
| Doughty and Rodgers (2000) | Age at menarche | 1,338 kinship pairs | $a^2 = 0.54$ and $e^2 = 0.46$ |
| Ge et al. (2007) | Pubertal timing using PDS | Sample 1: 718 same-sex twin and sibling pairs aged 9–18 years<br>Sample 2: 1,680 same-sex twin and sibling pairs aged 12–18 years | *Males*<br>Sample 1: $a^2 = 0.45$, $c^2 = 0.01$, and $e^2 = 0.55$<br>Sample 2: $a^2 = 0.40$, $c^2 = 0.05$, and $e^2 = 0.55$<br>*Females*<br>Sample 1: $a^2 = 0.50$, $c^2 = 0.01$, and $e^2 = 0.49$<br>Sample 2: $a^2 = 0.46$, $c^2 = 0.06$, and $e^2 = 0.48$ |
| Kaprio et al. (1995) | Age at menarche | 391 female twin pairs aged 16 years | $a^2 = 0.37$, $d^2 = 0.37$, and $e^2 = 0.26$ |
| Mustanski et al. (2004) | Latent variable of pubertal development | 1,891 twin pairs assessed at ages 11 and 14 years | $a^2 = 0.88$ |
| Van den Akker et al. (1987) | Age at menarche | Sample 1: 364 female twin pairs<br>Sample 2: 99 female twin pairs<br>Sample ages ranged from 18 to 45 years | Sample 1: $a^2 = 0.70$<br>Sample 2: $a^2 = 0.54$ |
| van den Berg et al. (2006) | Self-reported pubertal development using tanner stages and age at menarche | 184 female twin pairs, aged 12 years | Menarche (M): $a^2 = 0.30$, $c^2 = 0.47$, and $e^2 = 0.23$<br>Breast Development (BD): $a^2 = 0.67$, $c^2 = 0.17$, and $e^2 = 0.16$<br>Pubic Hair (PH): $a^2 = 0.72$, $c^2 = 0.20$, and $e^2 = 0.08$<br>*Correlations*<br>BD-PH: $r_A = 0.77$, $r_C = 0.27$, $r_E = 0.37$<br>BD-M: $r_A = 0.70$, $r_C = 0.72$, $r_E = 0.22$<br>PH-M: $r_A = 0.73$, $r_C = 0.23$, $r_E = 0.21$ |
| van den Berg and Boomsma (2007) | Age at menarche | 4,995 individual twins (mean age 25 years), 1,296 sisters (mean age 30 years), and 2,946 mothers (mean age 46 years) | $a^2 = 0.70$ |
| **Studies of testosterone** | | | |
| Harris et al. (1998) | Total plasma testosterone levels | 160 adolescent twin pairs aged 14–21 years and their parents | Young-adult males:<br>$a^2 = 0.66$<br>Adolescent and Adult females:<br>$a^2 = 0.41$ |
| Kuijper et al. (2007) | Plasma testosterone | 128 adult male twins and siblings aged 15–68 years | $a^2 = 0.56$ and $e^2 = 0.44$ |

**Table 1** continued

| | Phenotype | Sample characteristics | Results |
|---|---|---|---|
| Studies of pubertal development and testosterone | | | |
| Harden et al. (2014) | Salivary testosterone and self-reported pubertal status on the PDS, controlled for age | 117 twin pairs aged 13–19 years | *Males* Testosterone: $a^2 = 0.55$, $c^2 = 0.02$, and $e^2 = 0.43$<br>Pubertal status: $a^2 = 0.42$ and $e^2 = 0.58$<br>*Females* Testosterone: $a^2 = 0.12$, $c^2 = 0.53$, and $e^2 = 0.36$ |
| Hoekstra et al. (2006) | Salivary testosterone and self-reported androgen-dependent puberty ratings using the Tanner Scales | 183 twins pairs aged 12 years (males and females combined) | Testosterone: $a^2 = 0.52$, $e^2 = 0.47$<br>Pubertal status (unique variance): $a^2 = 0.45$, $c^2 = 0.37$, and $e^2 = 0.03$ |
| Koenis et al. (2013) | Salivary testosterone levels and tanner stage determined by physical examination | 112 twin pairs assessed at age 9 years and 89 twin pairs re-assessed at age 12 years | *Males at age 9 years*<br>Testosterone: $H^2 = 0.64$ and $e^2 = 0.36$<br>Penile development (PD): $a^2 = 0.09$, $c^2 = 0.87$, and $e^2 = 0.04$<br>Testicle development (TD): $a^2 = 0.40$, $c^2 = 0.51$, and $e^2 = 0.09$<br>Pubic hair (PH): $a^2 = 0.81$, $c^2 = 0.07$, and $e^2 = 0.12$<br>*Males at age 12 years*<br>Testosterone: $H^2 = 0.78$ and $e^2 = 0.22$<br>PD: $a^2 = 0.09$, $c^2 = 0.30$, and $e^2 = 0.62$<br>TD: $a^2 = 0.72$, $c^2 = 0.18$, and $e^2 = 0.10$<br>PH: $a^2 = 0.33$, $c^2 = 0.49$, and $e^2 = 0.19$<br>Testosterone and PD: $r_G = 1.00$<br>Testosterone and TD: $r_G = 0.58$<br>Testosterone and PH: $r_G = 1.00$<br>*Females at age 9 years*<br>Testosterone: $H^2 = 0.70$ and $e^2 = 0.30$<br>Breast development (BD): $a^2 = 0.72$, $c^2 = 0.23$, and $e^2 = 0.05$<br>Pubic hair (PH): $a^2 = 0.29$, $c^2 = 0.67$, and $e^2 = 0.04$<br>*Females at age 12 years*<br>Testosterone: $H^2 = 0.51$ and $e^2 = 0.49$<br>BD: $a^2 = 0.21$, $c^2 = 0.69$, and $e^2 = 0.10$<br>PH: $a^2 = 0.80$, $c^2 = 0.06$, and $e^2 = 0.14$<br>Testosterone and BD: $r_G = 1.00$<br>Testosterone and PH: $r_G = 0.35$ |

testosterone was explained by genetic factors in males, but they found no phenotypic association between pubertal timing and testosterone in females.

