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# Hair and Salivary Testosterone, Hair Cortisol, and Externalizing Behaviors in Adolescents

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## Abstract

Although testosterone is associated with aggression in the popular imagination, previous research on the links between testosterone and human aggression has been inconsistent. This inconsistency might be because testosterone's effects on aggression depend on other moderators. In a large adolescent sample ($N$ = 984, of whom 460 provided hair samples), we examined associations between aggression and salivary testosterone, hair testosterone, and hair cortisol. Callous-unemotional traits, parental monitoring, and peer environment were examined as potential moderators of hormone-behavior associations. Salivary testosterone was not associated with aggression. Hair testosterone significantly predicted increased aggression, particularly at low levels of hair cortisol (i.e., Testosterone × Cortisol interaction). This study is the first to examine the relationship between hair hormones and externalizing behaviors and adds to the growing literature that indicates that androgenic effects on human behavior are contingent on aspects of the broader endocrine environment—in particular, levels of cortisol.

## Keywords

hair hormones, Testosterone × Cortisol, rule breaking, aggression, salivary testosterone

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Testosterone is a sex hormone produced primarily by the testes in males and a combination of the adrenal glands, ovaries, and circulating androstenedione in females. During adolescence, males and females experience a sharp increase in average circulating levels of testosterone (Braams, van Duijvenvoorde, Peper, & Crone, 2015). In addition to its importance for normal reproductive development, testosterone is also of interest because of its effects on social behavior. In particular, testosterone is thought to increase behaviors that enhance or maintain social status, most notably aggression and risk taking (Eisenegger, Haushofer, & Fehr, 2011; Mazur & Booth, 1998). Among adolescents, these *externalizing behaviors* are among the leading causes of morbidity and mortality (Shepherd, Farrington, & Potts, 2004). The behavioral effects of testosterone are potentially driven by testosterone's documented effects on multiple neurobiological systems, including (a) reduced orbitofrontal cortex-amygdala coupling during affective tasks, resulting in poor emotion regulation; (b) enhanced activity in the ventral striatum and nucleus accumbens, resulting in increased reward sensitivity and risk taking; and (c) downregulation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in greater stress resilience (Braams et al., 2015; Volman, Toni, Verhagen, & Roelofs, 2011; reviewed in Eisenegger et al., 2011).

Experimental studies in humans and animals indicate the importance of testosterone for aggressive and status-seeking behavior, but the meta-analytic effect size of this association in humans for endogenous individual differences in testosterone is small ($r$ = .08; Archer,

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Graham-Kevan, & Davies, 2005). Individual studies report highly variable findings, including positive, negative, and null associations. Although these inconsistencies likely stem, in large part, from sampling variability that is particularly pronounced among studies with small sample sizes, it is also possible that testosterone's effect on aggression is dependent on physiological, psychological, or social contexts.

## Hypothesized Moderators of Testosterone-Aggression Associations

A model of hormone-behavior relationships that includes a single hormone is likely overly focused on one piece of an interconnected endocrine system. Cortisol—the primary output of the HPA axis—may be critical for understanding the behavioral effects of testosterone (Mehta & Prasad, 2015). Cortisol has been found to increase punishment sensitivity via heightened expression of the corticotropin-releasing hormone gene (Schulkin, 2003) and has been associated with avoidant and freezing behavior (Roelofs, Elzinga, & Rotteveel, 2005). Testosterone has been found to be more strongly associated with aggression, dominance, and risk taking among individuals with lower cortisol than with those with higher cortisol (Mehta & Josephs, 2010; Mehta, Welker, Zilioli, & Carré, 2015; Popma et al., 2007), suggesting that cortisol functions to suppress androgenic effects on behavior. These findings are consistent with the *dual-hormone hypothesis* that testosterone and cortisol jointly regulate status-seeking tendencies (e.g., Mehta & Josephs, 2010; Mehta & Prasad, 2015). However, findings in the opposite direction have been reported for related traits (e.g., psychopathy; Welker, Lozoya, Campbell, Neumann, & Carré, 2014).

Another explanation for heterogeneity in prior studies of testosterone-behavior associations is that many studies do not account for environmental factors that "permit, stimulate, suppress, or set the stage" for how testosterone affects behavior (Booth, Granger, Mazur, & Kivlighan, 2006, p. 170). Levels of parental monitoring and peer deviance are environmental factors that may guide androgenic effects on aggressive and risk-taking behaviors. Parental monitoring functions as a protective factor against a number of maladaptive adolescent outcomes (e.g., delinquent behavior; Mann, Kretsch, Tackett, Harden, & Tucker-Drob, 2015) and, therefore, may downregulate the effect of testosterone on aggression. In contrast to the mitigating effects of parental monitoring, levels of peer deviance may amplify an association between testosterone and externalizing. Peer deviance is a risk factor for externalizing behavior (Moffitt, 1993), and some studies indicate Peer Group × Testosterone interactions on externalizing. For instance, Rowe, Maughan, Worthman, Costello, and Angold (2004) reported that testosterone was associated with dominant characteristics for male adolescents in positive peer groups, while testosterone predicted non-aggressive symptoms of conduct disorder among participants with more deviant peers.