The present study sought to add to the current understanding of the etiology of individual differences in the variation and covariation in sex hormones and puberty by examining two puberty-relevant hormones as well as external manifestations of pubertal development. We used salivary assays of testosterone and DHEA and multi-informant reports of pubertal development to (1) identify the extent of genetic and environmental influences on hormonal and physical markers of pubertal development; (2) characterize the extent to which these genetic and environmental influences overlap among measures (i.e., do testosterone and DHEA covary independent of their association with pubertal development or timing, and is this covariation due to genetic or environmental influences?); and (3) determine whether the magnitude of these influences differ for males and females. Since little is known about the genetic and environmental etiology of testosterone, DHEA, and pubertal status, much less the covariation among them, these analyses are, by necessity, exploratory in nature.

## Methods

### Subjects

Participants were drawn from a longitudinal study of child and adolescent psychopathology (Schmidt et al. 2012). Twins were identified from Wisconsin state birth records; 87 % of nearly 7,000 confirmed contacts responded favorably and were recruited to a research panel when their

twins were infants/toddlers (Schmidt et al. 2012). Families were invited to participate in an extensive in-home assessment when twins were approximately age 7 years. This sample was followed-up again in early-to-mid adolescence. Data collection in adolescence involved behavioral assessments completed by parents and offspring, executive functioning tasks, pubertal status assessments, and neuroendocrine measures including cortisol, testosterone, and DHEA. All protocols were approved by the University of Wisconsin-Madison IRB, and parents completed a consent form prior to participation at each assessment; adolescents provided assent. Families were paid for their participation.

Participants were 12–16 years old at the time of assessment; average age was 13.2 (SD = 1.4) years for 359 males and 13.0 (SD = 1.4) years for 411 females. We present data on a total of 385 twin pairs: 85 monozygotic female pairs (MZF), 53 monozygotic male pairs (MZM), 62 dizygotic female pairs (DZF), 68 dizygotic male pairs (DZM), and 117 dizygotic opposite-sex pairs (DZOS). Mothers had an average education of 15.0 years and fathers had an average education of 14.4 years. Median annual family income was $70,000–$80,000. The majority of families were White (91 %), 3 % African-American, 5 % non-black Hispanic, and 5 % other, consistent with the demographics of the state as a whole. There were no sex differences in age, parent education or family income (see Table 2).

### Zygosity

Zygosity was classified during each assessment wave using the Zygosity Questionnaire for Young Twins (Goldsmith 1991), which has demonstrated over 95 % agreement with

**Table 2** Sample size and descriptive statistics for testosterone, DHEA, and pubertal status

|  | Males | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|
|  | N | M | SD | N | M | SD | t | df |
| Testosterone[b] (raw) | 312 | 70.1 | 44.1 | 349 | 52.0 | 25.8 | 6.9** | 490.7 |
| Testosterone[a,b] | 306 | 0.1 | 0.7 | 340 | −0.01 | 0.5 | 5.2** | 557.4 |
| DHEA[b] (raw) | 313 | 152.7 | 126.7 | 341 | 194.2 | 154.8 | 3.7** | 643.1 |
| DHEA[a,b] | 310 | −0.15 | 0.8 | 331 | 0.13 | 0.8 | 4.9** | 627.8 |
| Pubertal status | 311 | 2.8 | 1.0 | 362 | 3.2 | 0.9 | 6.3** | 619.2 |
| Age in years | 335 | 13.2 | 1.4 | 370 | 13.0 | 1.4 | 1.6 | 703 |
| Mother years of education | 331 | 15.0 | 2.3 | 363 | 14.9 | 2.3 | 0.4 | 692 |
| Father years of education | 325 | 14.2 | 2.2 | 353 | 14.5 | 2.4 | 1.3 | 676 |
| Family income level | 316 | 11.5 | 3.5 | 353 | 11.3 | 3.6 | 0.8 | 667 |

* $p < 0.05$, ** $p < 0.01$

[a] Hormone concentrations after log-transforming and residualizing on minutes since waking. All models were fit to transformed, residualized scores

[b] The Satterthwaite $t$ test is reported due to inequality of variances between males and females

genotypic zygosity determination (Forget-Dubois et al. 2003). Cases of ambiguous zygosity were resolved via hospital placenta(e) reports (an unambiguous monochorionic placenta indicated monozygosity) and follow-up zygosity questionnaires completed by parents, twins, and trained research assistants. Genotyping photographs and video images were utilized for 54 families for whom questionnaire information was not definitive. One family was excluded from the behavior genetic models described in the Data Analysis section due to unresolved ambiguous zygosity status.

## Testosterone and DHEA

Prior to an in-home visit, parents were mailed prelabelled Salivette collection tubes (Starstedt) and instructions to collect saliva from their offspring on two consecutive days, approximately 30 min after waking; 95 % of morning samples were collected between 6:30 AM and 10:00 AM ($M = 31$ min since waking, $SD = 21.2$ min). Families were instructed not to eat or drink 1 h prior to saliva collection and to store samples in the freezer immediately after collection. The research team subsequently collected the samples during the in-home visit and transferred them back to the laboratory on ice. Samples were stored at $-80$ °C until assayed for testosterone and DHEA. Parents recorded the date and time of collection in addition to waking time, medication use and general health for each twin on each collection day. Enzymeimmunoassays were completed by Middleton Research Biodiagnostic Laboratory (Middleton, WI) using Salimetrics' kits (State College, PA). Samples were measured in duplicate; duplicates that varied by more than 7 % were repeat tested. For DHEA, the range of sensitivity was from 5 to 1,000 pg/ml. The average intra-assay coefficient of variation (CV) was 5.6 % and the average interassay CV was 8.2 %. For testosterone, the range of sensitivity was from 1 to 600 pg/ml. Average intra- and interassay CVs were 4.6 and 8.3 %, respectively.

Hormone levels follow a diurnal rhythm, and both the level and diurnal pattern in sex hormones can be affected by medication use (Hibel et al. 2007). Therefore, participants were excluded from analyses if they failed to report sample collection time or wake times or if they reported using medication known to affect hormone levels such as corticosteroids or hormonal birth control on sample collection days. Out of 385 families, 361 (94 %) provided saliva samples for assaying. Saliva samples were successfully assayed for testosterone or DHEA for at least one twin in 357 (99 %) twin pairs. Seventeen pairs were missing sample collection times and/or wake times on collection days for both twins. Six pairs reported using corticosteroids or hormonal birth control on sample collection days. These exclusions resulted in a total sample of

332 twin pairs: 73 monozygotic (MZF) female; 51 dizygotic (DZF) female; 45 monozygotic (MZM) male; 60 dizygotic (DZM) male; 103 opposite-sex (DZOS) pairs. Twenty-four percent of participants reported using other non-steroidal medications on sample collection days and 13 % reported experiencing some symptoms of illness (cough, runny nose, stomach pain, etc.). Neither medication use nor symptoms of illness were related to DHEA or testosterone concentrations and were not considered further. Testosterone and DHEA were positively skewed and log-transformed prior to analyses. To control for the inherent variations in testosterone and DHEA due to variations in sample collection times, testosterone and DHEA concentrations were regressed on sample collection time converted into minutes-since-waking. The unstandardized residuals were subsequently averaged across the two sample collection days prior to analyses.

## Pubertal status

Puberty was assessed using a composite score derived from mother- and self-reports of Pubertal Development Scale (PDS; Petersen et al. 1988) and self-report via the picture-based interview about puberty (PBIP; Dorn and Susman 2002) collected during the home visit. The PDS consists of five questions about physical development (e.g. growth of body hair or breast development) scored from 1 (*has not begun*) to 4 (*is complete*). Trained interviewers administered the PBIP during the in-home visit. Interviewers used a script and photographs to walk the adolescents through the stages of pubertal development. The interviewer then left the room and the participant rated his or her stage of development. Female interviewers administered the PBIP to both males and females while male interviewers only administered the instrument to males. Ratings from the multiple informants was composited using a method similar to that of Ellis et al. (2011). PDS scores were recoded to capture both gonadal and adrenal signs of physical development (Shirtcliff et al. 2009). For both males and females, the adrenal score reflected pubic/body hair and skin changes. For females, gonadal scores reflected growth spurt, breast development, and menarche. For males, gonadal scores reflected growth spurt, deepening voice, and facial hair growth. Mother and twin reports on the PDS were averaged to form separate gonadal and adrenal scores. Correlations between mother- and twin-reported PDS scores were high for both males and females ($r_s$ ranged from 0.66 to 0.72). Next, gonadal scores from the PDS were averaged with twin report of breast (for females) or genital (for males) development during the PBIP and adrenal scores were averaged with twin ratings of pubic hair development from the PBIP resulting in a *continuous* score ranging from 1.0 (pre-pubertal) to 5.0 (post-pubertal).

Both mother and twin-reported PDS scores were highly correlated with twin-reported breast/genital and pubic development ($r_s$ ranged from 0.53 to 0.77). Although gonadal and adrenal development typically begin at different ages, produce different changes in the body (Ellis 2004), and are differentially related to DHEA and testosterone (Bordini and Rosenfield 2011), gonadal and adrenal score were highly correlated ($r = 0.86$ in males and 0.75 in females). The pattern of results from model fitting were the same for adrenal and gonadal scores; thus, for simplicity only results from the combined pubertal status are reported here.

Data analysis

The univariate biometric twin model relies on the assumption that the phenotypic variance in a single trait of interest can be attributed to variation in unmeasured latent additive genetic influences (A), shared environmental influences (C) that vary between families, and non-shared environmental influences (E) that vary among families. The E term also includes measurement error. Because MZ twins share (effectively) 100 % of their genes and DZ twins share on average 50 % of their genes, the additive genetic effect is modeled with a correlation of 1.0 in MZ twin pairs and 0.5 in DZ twin pairs. Thus, larger MZ twin than DZ twin correlations are indicative of additive genetic influences. Because members of both MZ and DZ twin pairs raised together are expected to be influenced by the shared environment to the same degree, the shared environmental effect is modeled with a correlation of 1.0 regardless of zygosity. Shared environmental influences are indicated by DZ correlations greater than half the MZ correlations. Non-shared environmental effects, or those environmental factors that serve to make twins different, are modeled as uncorrelated, and are indicated by MZ correlations less than 1.0. Standardized estimates for A, C and E, reflect the proportion of the total phenotypic variance that is accounted for by additive genetic variation or heritability ($a^2$), shared environmental variation ($c^2$) and non-shared environmental variation ($e^2$) and sum to 1.0.

The univariate biometric model can be extended in two important ways. First, the model can incorporate sex differences. In the *quantitative* sex differences model, twin pairs are grouped by sex as well as zygosity so that opposite-sex twin pairs are included as a separate twin pair type. The model assumes that the same genetic, shared, and non-shared environmental factors influence the phenotype in males and females, but the magnitude of influence is allowed to vary by sex (Neale and Cardon 1992). In this model, opposite-sex twins are assumed to have a genetic correlation of 0.5 as same-sex DZ twin pairs do. However, this assumption can be relaxed in a *qualitative* sex differences model. Qualitative sex differences (i.e. sex specific genetic factors) are indicated when the genetic correlation between opposite-sex twin pairs is estimated to be less than the genetic correlation for same-sex twins pairs (e.g. 0.5 as noted above). Sex specific genetic factors are implicated when the DZ opposite-sex phenotypic correlations are lower than DZ same-sex phenotypic correlations.

Second, the univariate model can be extended by incorporating multiple phenotypes, and estimating the genetic and environmental contributions to the *covariance* between two or more traits. The total phenotypic *covariation* can be attributed to underlying genetic, shared environment and non-shared environmental *covariation* in a manner analogous to the phenotypic variation described above. The bivariate Cholesky model, for instance, provides estimates of A, C, and E influences on one trait that also influence a second trait, thus creating their covariance, as well as estimates of A, C, and E influences that are unique to the latter trait. Thus, variance in the second trait in the model may be due wholly to factors that also influence the former trait, variance specific to the latter trait only, or some combination thereof. In addition to this bivariate decomposition, we can also examine genetic and environmental correlations between the traits. Genetic correlation between traits should not be confused with the genetic correlation between relatives; the former is estimated by the model while the latter is a fixed quantity. The genetic correlation between any two traits, or $r_A$, considers only the genetic variances and covariances and is derived by standardizing the genetic covariance (i.e. dividing the genetic covariance between two traits by the product of the genetic variance of each trait). The genetic correlation indicates the extent to which two traits share overlapping genetic influences. Thus, if the same set of genes influences two traits $r_A$ will be high even if the total phenotypic covariation is relatively low because the genetic covariance is a high portion of the total genetic variance. Two traits may be influenced by the same set of shared environmental or non-shared environmental factors as well, and we can estimate shared ($r_C$) and non-shared environmental ($r_E$) correlations in an analogous fashion.