Finally, personality traits may also moderate the behavioral effects of hormones. As described in DeYoung and Clark's (2012) exposition of Gene × Trait interactions, personality traits reflect "a characteristic pattern of psychological function . . . which has its origin in the cumulative effects of both the genome and the environment, [and] . . . therefore describes variation in the broad organismic context in which any single gene operates" (p. 1307). Like the effects of a single gene, the effects of a single hormone may depend on the "organismic context" of the individual, that is, personality. Consistent with this hypothesis, previous research in adolescents has found that testosterone is associated with externalizing behavior only among youth with personality risks, specifically, low conscientiousness and low agreeableness/high disagreeableness (Tackett, Herzhoff, Harden, Page-Gould, & Josephs, 2014). Callous-unemotional traits—temperamental tendencies toward limited empathy and constricted emotionality—are associated with chronic levels of antisocial behavior and aggression (e.g., Frick, Ray, Thornton, & Kahn, 2014) and capture personality traits characterized by low agreeableness and low conscientiousness (Mann, Briley, Tucker-Drob, & Harden, 2015). These findings suggest that youth with high testosterone levels may be at particular risk for externalizing behaviors if they are also high in callous-unemotional traits.

## State and Trait Variation in Testosterone as Measured in Saliva and Hair

Previous research on testosterone-behavior associations has typically measured hormone levels using a single salivary or blood sample. Single salivary samples collected at the same time of day evince moderate levels of stability whether they are collected 2 days ($r$ = .62; Harden et al., 2016) or 8 weeks ($r$ = .52; Dabbs, 1990) apart. Single samples collected at the same position in the diurnal curve, therefore, appear to pick up on a large component of traitlike individual differences in secretory patterns and diurnal change but are also likely to reflect some component of state fluctuations, including reactivity to the current environment. Aggregate measures of testosterone that attempt to capture more of the traitlike component of testosterone may provide more robust predictors of general behavioral repertoire.

Although only a few studies have used repeated testosterone sampling to examine associations with

externalizing behavior, existing research is consistent with the prediction that aggregate measures of testosterone are robust predictors of relatively stable behavioral dispositions. For example, among high externalizing males, salivary testosterone aggregated longitudinally across the ages of 13 to 21 years predicted the likelihood of becoming delinquent convicts (van Bokhoven et al., 2006). In a separate study, diurnal change in salivary testosterone, estimated from multiple salivary samples, was negatively associated with problem behaviors in females; in contrast, associations between problem behaviors and individual hormone samples from the same data set were relatively attenuated and inconsistent (Granger et al., 2003). Both of these studies estimated trait levels of testosterone by collecting multiple salivary or blood samples separated in time. This approach is promising but comes with challenges associated with participant compliance (e.g., providing samples at the requested times, missing sampling time points, and attrition). Hair sampling, a simpler alternative that can be carried out in a single visit, provides a putative measure of systemic, free hormone secretion over a period of several months (Dettenborn et al., 2012). Although no studies to date have used hair testosterone as a predictor of externalizing behaviors, positive associations between hair testosterone and other psychiatric outcomes (e.g., borderline personality disorder; Dettenborn et al., 2016) have been reported.

## Goals of the Current Study

In the present study, we used a population-based sample of adolescents to examine the associations between hair and salivary testosterone levels and self-reported repertoires of aggressive and rule-breaking behavior. We examined how hormone-behavior relationships differed as a function of specimen source (hair vs. saliva) and tested a number of hormonal (i.e., cortisol), family, peer, and personality moderators that have been advanced in previous research. We hypothesized that hair testosterone would be a more robust predictor of rule breaking and aggression at low levels of hair cortisol. We further predicted that this relationship would be particularly strong at low levels of parental monitoring and peer prosociality and at high levels of callous-unemotional traits and peer deviance.

## Method

### *Participants*

Twins in the Austin and Houston, Texas, area were identified using public school rosters and recruited via telephone calls and mailings. Five participants reported endocrine abnormalities and were excluded from the results. Participants ranged in age from 13.5 to 20.1 years ($M$ = 15.91, $SD$ = 1.39). The final sample consisted of 891 individuals (435 female) from 443 unique families enrolled in the Texas Twin Project (Harden, Tucker-Drob, & Tackett, 2013). Ninety-three of these individuals had data on two occasions, for a total of 984 observation points. Fifty-seven percent of participants identified as non-Hispanic White, 20% identified as Hispanic or Latino, 13% identified as African American, and 10% identified as another race or ethnicity. Of the participating families, 31.5% reported receiving some form of public assistance, including food stamps, since the twins' birth. Different research assistants assessed twins in separate rooms, and participants completed potentially sensitive survey questions on the computer. Participants were informed that the study was granted a federal certificate of confidentiality to promote honest reporting without concern of legal repercussions. Study procedures were approved by the university institutional review boards, and all participants provided both verbal and written consent prior to participation.

### *Measures*

***Rule breaking and aggression.*** Externalizing behaviors were assessed using youth self-report on 25 items from the shortened form of the Child Behavior Checklist (Lizotte, Chard-Wierschem, Loeber, & Stern, 1992). All items were rated on a 3-point scale (0 = *not true*, 2 = *very true or often true*). Construction of scale scores was informed by prior exploratory and confirmatory factor analyses (Harden et al., 2015). Aggression scores were created by taking the average of 13 items (e.g., "I physically attack people"), and nonaggressive-rule-breaking scores were created by taking the average of 12 items (e.g., "I break rules at home, school, or elsewhere").