We first fit separate univariate biometric models to testosterone, DHEA, and pubertal status, which provided independent estimates of the genetic, shared environment, and non-shared environment influences on each trait. We tested for *qualitative* sex differences in the univariate models by comparing a model that allowed the opposite-sex DZ correlation to be freely estimated to a model that constrained the opposite-sex DZ correlation to be 0.5 and determining if the latter resulted in a significant loss in overall model fit. The test for qualitative sex differences should be considered exploratory, as very large sample sizes are required to detect a significant deviation in the DZ

opposite-sex genetic correlation from the DZ same-sex genetic correlation of 0.5 (Galsworthy et al. 2000). We tested for *quantitative* sex differences by constraining path estimates in the twin models to be identical for males and females and determining if this significantly worsened overall model fit compared to a model that allowed these estimates to vary between sexes.

We then fit a bivariate Cholesky model of testosterone and DHEA to examine the genetic and environmental influences common to both hormones. Finally we fit a trivariate Cholesky model with variables ordered as pubertal status, testosterone, and DHEA. This model allowed us to examine the sources of covariation in testosterone and DHEA after controlling for their covariation with pubertal status. For each multivariate analysis, we also tested for quantitative sex differences. We tested for changes in model fit via the log-likelihood ratio test. Difference in the log-likelihood ratio between nested models yields a Chi square test of the more parsimonious model. A non-significant change in chi-quare ($\chi^2_{\text{diff}}$) indicates that the nested model fits the data as well as the full model with fewer parameters and is thus preferred over the full model. All models were fit using the R software package OpenMx (Boker et al. 2011; OpenMx 2012) using full information maximum likelihood.

# Results

Table 2 shows descriptive statistics for DHEA, testosterone, and pubertal status for males and females, separately. Note that the Satterthwaite test statistic is reported when variances are unequal between males and females. Males had significantly higher testosterone concentrations ($t(490.7) = 6.9$, $p < 0.001$), lower DHEA concentrations ($t(643.1) = 3.7$, $p < 0.001$), and less advanced pubertal status ($t(619.2) = 6.3$, $p < 0.001$) than females. Testosterone and DHEA were equally highly correlated with each other in males ($r = 0.62$) and females ($r = 0.63$). As expected given their respective roles in pubertal development in males, testosterone was more highly correlated with pubertal status than was DHEA ($r = 0.71$ and 0.35, respectively). In females, however, DHEA was more highly correlated with pubertal status than was testosterone ($r = 0.44$ and 0.18, respectively). Separate phenotypic cross-twin and cross-twin, cross-trait correlations for testosterone, DHEA and pubertal status for each zygosity/sex group are shown in Table 3. In general, opposite-sex DZ correlations were lower than same sex DZ correlations, particularly for testosterone, suggesting possible qualitative sex differences. DZ cross-twin correlations were greater than half the MZ cross-twin correlations, suggesting that shared environmental factors have a strong influence on variation in both pubertal status and hormone

concentration, particularly among males. Similarly, DZ cross-twin, cross-trait correlations were close to MZ cross-twin, cross-trait correlations, particularly for males, suggesting shared and non-shared environmental factors contributed to the *covariation* between testosterone, DHEA and pubertal status as well.

## Univariate models

Standardized parameter estimates (and their 95 % confidence intervals) from fitting the three separate univariate models are shown in Table 4. Fitting the *qualitative* sex differences models[1] resulted in an estimated opposite-sex DZ genetic correlation of 0.0 for testosterone, 0.3 for DHEA, and 0.5 for puberty, but fixing the opposite-sex DZ genetic correlation to 0.5 did not result in a significant loss in model fit (testosterone: $\chi^2_{\text{diff}} = 1.6$, 1df, $p = 0.21$; DHEA: $\chi^2_{\text{diff}} = 0.22$, 1df, $p = 0.64$; puberty: $\chi^2_{\text{diff}} = 0$, 1df, $p = 1.0$). Therefore we did not consider qualitative sex differences further. For males, the majority of the variation in testosterone was due to shared environmental influences ($c^2 = 0.69$), the majority of variation in DHEA was due to genetic factors ($a^2 = 0.68$), and the majority of the variation in pubertal status was due to shared environmental influences ($c^2 = 0.72$). In contrast, in females, the variation in testosterone was due primarily to genetic factors ($a^2 = 0.63$) with very little shared environmental variation ($c^2 = 0.07$). Variation in DHEA and pubertal status in females were attributable to genetic, shared environmental, and non-shared environmental factors in approximately equal measure. We found significant *quantitative* sex differences for testosterone ($\chi^2_{\text{diff}} = 42.6$, 3df, $p < 0.001$) and pubertal status ($\chi^2_{\text{diff}} = 11.2$, 3df, $p = 0.01$). Although the point estimates for the genetic and environmental influences on DHEA appeared to be quite different for males and females (e.g., $a^2 = 0.68$ vs 0.33), the model equating parameters across sexes fit the data as well as the quantitative sex differences model ($\chi^2_{\text{diff}} = 4.3$, 3df, $p = 0.24$). Together, these results suggest that although the same genetic and environmental factors influence individual differences in males and females, there are substantial sex differences in the relative contribution of these influences on the overall phenotypic variance of testosterone and pubertal status, with shared environment accounting for more of the phenotypic variation in males than in females.

## Multivariate models

Standardized estimates (and their 95 % confidence intervals) from fitting the bivariate Cholesky model are shown

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[1] See Supplemental Table 1.

**Table 3** Twin intraclass and cross-trait correlations for testosterone, DHEA, and pubertal status

| | N | Testosterone | DHEA | Cross-trait $r_{T-D}$ | Pubertal status | Cross-trait $r_{P-T}$ | Cross-trait $r_{P-D}$ |
|---|---|---|---|---|---|---|---|
| MZF | 85 | 0.76 | 0.78 | 0.56 | 0.85 | 0.18 | 0.43 |
| DZF | 62 | 0.57 | 0.55 | 0.35 | 0.55 | 0.20 | 0.27 |
| MZM | 53 | 0.83 | 0.75 | 0.48 | 0.83 | 0.64 | 0.23 |
| DZM | 68 | 0.76 | 0.44 | 0.50 | 0.80 | 0.60 | 0.39 |
| DZOS | 117 | 0.30 | 0.43 | 0.26 | 0.71 | 0.57 | 0.38 |

*MZF* monozygotic female twin pairs, *DZF* dizygotic female twin pairs, *MZM* monozygotic male twin pairs, *DZM* dizygotic male twin pairs, *DZOS* opposite sex twin pairs, *T* testosterone, *D* DHEA, *P* pubertal status

**Table 4** Standardized $a^2$, $c^2$, $e^2$ estimates (95 % confidence interval) for testosterone, DHEA, and pubertal status from fitting a univariate model

| | Comparison to equating parameters across sexes | | | Males | | | Females | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\chi^2_{\text{diff}}$ | df | $p$ | $a^2$ | $c^2$ | $e^2$ | $a^2$ | $c^2$ | $e^2$ |
| Testosterone | 42.6 | 3 | <0.001 | 0.13 | 0.69 | 0.16 | 0.63 | 0.07 | 0.29 |
| | | | | (0.0–0.39) | (0.45–0.81) | (0.10–0.29) | (0.0–0.77) | (0.0–0.64) | (0.21–0.40) |
| DHEA | 4.3 | 3 | 0.24 | 0.68 | 0.08 | 0.24 | 0.33 | 0.42 | 0.24 |
| | | | | (0.27–0.83) | (0.0–0.45) | (0.15–0.38) | (0.01–0.79) | (0.0–0.71) | (0.17–0.34) |
| Pubertal status | 11.2 | 3 | 0.01 | 0.10 | 0.72 | 0.17 | 0.41 | 0.44 | 0.15 |
| | | | | (0.0–0.28) | (0.55–0.83) | (0.12–0.24) | (0.25–0.57) | (0.29–0.58) | (0.10–0.21) |

$a^2$, $c^2$, $e^2$ refer to the standardized genetic, shared environment and non-shared environmental variance respectively

**Table 5** Standardized $a^2$, $c^2$, $e^2$ estimates (95 % confidence intervals) of variance/covariance in testosterone and DHEA and estimated genetic and environmental correlations from fitting a bivariate Cholesky model

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | $a^2$ | $c^2$ | $e^2$ | $a^2$ | $c^2$ | $e^2$ |
| **Variance** | | | | | | |
| Testosterone | 0.24 (0.0–0.51) | 0.57 (0.32–0.77) | 0.19 (0.12–0.29) | 0.36 (0.10–0.73) | 0.42 (0.03–0.65) | 0.35 (0.09–0.62) |
| DHEA | 0.45 (0.17–0.64) | 0.28 (0.13–0.50) | 0.27 (0.17–0.43) | 0.39 (0.14–0.62) | 0.38 (0.15–0.62) | 0.22 (0.17–0.30) |
| **Covariance** | 0.16 (−0.09 to 0.47) | 0.62 (0.36–0.83) | 0.20 (0.10–0.37) | 0.20 (−0.05 to 0.69) | 0.56 (0.09–0.81) | 0.23 (0.15–0.39) |
| | $r_A = 0.30$ | $r_C = 0.96$ | $r_E = 0.55$ | $r_A = 0.36$ | $r_C = 0.93$ | $r_E = 0.67$ |

Proportion of phenotypic variance accounted for by A, C, and E are shown in the top panel; proportion of phenotypic covariance accounted for by A, C, and E are shown in the bottom panel; $a^2$, $c^2$, $e^2$ refer to the standardized genetic, shared environment and non-shared environmental variance or covariance; $r_A$, $r_C$, and $r_E$ refer to the genetic, shared environment, and non-shared environment correlations

in Table 5. We found evidence for quantitative sex differences, as parameter estimates could not be equated across sexes without a significant loss in fit ($\chi^2_{\text{diff}} = 36.6$, 9df, $p < 0.001$). Nevertheless, the pattern of genetic and environmental influences on the covariation between testosterone and DHEA was quite similar for males and females. For males, the majority of covariation between testosterone and DHEA was attributable to shared environmental influences common to both hormones ($c^2 = 0.62$), with modest covariation due to genetic ($a^2 = 0.16$) and non-shared environmental ($e^2 = 0.22$)

factors common to both hormones. Likewise, in females shared environmental factors common to testosterone and DHEA accounted for most of the phenotypic covariation ($c^2 = 0.56$) with the remaining covariation equally attributable to genetic ($a^2 = 0.20$) and non-shared environmental ($e^2 = 0.22$) factors. Although small in magnitude, the genetic and non-shared environmental covariation could not be dropped from the model without a significant loss in fit ($\chi^2_{\text{diff}} = 7.9$, 2df, $p = 0.02$, $\chi^2_{\text{diff}} = 65.0$, 2df, $p < 0.001$, respectively). Genetic correlations were modest for both males and females ($r_A = 0.30$ and $0.36$) while the shared

**Table 6** Standardized $a^2$, $c^2$, $e^2$ estimates (95 % confidence intervals) of variance/covariance in pubertal status, testosterone, DHEA and estimated genetic and environmental correlations from fitting a trivariate Cholesky model

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | $a^2$ | $c^2$ | $e^2$ | $a^2$ | $c^2$ | $e^2$ |
| Pubertal status | 0.11 (0.01 to 0.32) | 0.69 (0.51 to 0.81) | 0.18 (0.13 to 0.35) | 0.42 (0.26 to 0.68) | 0.44 (0.28 to 0.58) | 0.13 (0.01 to 0.19) |
| Testosterone | 0.31 (0.0 to 0.57) | 0.51 (0.24 to 0.77) | 0.18 (0.12 to 0.36) | 0.44 (0.52 to 0.82) | 0.32 (0.0 to 0.75) | 0.23 (0.16 to 0.37) |
| DHEA | 0.63 (0.03 to 0.79) | 0.13 (0.0 to 0.39) | 0.24 (0.16 to 0.36) | 0.59 (0.24 to 0.82) | 0.20 (0.0 to 0.54) | 0.21 (0.15 to 0.29) |
| Puberty-testosterone | 0.13 (−0.21 to 0.54) | 0.83 (0.44 to 1.1) | 0.03 (−0.04 to 0.11) | 0.57 (0.01 to 1.4) | 0.41 (−0.40 to 0.88) | 0.02 (−0.11 to 0.17) |
| | $r_A = 0.45$ | $r_C = 0.89$ | $r_E = 0.10$ | $r_A = 0.43$ | $r_C = 0.34$ | $r_E = 0.04$ |
| Puberty-DHEA | 0.24 (−0.33 to 0.86) | 0.80 (0.25 to 1.3) | −0.04 (−0.23 to 0.12) | 0.53 (0.18 to 1.1) | 0.43 (−0.01 to 0.75) | 0.04 (0.0 to 0.13) |
| | $r_A = 0.31$ | $r_C = 0.91$ | $r_E = -0.07$ | $r_A = 0.48$ | $r_C = 0.65$ | $r_E = 0.10$ |
| Testosterone-DHEA | 0.44 (−0.17 to 0.78) | 0.38 (0.01 to 0.68) | 0.18 (0.01 to 0.32) | 0.49 (−0.20 to 0.84) | 0.28 (−0.01 to 0.94) | 0.22 (0.14 to 0.32) |
| | $r_A = 0.58$ | $r_C = 0.85$ | $r_E = 0.48$ | $r_A = 0.63$ | $r_C = 0.72$ | $r_E = 0.65$ |