***Pubertal development.*** Pubertal development was measured using the Pubertal Development Scale (Petersen, Crockett, Richards, & Boxer, 1988). Males and females rated appearance of body hair, growth in height, and skin changes on a 4-point scale (1 = *not yet begun to change*, 4 = *finished changing*). Male participants additionally rated growth of facial hair and deepening of voice, while female participants rated growth of breasts and the onset of menstruation. The menstruation item was scored 1 for "no" and 4 for "yes." The pubertal development outcome was taken as the average of the five items.

***Peer deviance and prosociality.*** Peer behaviors were assessed using a self-report questionnaire adapted from Thornberry, Lizotte, Krohn, Farnworth, and Jang (1994). Twenty-two items asked what proportion of friends engage

in delinquent behaviors (e.g., skipping school) and prosocial behaviors (e.g., participating in after-school programs). All questions were rated on a 4-point scale (1 = *none of them*, 4 = *all of them*). Prosocial and deviance items were separately averaged to create two composite scores.

***Callous-unemotional traits.*** The Inventory of Callous-Unemotional Traits self-report questionnaire (Kimonis et al., 2008) was used to assess levels of callous-unemotional traits. The questionnaire consists of 24 items rated on a 4-point scale (1 = *disagree strongly*, 4 = *agree strongly*). Scale items are designed to measure callous, uncaring, unemotional, and careless traits, using statements such as "I am not concerned about the feelings of others" and "I do not feel remorseful when I do something wrong." Although subscale scores can be computed, a composite average was created to reflect current methodological recommendations.

***Parental monitoring.*** Parental monitoring was assessed using a self-report questionnaire adapted from Capaldi & Patterson (1989). Participants rated seven items that assessed parental knowledge (1 = *they don't know*, 3 = *they know a lot*) about friends and activities (e.g., "What you do with your free time"). An additional eight items assessed parental rules and restrictions (1 = *never*, 3 = *always*) over friends and activities (e.g., "If you have been out past curfew, do your parents require an explanation"). The 15 items were averaged to create composite scores.

***Salivary testosterone.*** Participants provided salivary samples via passive drool into a 2-ml vial after approximately 15 min of completing consent forms and answering basic questions relevant to sample collection (e.g., what time they woke up that day). Samples were collected at one of three appointment times: 9:00 a.m. to 10:00 a.m. (29% of participants), 12:00 p.m. to 1:00 p.m. (49% of participants), or 2:00 p.m. to 3:00 p.m. (22% of participants). All participants were instructed to avoid eating or drinking anything 2 hr prior to the lab visit, to avoid smoking 4 hr before the study, and to avoid flossing on the morning of the visit. Saliva samples were immediately frozen on site at ≤ −30° C for a maximum of 12 months prior to being shipped on dry ice to Clemens Kirschbaum's laboratory at the Technical University of Dresden for analyses. Salivary testosterone concentrations were measured using commercially available chemiluminscense immunoassays (IBL International, Hamburg, Germany). The lower limit of sensitivity for the assay was 1.8 pg/mL, while extremely high values were estimated from a standard curve. The intra-assay and interassay coefficients of variation were < 8% and < 11%, respectively.

***Hair testosterone and cortisol.*** Hair samples approximately 3 mm in diameter and at least 3 cm in length were obtained from the posterior vertex position (i.e., the center of the back of the head). One cortisol value and one testosterone value that were estimated at more than 40 standard deviations above the sample means were excluded. The final hair sample included 460 participants (134 males). Fewer participants contributed hair data than saliva data because (a) the study began collecting hair data approximately 1 year after beginning saliva collection, and (b) many male participants had hair shorter than the 3-cm requirement. Hair hormones appear to be robust to a number of potential confounds, including contraceptive use, smoking, and frequency of hair washing (Dettenborn et al., 2012). However, participants were still instructed not to use any hair products that were not rinsed out of the hair on the day of the appointment.

The 3-cm hair segment closest to the scalp was analyzed as a marker for average testosterone and cortisol secretion over the most recent 3-month period. Whole nonpulverized hair was washed in isopropanol following wash and steroid extraction procedures describe elsewhere (Gao et al., 2013). The inter- and intra-assay coefficients of variation have been established as less than 10% for testosterone and cortisol in other samples (Gao et al., 2013). Internal consistency estimates for cortisol analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) have been reported above .96 (Stalder et al., 2012) and have yet to be established for hair testosterone. A subset of hair samples ($n$ = 27) collected in the first year were analyzed in duplicate, with a reliability estimate of .91 for hair testosterone and .89 for hair cortisol. However, only 8 of the 27 participants analyzed in duplicate were from the current sample. The current participant sample was also unique in that it included genetically identical participants who can provide an indirect index of within-sample reliability. These results suggested that hair and salivary markers provided a reasonably reliable estimate of hormone levels (see Table S1 in the Supplemental Material available online).