Proportion of phenotypic variance accounted for by A, C, and E are shown in the top panel; proportion of phenotypic covariance accounted for by A, C, and E are shown in the bottom panel; $a^2$, $c^2$, $e^2$ refer to the standardized genetic, shared environment and non-shared environmental variance; $r_A$, $r_C$, and $r_E$ refer to the genetic, shared environment, and non-shared environment correlations

environmental correlations were extremely high ($r_C = 0.96$ and 0.93). Non-shared environmental correlations were also relatively high ($r_E = 0.55$ and 0.67). These results suggest that the covariance between testosterone and DHEA in males and females is largely due to shared and non-shared environmental factors common to both hormones. High shared and non-shared environmental correlations indicate few environmental influences specific to either hormone; low genetic correlations indicate that genetic influences tend to be hormone-specific.

Standardized estimates (and their 95 % confidence intervals) from fitting the trivariate Cholesky are shown in Table 6. The top panel of Table 5 shows the phenotypic variance in pubertal status, testosterone and DHEA attributable to all genetic, shared environment and non-shared environmental sources (i.e., common and unique). The bottom panel of Table 5 shows the proportion of covariance among the three variables accounted for by underlying genetic, shared environmental, and non-shared environmental covariation. As in the bivariate model, parameter estimates could not be equated across sexes without a significant loss in fit ($\chi^2_{diff} = 86.5$, 18df, $p < 0.001$). In males, the covariation between pubertal status and sex hormones was due primarily to shared environmental factors common to all three traits, and in females the covariation between pubertal status and sex hormones was primarily due to genetic factors common to all three traits. For males, 44 % of the total phenotypic covariation between testosterone and DHEA was due to factors that both hormones shared with pubertal status; for females, 27 % of the total phenotypic covariation between testosterone and DHEA was due to factors that both shared with pubertal status. Dropping the non-significant genetic

(male), shared environment (female) and non-shared environmental (male and female) covariation between puberty and the two sex hormones resulted in a non-significant change in fit ($\chi^2_{diff} = 11.4$, 8df, $p = 0.18$). Genetic covariance specific to testosterone and DHEA in both males and females could also be dropped without a significant loss in fit ($\chi^2_{diff} = 13.2$, 10df, $p = 0.21$). However, dropping shared environmental or non-shared environmental covariance specific to testosterone and DHEA did result in a significant loss in fit ($\chi^2_{diff} = 18.3$, 10df, $p = 0.05$ and $\chi^2_{diff} = 72.2$, 10df, $p < 0.001$, respectively). The standardized parameter estimates from the best fitting model are shown in Fig. 1. The total genetic and environmental correlations between testosterone and DHEA based on the best fitting model were, for males $r_A = 0$, $r_C = 0.95$ and $r_E = 0.58$ and for females, $r_A = 0.46$, $r_C = 0.97$ and $r_E = 0.66$. Taken together, these results suggest that the relationship between testosterone and DHEA is more closely related to pubertal status in males than females, that different factors are responsible for the covariation between puberty and sex hormones in males (mostly shared environment) and females (mostly genetic), and that covariation specific to testosterone and DHEA is due to a combination of shared and non-shared environmental covariation in both males and females.

We repeated the trivariate Cholesky with pubertal *timing*[2] (pubertal status regressed on age). Unlike pubertal status, variation in pubertal timing could be attributed primarily to genetic variation in both males and females ($a^2 = 0.54$ and 0.77 respectively) with the remaining variation attributable to non-shared environmental factors. In

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[2] See Supplemental Table 2.

**Fig. 1** Standardized *squared path* estimates from the best fitting trivariate Cholesky model shown separately for males (**a**) and females (**b**). *A* refers to additive genetic influences, *C* refers to shared environmental influences, and *E* refers to non-shared environmental influences. The following covariances have been dropped from the best fitting model: genetic covariation between pubertal status and sex hormones (male), shared environmental covariation between pubertal status and sex hormones (female), non-shared environmental covariation between pubertal status and sex hormones (male and female), additive genetic covariance specific to sex hormones (male and female)
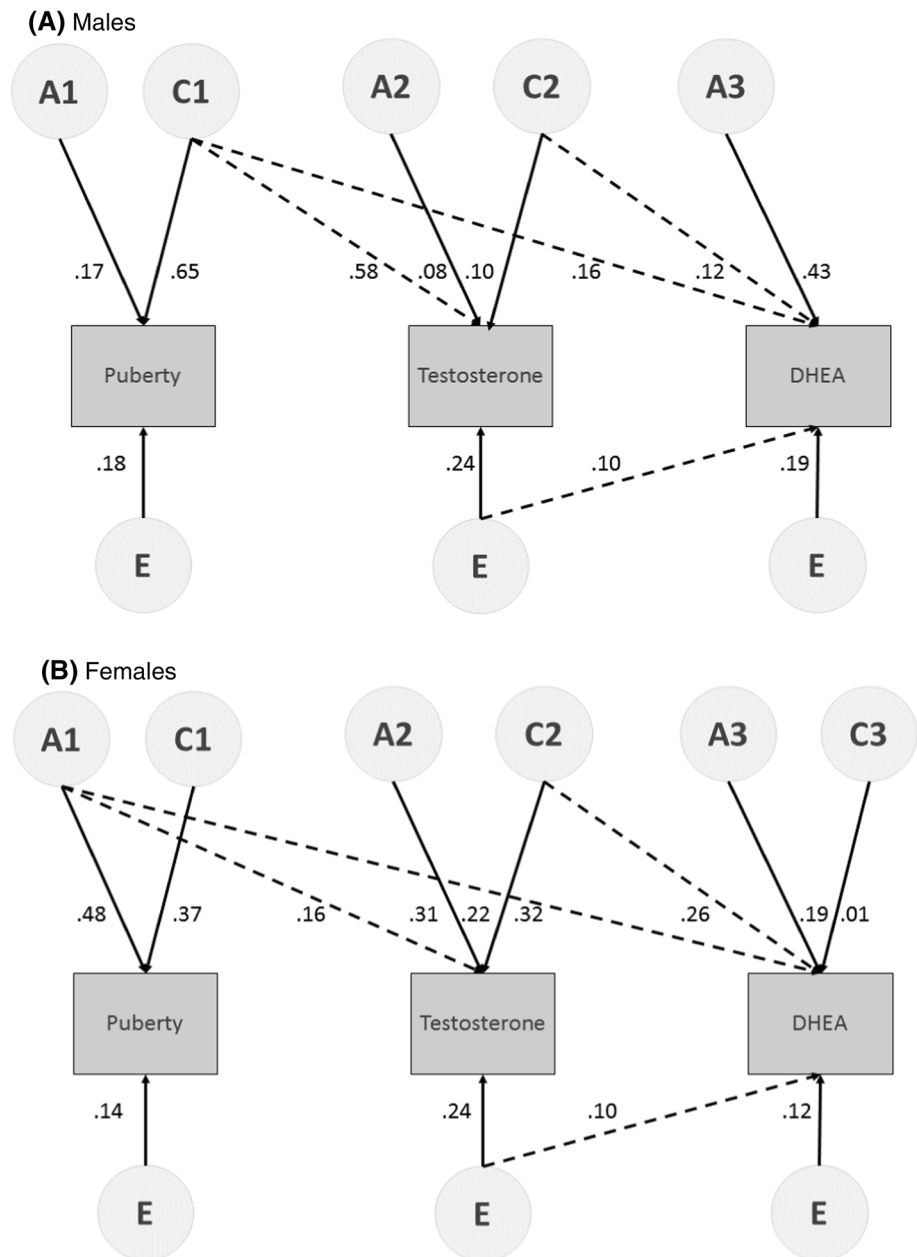


**(A)** Males

**(B)** Females

males, 54 % of the covariation between testosterone and DHEA was due to factors that both share with pubertal timing. In females, only 5 % of the covariation between testosterone and DHEA was due to factors that both share with pubertal timing. Beyond that, the pattern of covariation between puberty, testosterone and DHEA remained unchanged. In males, covariation between testosterone and DHEA could be attributed to shared environmental factors that also influenced pubertal timing. In females, covariation between testosterone and DHEA could be attributed to genetic factors that also influenced pubertal timing. In both sexes, covariance specific to testosterone and DHEA was due to underlying shared environmental covariance

($c^2 = 0.60$ for males and 0.65 for females) and non-shared environmental covariance ($e^2 = 0.21$ for males and 0.23 for females).

## Discussion

This is the largest adolescent twin study to include hormonal assays, and the first study to examine genetic and environmental factors underlying the covariation in two puberty-related hormones controlling for pubertal status. Our results do little to reconcile divergent findings in the literature, but they do contribute to a broader discussion

regarding the relative contributions of genetic and environmental forces to pubertal maturation, and the utility of hormones such as testosterone and DHEA as pubertal biomarkers. Consistent with prior research (Huang et al. 2009; Matchock et al. 2007; Shirtcliff et al. 2009) and theory (Dorn et al. 2006), we found that sex hormones were indeed related to puberty, but we also found covariation specific to testosterone and DHEA, suggesting that these hormones contain information beyond secondary sexual characteristics.

Findings of moderate genetic influences on individual differences in testosterone and pubertal status in females is in keeping with prior studies (see Table 1). Although we were unable to detect significant qualitative sex differences, the low opposite-sex DZ correlations for testosterone are in keeping with findings from Harden et al. (2014). Likewise, our findings of high heritability for pubertal timing for both males and females align with estimates from other studies (Doughty and Rodgers 2000; Ge et al. 2007; Harden et al. 2014; Treloar and Martin 1990). Unlike prior studies, we found large shared environmental influences on individual variation in testosterone and pubertal status in males. In addition, we found strong shared environmental influences on the covariance between pubertal status and sex hormones in boys, and strong genetic influences on the covariance between pubertal status and sex hormones in females. Few other studies have explicitly modeled the covariance between pubertal development and its hormonal underpinnings. Our results are consistent with the extant literature in that we found genetic effects of puberty and sex hormone covariation in females similar to Koenis et al. (2013) and Hoekstra et al. (2006), who examined parallel age-groups as the present study. On the other hand, our results are inconsistent with the extant literature in that only modest genetic effects on variation in pubertal status and testosterone were observed within males.

There are both conceptual and practical reasons that our study found large shared and non-shared environmental effects within males. Conceptually, within males, puberty and/or testosterone release is mediated more by environmental or stress-related processes than in females. Compared to females, testosterone release in males shows greater absolute rises in response to stress (Rejeski et al. 1989) as well as being more responsive to challenging or competitive contexts (Booth et al. 1989; Zilioli and Watson 2014). Therefore, it is not unreasonable to expect some significant environmental influences in males. Our results also emphasize that maturational events that culminate in adult-like development in males and females are not necessarily driven by the same underlying processes. Similar conclusions were reached by Dorn et al. (2006), and the present study adds genetic and environmental decompositions to that idea. On a practical level, the male DZ

phenotypic correlations in our study were relatively high compared to male DZ correlations reported in comparable studies and as such may be an artifact of this sample. However, Koenis et al. (2013) reported modest to large point estimates for shared environmental influences on pubertal status in males at age 12 years, although the estimates fell short of reaching statistical significance. Also, age 12 or 13 is frequently targeted for studies on puberty or sex hormone maturation because variability in maturation is widest at this age, ranging from no development to adult-like development (Ellis et al. 2011; Shirtcliff et al. 2009); therefore, studies with older samples may suffer a restriction in range. Other potential methodological issues are noted in the section on limitations. Because this is a nascent area of research, more studies with larger sample sizes are necessary before reaching firm conclusions about the magnitude of genetic and environmental influences on puberty and sex hormones.

It is interesting that genetic forces accounted largely for the overlap of puberty with testosterone and DHEA for females as it lends credence to the argument that at least some markers of puberty in females are highly genetic (Mustanski et al. 2004; van den Berg et al. 2006) and sex hormones may underlie these heritability estimates as has been frequently speculated within the pubertal literature (see review by Ellis 2004). Although estradiol and progesterone have been emphasized as pubertal biomarkers for females (Finkelstein et al. 1999), perhaps because testosterone and DHEA are primarily of adrenal rather than gonadal origin within females (Grumbach 2002), our findings add to the evidence that testosterone and DHEA are relevant biomarkers of puberty for both males and females (Huang et al. 2009; Matchock et al. 2007; Shirtcliff et al. 2009).