The lower limit of sensitivity for both hair testosterone and cortisol was 0.1 pg/ml (Gao et al., 2013). Although no samples were estimated below this limit for cortisol, 8 samples were below the sensitivity threshold for male testosterone, and 101 samples were below the limit for females. A binomial regression indicated that age was a significant predictor, log odds = −.20, 95% confidence interval (CI) = [−.37, −.03], $p$ = .019, of whether testosterone concentrations were below the sensitivity threshold (0 = no, 1 = yes); specifically, younger participants were more likely to have below-threshold values. Participants with below-threshold values were, therefore, given a value of 0.1 pg/ml, because excluding these participants altogether would have omitted informative data and potentially biased results.

### *Analyses*

Distributions for hormone, externalizing, parental monitoring, and peer outcomes were skewed and, therefore, log-transformed to approximate normal distributions. Hair hormone and salivary levels were then residualized for batch year and race (see Table S2 in the Supplemental Material for covariate effects) to control for potential confounds. Batch year was a dummy-coded variable created to reflect the year the samples were sent for assay. Salivary testosterone levels were additionally residualized for time since waking—computed as the minutes between waking that morning and the time of saliva collection. All results are reported with respect to standardized outcomes. Focal predictors—excluding age and sex—were standardized prior to conducting analyses or creating interaction terms. Age was mean-centered for all analyses, and female participants were the reference group for the sex variable. All analyses were conducted in Mplus (Version 7.4; Muthén & Muthén, 2012). This article does not report genetic effects. All analyses accounted for the nonindependence of observations within twin pairs using a sandwich estimator implemented using the complex survey capabilities in Mplus. Full information maximum likelihood was used for all models so that the entire data set was included, even for participants with missing hair data. The moderating effects of callous-unemotional traits, parental monitoring, peer deviance, and peer prosociality were examined in separate models.

General recommendations for sample sizes within structural equation models is 10 participants per freely estimated parameter (Hair, Anderson, Tatham, & Black, 1998). The callous-unemotional traits model was the most complex, containing 81 estimated parameters, for a recommended sample size of 810. The current sample ($N$ = 984) was, therefore, of an adequate size for inferential analyses. Data collection for the Texas Twin Project is largely conducted over the summer, with 200 to 300 adolescent participants run each year. We decided to proceed with the current analyses after the fifth summer of data collection when the number of participants was sufficient to examine the more complex models.

## Results

### *Preliminary analyses*

As a first step, hormonal outcomes were regressed on relevant covariates (age, age$^2$, sex, puberty, and Age × Sex interactions). In an initial model, the Age × Sex interaction and quadratic effect of age (i.e., Age × Age interaction) were estimated as nonsignificant for both hair outcomes, as was the Age$^2$ × Sex effect for all outcomes. These effects were subsequently dropped so as to produce more interpretable main effects. Results indicated that both salivary and hair testosterone concentrations were significantly higher in male participants, but hair cortisol concentrations did not differ by sex (Table 1). For salivary testosterone, the sex difference increased

**Table 1.** Unstandardized Parameter Estimates and Residual Covariances for the Model of Hormonal Outcomes

| Predictor | Salivary testosterone | Hair testosterone | Hair cortisol |
|---|---|---|---|
| | Parameter estimates | | |
| Sex | 1.36 [1.23, 1.48]*** | 0.78 [0.56, 0.99]*** | 0.04 [–0.20, 0.27] |
| Age | 0.04 [–0.03, 0.11] | 0.03 [–0.04, 0.10] | 0.04 [–0.04, 0.13] |
| Age × Sex | 0.15 [0.07, 0.23]*** | | |
| Age$^2$ | –0.03 [–0.06, –0.01]** | | |
| Pubertal development | 0.13 [0.06, 0.20]** | 0.05 [–0.06, 0.16] | 0.03 [–0.07, 0.14] |
| | Residual covariances | | |
| Hair testosterone | 0.03 [–0.05, 0.11] | | |
| Hair cortisol | –0.02 [–0.09, 0.05] | 0.08 [–0.03, 0.18] | |

Note: Hormonal outcomes were standardized prior to model fitting. Values in brackets are 95% confidence intervals. Age was mean-centered, and participant sex was dummy-coded (0 = female, 1 = male) prior to the construction of product or quadratic terms and model fitting. Thus, because of the inclusion of an Age × Sex term, the main effect of sex represents the effect of sex at the mean sample age, and the main effect of age represents the effect of age for the reference sex (females). Pubertal development was standardized prior to model fitting. In an initial model, the Age × Sex interaction and quadratic effect of age (i.e., Age × Age interaction) were estimated as nonsignificant for both hair outcomes, as was the Age$^2$ × Sex effect for all outcomes; these effects were dropped to produce more interpretable main effects. Residual covariances among the hormonal outcomes were freely estimated. The model was run for the full sample, including the subset of participants ($n$ = 460) for whom hair hormones were available. Asterisks indicate significant differences from zero (**$p$ < .01, ***$p$ < .001; all $p$s two-tailed).

with age. On the basis of the observed sex differences in testosterone but not cortisol, and following previous research (e.g., Mehta & Josephs, 2010), we standardized testosterone outcomes within sex for the remaining analyses, while cortisol continued to be standardized across sex.

Zero-order and age-partial correlations and descriptive statistics using the within-sex standardized testosterone outcomes are provided in Table 2, and age-partial correlations by sex are provided in Table S3 in the Supplemental Material. It is of note that the age-partial correlation between hair and salivary testosterone phenotypes was low ($r$ = .05, 95% CI = [−.06, .17], $p$ = .368), an effect that was consistent when the correlation was estimated separately for males ($r$ = .08, 95% CI = [−.11, .28], $p$ = .407) and females ($r$ = .04, 95% CI = [−.08, .18], $p$ = .577). Results indicated that although hair and salivary testosterone were not significantly associated with rule breaking and aggression, the associations were significantly different across hair and saliva for rule breaking ($\Delta r$ = .12, 95% CI = [< .01, .25], $p$ = .048) and aggression ($\Delta r$ = .14, 95% CI = [.02, .27], $p$ = .023).

## Hormones and externalizing

The next goal was to examine associations between hormones, age, sex, puberty, and aggressive and rule-breaking forms of externalizing. The following description of hormone and externalizing variables regressed on predictors applies to all subsequently presented structural equation models. Hair cortisol was regressed on age, sex, and puberty. Hair and salivary testosterone were regressed on age and puberty, but because they were already sex-standardized, were not regressed on sex. On the basis of the results reported above, which indicated sex moderation of age trends in salivary testosterone, we regressed salivary testosterone on an Age × Sex interaction. Externalizing outcomes were regressed on age, puberty, sex, Age × Sex, hair testosterone, salivary testosterone, hair cortisol, and a Hair Testosterone × Hair Cortisol interaction. The residual covariance between aggression and nonaggressive rule breaking was also estimated. Salivary testosterone was not significantly associated with rule breaking or aggression. There was a main effect of hair testosterone on rule breaking ($b$ = 0.10, 95% CI = [< −0.01, 0.20], $p$ = .063; Table 3) and a significant main effect of hair testosterone on aggression ($b$ = 0.11, 95% CI = [0.01, 0.21], $p$ = .028).

Because the model included higher-order effects (i.e., Testosterone × Cortisol interactions), these main effects should be interpreted as the effect of hair testosterone estimated at (standardized) cortisol levels equal to 0 (i.e., the sample mean). There was a significant Hair Testosterone × Hair Cortisol interaction predicting aggression ($b$ = −0.12, 95% CI = [−0.21, −0.03], $p$ = .011; Fig. 1). At high levels of cortisol (+1 *SD*), the simple slope of hair testosterone had no discernible effect on aggression ($b$ = −0.02, 95% CI = [−0.10, 0.07], $p$ = .945), but hair testosterone predicted increasing levels of aggression ($b$ = 0.22, 95% CI = [0.04, 0.39], $p$ = .008) when cortisol was low (−1 *SD*). Put another way, the highest levels of aggression were observed for participants with low cortisol and high testosterone concentrations.

Interactions with sex were also tested for all hormonal predictors and the Testosterone × Cortisol interaction but were not significant ($p$s ≥ .224). However, results are provided separately by sex in Figure 1 and in Table S4 in the Supplemental Material. Although significance levels were attenuated within males and females, which may be a reflection of reduced power, the direction of effects was equivalent across sexes. Sensitivity analyses excluding participants below the 0.1 pg/ml threshold for hair testosterone also indicated the same general pattern of findings, with two notable exceptions (see Table S5 in the Supplemental Material). First, the interaction between testosterone and cortisol was significant for rule breaking. Second, at high levels of cortisol there was a small negative effect of testosterone on both externalizing outcomes (see Fig. S1 in the Supplemental Material). Interactions between salivary testosterone and time since waking were also examined, but they revealed nonsignificant effects for rule breaking ($b$ = −0.03, 95% CI = [−0.06, 0.01], $p$ = .113) and aggression ($b$ = −0.02, 95% CI = [−0.05, 0.01], $p$ = .303).

## Callous-unemotional traits

The remaining goals were to examine potential moderators of hormone effects on rule breaking and aggression. All remaining models included externalizing outcomes regressed on moderator main effects and interactions between the moderator of interest and all hormonal predictors, including a three-way Moderator × Hair Testosterone × Hair Cortisol interaction. In addition, the moderator and interaction terms with hormones were regressed on age, puberty, sex, and an Age × Sex interaction. There were significant main effects of callous-unemotional traits; participants higher in callous-unemotional traits reported elevated levels of rule breaking ($b$ = 0.44, 95% CI = [0.36, 0.52], $p$ < .001) and aggression ($b$ = 0.32, 95% CI = [0.25, 0.40], $p$ < .001; Table S1). Callous-unemotional traits did not significantly moderate the effect of any hormones.