There is a distinct pattern of covariation between DHEA and testosterone throughout adolescence (Ruttle et al. 2013) with implications for behavior (Johnson et al. 2014). Across both sexes, covariation between testosterone and DHEA due to factors outside those that influence pubertal status could be attributed to environmental covariance. Taken as a whole, our results suggest that covariation between testosterone and DHEA is largely indicative of hormonal sensitivity to environmental inputs. Finding robust shared environmental effects provides an important linkage between research that finds environmental effects on hormone concentrations and genetically-informed research. A growing literature has shown that testosterone and DHEA levels are influenced by experiences within the family, and these family environment effects may be reflected within high shared environmental correlations as the family ecology is largely common to both twins. That is, those family experiences that increase testosterone levels may also increase DHEA levels. We speculate that family stressors or challenges may be salient

environmental factors given that both of these sex hormones are reactive to stress and challenge (reviewed in Shirtcliff and Ruttle 2010). For example, testosterone and DHEA reactivity to parent–child conflict is most evident within families characterized by high negative emotionality (Marceau et al. 2012) while relationships between these hormones and problematic outcomes appear diminished within high quality family environments (Booth et al. 2003; Halpern et al. 1994; Halpern et al. 1997). It is also possible that emotional experiences within the family are salient, given that testosterone varies according to fear (Hermans et al. 2006), anger (Wirth and Schultheiss 2007), and reward cues (Op de Macks et al. 2011); similarly, DHEA may be related to emotions, mood, and well-being (Ceballos and Alabsi 2006; Van Niekerk et al. 2001; Wolf and Kirschbaum 1999).

Limitations

There are a number of limitations that deserve highlighting. Although we have emphasized the sex differences in our findings, these results must be viewed with caution for two reasons. First, the sample size within each sex and zygosity grouping was somewhat low, as reflected in the wide confidence intervals. Second, larger studies are needed to detect differences in the set of genes that influence individual differences in hormones in males and females (i.e. qualitative sex differences). It is particularly important to understand the exact nature of sex differences in hormones before moving to molecular genetic analyses. Finally, because females mature at an earlier age than males, pubertal status is inextricably tied to sex. Thus, sex differences in the magnitude of genetic and environmental influences may simply reflect differences in the relationship between puberty, testosterone, and DHEA at different points in development. Disentangling sex and maturational effects is particularly tricky. Future studies should include a large sampling of adolescents at each stage of pubertal development in order to detect differences in underlying genetic and environmental influences across development, but even then males and females at the same stage of puberty are likely to be different ages and experiencing maturation within different social contexts.

Shared environmental influences of the magnitude we found on testosterone and DHEA are somewhat unusual, although the shared environment does tend to be more influential in younger samples. Similarly, non-shared environmental covariance between traits is often small or non-existent, a reflection of the extent to which non-shared environment includes measurement error. Testosterone and DHEA concentrations were measured in the same assay, which may have inflated our estimates of environmental influences if twin pairs were assayed on the same plates,

although one would expect to find more consistency in the covariation due to shared environment across sexes if this were the case. Estimates of non-shared environmental covariation may nevertheless have been inflated due to systematic variations in the assays. We did not collect any information on medication use prior to sample collection days. Several medications used to treat common childhood behavior problems influence sex hormone levels (Hibel et al. 2007; Lee et al. 2008), although it is unknown to what extent these effects persists beyond medication use. Similarly, we did not obtain information on menstrual cycles in females, although roughly half the sample reported having started menstruating. Both could potentially lead to inflated estimates of non-shared environmental covariation.

Questions may also be raised as to the extent to which the results of twin studies of hormones and puberty may be generalizable to the population as a whole. Twins are more likely to be born premature or experience birth complications than singletons (Luke et al. 1991; Spellacy et al. 1990) and there is some evidence that premature birth and low birth weights are associated with precocious puberty (Ibanez et al. 2000; Wehkalampi et al. 2011). In general, however, studies have found that by early- to mid-childhood twins are generally representative of singletons on wide variety of physical and behavioral measures (Rutter and Redshaw 1991; Van den Oord et al. 1995). Thus it is unclear to what extent twin status may affect puberty or hormone concentration. More importantly, twin studies in general may inflate heritability estimates (Ellis 2004) because heritability estimates are bound by the extent to which there is variance across environments, which are often truncated in genetically-informed studies. As Ellis (2004) points out, heritability estimates are often lower in dyads with divergent environments (e.g., mother-daughter) compared to dyads with more proximate environments (e.g., sister–sister) (Malina et al. 1994). Evidence from multiple sources such as adoption studies or extended twin-family designs are needed to fully elucidate the role of genetic and environmental influences on individual differences in sex hormones levels and pubertal development.

Finally, these analyses do not account for possible gene-by-environment interactions. If unaccounted for, interactions between genetic influences and shared environmental factors, such as SES or father presence/absence, result in higher MZ correlations relative to DZ correlations and thus inflate estimates of heritability, while interactions between genetic and non-shared environmental factors reduce the overall twin similarity and inflate estimates of non-shared environment (Purcell 2002). It is not possible to determine the extent to which these interactions, if they exist, may be biasing the results of the current study. Several methods have been proposed to test for interactions between *measured* environmental moderators and the unmeasured latent

genetic, shared, and non-shared environment influences on phenotypic variation (Jaffee and Price 2007; Purcell 2002; Rathouz et al. 2008). Therefore, future studies should include measures of environmental factors known to affect hormone levels and/or pubertal development or timing.

## Conclusion

This study adds to the literature on the genetic and environmental influences on sex hormones and their relation to pubertal maturation. Despite the important role that sex hormones play in pubertal development during adolescence, we found significant variation and covariation specific to testosterone and DHEA. In other words, sex hormones provided unique information beyond simply reflecting the physical characteristics of pubertal maturation. On a practical level, then, the present study has implications for research which examines pubertal maturation as an indirect index of hormonal events, as this practice may be an incomplete strategy for elucidating underlying biological mechanisms for maturational events. On the whole, our results suggest that testosterone and DHEA and the covariance unique to them are influenced by environmental forces and we speculate that shared experiences within the family may be particularly powerful at influencing hormone concentrations and, potentially, influencing biosocial risk for problems related to these hormones.

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