**Table 2.** Descriptive Statistics and Zero-Order and Partial Correlations

| Variable | *M* (*SD*) | Range | Correlations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1. Age (years) | 15.91 (1.39) | 13.57–20.11 | .23*** | .07 | .09 | .01 | .09* | −.27*** | .25*** | .01 | −.10** | .39*** |
| 2. Salivary testosterone (pg/ml) | 67.08 (64.79) | 2.10–616.25 | — | .07 | −.01 | −.06 | −.01 | −.13*** | .09** | .01 | .01 | .22*** |
| 3. Hair testosterone (pg/ml) | 1.76 (6.71) | 0.1–96.6 | .05 | — | .09 | .09 | .09 | −.04 | .02 | −.02 | .05 | .05 |
| 4. Cortisol (pg/ml) | 10.32 (33.89) | 0.31–424.80 | −.03 | .09 | — | −.07 | −.01 | −.04 | −.05 | .10* | −.05 | .06 |
| 5. Aggression | 0.39 (0.26) | 0–1.62 | −.05 | .09 | −.07 | — | .52*** | −.11** | .30*** | −.27*** | .32*** | −.09** |
| 6. Rule breaking | 0.28 (0.25) | 0–1.5 | −.04 | .08 | −.02 | .52*** | — | −.35*** | .57*** | −.42*** | .44*** | −.04 |
| 7. Parental monitoring | 2.59 (0.30) | 1.07–3.00 | −.06 | −.01 | −.02 | −.11** | −.32*** | — | −.29*** | .32*** | −.30*** | −.03 |
| 8. Peer deviance | 1.30 (0.30) | 1–3.3 | .03 | .01 | −.07 | .30*** | .55*** | −.24*** | — | −.42*** | .24*** | .07* |
| 9. Peer prosociality | 2.92 (0.44) | 1–4 | .01 | −.03 | .10* | −.27*** | −.42*** | .33*** | −.43*** | — | −.43*** | .14*** |
| 10. Callous-unemotional traits | 1.89 (0.32) | 1.13–3.08 | .03 | .06 | −.05 | .32*** | .45*** | −.34*** | .28*** | −.44*** | — | −.20*** |
| 11. Puberty | 3.19 (0.59) | 1–4 | .13*** | .03 | .03 | −.08** | −.06* | .09* | −.04 | .15*** | −.17*** | — |
| 12. Hair testosterone – salivary testosterone | | | | | .12 | .14* | .12* | .06 | −.02 | −.02 | .03 | −.10 |

Note: Descriptive statistics are reported in raw units, and correlations are between transformed variables. Testosterone outcomes were standardized within sex. Zero-correlations are reported above the diagonal, and partial correlations with respect to age are reported below the diagonal. The bottom row reports the difference in the age-partial correlations across hair testosterone and salivary testosterone. Correlations were computed using the full sample, including for hair hormones that were available on a subset of participants ($n = 460$). Asterisks indicate significant differences from zero (*$p < .05$, **$p < .01$, ***$p < .001$; all $p$s two-tailed).

**Table 3.** Unstandardized Parameter Estimates From the Primary Model of Aggression and Rule Breaking

| Predictor | Aggression | Rule breaking |
|---|---|---|
| Salivary testosterone | –0.05 [–0.12, 0.03] | –0.05 [–0.12, 0.03] |
| Hair testosterone | 0.11 [0.01, 0.21]* | 0.10 [< –0.01, 0.20] |
| Hair cortisol | –0.07 [–0.17, 0.04] | –0.02 [–0.12, 0.09] |
| Hair Testosterone × Hair Cortisol | –0.12 [–0.21, –0.03]* | –0.05 [–0.14, 0.03] |
| Age | 0.06 [–0.01, 0.13] | 0.05 [–0.01, 0.12] |
| Puberty | –0.09 [–0.17, –0.01]* | 0.01 [–0.07, 0.09] |
| Sex | 0.04 [–0.12, 0.20] | 0.33 [0.16, 0.49]*** |
| Age × Sex | –0.04 [–0.14, 0.06] | 0.05 [–0.05, 0.14] |
| Multiple $R$ | 0.21 [–0.04, 0.31] | 0.22 [0.10, 0.30]** |
| Multiple $R^2$ | 0.04 [< –0.01, 0.09] | 0.05 [0.01, 0.09]** |

Note: The residual covariance between aggression and rule breaking was 0.50, 95% confidence interval (CI) = [0.41, 0.58], $p < .01$. Salivary testosterone, hair testosterone, hair cortisol, and puberty were standardized, age was centered at its mean, and sex was dummy-coded (0 = female, 1 = male) prior to computing product terms and being entered in the model. Aggression and rule breaking were also standardized prior to model fitting. Values in brackets are 95% CIs. Because of the inclusion of a Testosterone × Cortisol interaction, the main effects of hair testosterone and cortisol represent the model-implied effects at the sample average of cortisol and testosterone, respectively. Because of the inclusion of an Age × Sex interaction, the main effect of sex was estimated at the sample average age, and the main effect of age was estimated for the reference sex (females). The residuals for the hair hormones, after controlling for covariates, were freely correlated. The model was run for the full sample, including the subset of participants ($n = 460$) for whom hair hormones were available. Asterisks indicate significant differences from zero (*$p < .05$, **$p < .01$, ***$p < .001$; all $p$s two-tailed).

### *Parental monitoring and peer environment*

Results from a parental-monitoring moderation model are provided in Table S2. There was a significant main effect of parental monitoring predicting decreased levels of rule breaking ($b = –0.36$, 95% CI = [–0.43, –0.28], $p < .001$) and aggression ($b = –0.13$, 95% CI = [–0.21, –0.05], $p = .002$). Results from a peer-deviance moderation model are provided in Table S3. There was a significant main effect of peer deviance predicting higher levels of rule breaking ($b = 0.58$, 95% CI = [0.51, 0.65], $p < .001$) and aggression ($b = 0.33$, 95% CI = [0.25, 0.40], $p < .001$). There was also an interaction between salivary testosterone and peer deviance predicting aggression ($b = –0.10$, 95% CI = [–0.18, –0.03], $p = .006$; Fig. S2 in the Supplemental Material). Interaction findings were in the reverse direction as hypothesized; salivary testosterone was associated with decreased aggression at high levels of peer deviance but was not associated with aggression at low levels of peer deviance. There was also a three-way interaction between peer deviance, hair testosterone, and hair cortisol predicting rule breaking ($b = 0.16$, 95% CI = [0.03, 0.29], $p = .028$; Fig. S3 in the Supplemental Material). At low levels of peer deviance, testosterone predicted increased rule breaking at low levels of cortisol, while at high levels of peer deviance, testosterone predicted increased rule breaking at high levels of cortisol.

Results from a peer-prosociality moderation model are provided in Table S4. Peer prosociality predicted decreased levels of rule breaking ($b = –0.42$, 95% CI = [–0.52, –0.32], $p < .001$) and aggression ($b = –0.27$, 95% CI = [–0.35, –0.19], $p < .001$). In addition, there was another three-way interaction predicting rule breaking that mirrored the findings for peer deviance ($b = –0.18$, 95% CI = [–0.34, –0.02], $p = .048$; Fig. S3); at high levels of peer prosociality, testosterone predicted increased rule breaking at low levels of cortisol. That the three-way interactions were just within the significance threshold reduces confidence in their reproducibility. We therefore caution the reader to treat these particular interaction results as tentative.

## Discussion

This study extends testosterone and externalizing research by examining how this relationship changes across specimen source (saliva vs. hair), cortisol, peers, parents, and personality. There was a positive main effect of hair, but not salivary, testosterone on aggression, while neither form of testosterone was predictive of rule breaking. The main effect was still quite small, which is to be expected given the modest effect sizes

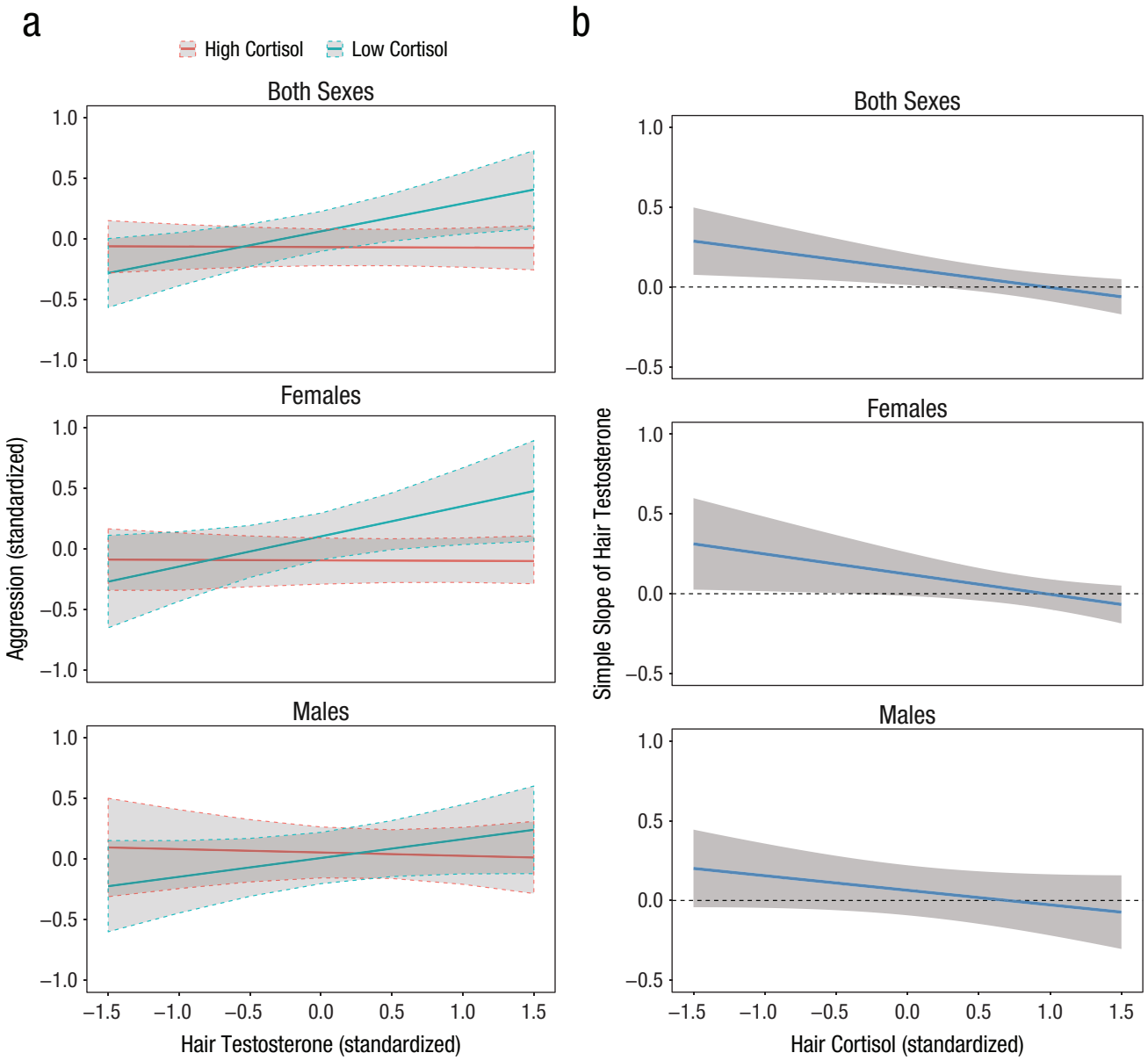


**Fig. 1.** Hair Testosterone × Hair Cortisol interactions. Predicted aggression for participants with low (−1 *SD*) and high (+1 *SD*) levels of cortisol (a) is shown as a function of hair testosterone. The simple slope of hair testosterone (b) is shown as a function of hair cortisol. In both panels, results are shown separately for females, males, and both sexes combined. Gray shading indicates 95% confidence intervals that controlled for the dependency between within-family observations.

reported in meta-analyses (e.g., Archer et al., 2005). One reason for these small effects may be that the majority of prior research evaluated the role of testosterone in isolation, whereas a growing literature suggests that the behavioral effects of testosterone are contingent on levels of cortisol.

Consistent with the dual-hormone hypothesis (e.g., Mehta & Josephs, 2010), the current results indicated that the effect of hair testosterone on aggression interacted with hair cortisol levels. At high levels of hair cortisol, hair testosterone was unrelated to aggression, whereas at low levels of cortisol, testosterone was positively related to aggression. In the sample excluding participants below the hair testosterone detection limit, this interaction also predicted rule breaking, and testosterone was negatively associated with behavior at high levels cortisol. This negative effect of testosterone at high cortisol levels has also been reported previously (Mehta & Josephs, 2010).

The interaction between testosterone and cortisol could be due to a number of neurobiological mechanisms, including a shared ability to affect neural mechanisms

involving threat and reward, cortisol's inhibitory effect on gonadal hormone synthesis, shared effects on DNA transcription, or an interactive effect on subsequent hormone release (reviewed in Mehta & Prasad, 2015). An alternative possibility is that these hormones are not interacting at a biological level of measurement but rather that they affect upstream psychological processes that interact to predict aggressive tendencies. For example, cortisol has been shown to increase avoidance tendencies (Roelofs et al., 2005), which may act to downregulate direct effects of testosterone on aggressive tendencies.

The current study indicated largely null interactions between putative nonhormonal moderators of testosterone associations with externalizing. The Salivary Testosterone × Peer Deviance interaction predicting aggression was in the opposite direction as hypothesized. We also identified 2 three-way interactions between testosterone, cortisol, and both peer deviance and peer prosociality. These interactions indicated that at high levels of peer deviance and low levels of peer prosociality, hair testosterone predicted increased rule breaking at high levels of hair cortisol. Future research should look to examine the replicability of these effects, as it is unclear what might explain them. It is possible that they are false discoveries.

## *Limitations and future research*

It is unclear whether hair testosterone is a more robust predictor of externalizing relative to salivary testosterone, as they were analyzed using different methods (immunoassay vs. LC-MS/MS), and the most robust effect, a Testosterone × Cortisol interaction, was examined only in hair. LC-MS/MS may also be a more valid measure of testosterone relative to immunoassays, particularly for individuals with low concentrations (e.g., Welker et al., 2016). In addition, the different sampling times for salivary testosterone may have attenuated behavioral associations. A weakness of the study was that analytic plans were not preregistered.

Future research should look to disentangle whether testosterone is associated with specific forms of aggression (e.g., proactive vs. reactive) that are combined in the Child Behavior Checklist used in the current study. A growing body of evidence suggests that trait levels of dominance moderate main effects of testosterone (Carré et al., 2016) and Testosterone × Cortisol interactions (Pfattheicher, 2017) predicting aggression, a finding that should be examined using hair samples. Future research should examine the reliability and validity of hair hormones using methods such as certified reference materials and duplicate samples. Finally, intraindividual variability captured by multiple salivary or blood samples should be examined as a separate predictor of externalizing behavior. High levels of within-person hormonal variation might be an indicator of a dysregulated endocrine system that has large downstream effects on behavior.

## *Conclusions*

The association between testosterone and externalizing is complex and methodological, and contextual differences across studies likely contribute to the heterogeneity of extant results. The present findings indicate that hair cortisol attenuates effects of hair testosterone on aggressive behavior. Designs that investigate the joint effects of multiple markers of an intertwined hormonal system may be key to understanding the neuroendocrine bases of externalizing behavior.

### Action Editor

Steven W. Gangestad served as action editor for this article.

### Author Contributions

K. P. Harden and E. M. Tucker-Drob developed the study concept and design. A. D. Grotzinger, M. W. Patterson, and F. D. Mann collected the data; data collection was supervised by K. P. Harden, E. M. Tucker-Drob, and J. L. Tackett. A. D. Grotzinger analyzed and interpreted the data under the supervision of K. P. Harden and E. M. Tucker-Drob. A. D. Grotzinger drafted the manuscript, and all authors provided critical revisions. All authors approved the final version of the manuscript for submission.

### Declaration of Conflicting Interests

The author(s) declared that there were no conflicts of interest with respect to the authorship or the publication of this article.

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### Supplemental Material

Additional supporting information can be found at http://journals.sagepub.com/doi/suppl/10.1177/0956797617742981

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