Psychoneuroendocrinology 99 (2019) xxx–xxx

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Dual-hormone regulation of psychopathy: Evidence from mass spectrometry

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ARTICLE INFO

Keywords:
Psychopathy
Hormones
Testosterone
Cortisol
Mass spectrometry
Liquid chromatography

ABSTRACT

Previous work suggests that testosterone and cortisol interactively predict psychopathy. This effect represents a reversal of the established dual-hormone hypothesis, whereby testosterone is positively correlated with psychopathic traits, but only among individuals with elevated cortisol concentrations. This study aims to replicate the dual-hormone moderation of psychopathy in two independent samples. Enzyme-linked immunoassays (ELISAs) were used to assess cortisol across both samples and testosterone in Sample 1 (n = 165, 100% males). To address recent criticism of ELISAs and potentially extend these findings to women, testosterone concentrations were determined by liquid chromatography tandem mass spectrometry (LC–MS/MS) in Sample 2 (n = 213, 44.1% males). We found conflicting evidence of the dual-hormone moderation of psychopathic traits. Although results were non-significant in Sample 1, a reversal of the dual-hormone hypothesis was found in Sample 2, in which testosterone was positively correlated with psychopathic traits, but only among individuals with high cortisol. This replication provides mixed support for less common reversals to the dual-hormone hypothesis. These findings emphasize the importance of using LC–MS/MS to measure testosterone and adds to the growing body of work on the relationship between hormones and psychopathology in general.

1. Introduction

People high in trait psychopathy tend to have low empathy, low anxiety, fearlessness, aggression, impulsivity and antisocial behaviors (Lykken, 1995; Hunt et al., 2005; Visser et al., 2012). At a glance, these characteristics have much in common with the behavioral and psychological correlates of the hormones testosterone and cortisol. Testosterone, a steroid hormone released by the hypothalamic-pituitary-gonadal (HPG) axis, has been associated with elevated aggression and impulsivity, but low empathy and anti-social behavior (Mazur and Booth, 1998; Archer et al., 2005; Hermans et al., 2006; Zilioli et al., 2015). Cortisol, a glucocorticoid hormone released by the hypothalamic-pituitary-adrenal (HPA) axis, is notably linked to anxiety, with several studies finding flattened diurnal cortisol fluctuations in those with high anxiety (e.g., Korte, 2001; Giese-Davis et al., 2004; Van den Bergh et al., 2008).

1.1. Previous research on testosterone, cortisol, and psychopathy

Due to the potential overlap between testosterone, cortisol, and trait psychopathy, research in this area has received increased empirical attention. This work has focused on individual effects of testosterone and cortisol, as well as interactive joint effects. Work in male criminal offenders reveals that testosterone concentrations have a positive relationship with anti-social personality disorder and socially deviant behavior (Stålenheim et al., 1998). People scoring high in psychopathic traits often have blunted diurnal rhythms of cortisol, as well as lower baseline cortisol levels, and reduced cortisol reactivity to stressors (see Shirtcliff et al., 2009 for a review). However, other work suggests no bivariate correlations between psychopathic traits and either testosterone or cortisol within a relatively large non-clinical sample (Glenn et al., 2011). Yet, when testosterone levels were high, the ratio of baseline testosterone to cortisol reactivity positively predicted individual differences in psychopathic traits in a predominantly male sample (Glenn et al., 2011). However, recent work has criticized using ratios to analyze two independent hormones, particularly due to statistical and interpretational difficulties with hormone ratios (see Sollberger and Ehlert, 2016). Cortisol also has an inverse relationship with the severity of psychopathic traits in violent male offenders (Holi et al., 2006). Consistent with these findings, psychopathic male prison inmates had significantly lower cortisol levels than non-psychopathic offenders in a sample of both male prison inmates and healthy male controls (Cima et al., 2008).

The lack of consistent bivariate associations between the two

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https://doi.org/10.1016/j.psyneuen.2018.09.006
Received 24 May 2018; Received in revised form 17 August 2018; Accepted 7 September 2018

hormones and psychopathy might be explained by recent research highlighting the dual-hormone hypothesis and psychopathy (Welker et al., 2014). Research has revealed that testosterone and cortisol may not have exclusively independent effects, but instead interactively modulate behavior (Terburg et al., 2009; Mehta and Josephs, 2010; Carré and Mehta, 2011; Mehta and Prasad, 2015). Indeed, work suggests the HPA and HPG axes have mutually inhibitory effects on each other, resulting in a potential co-regulation of social behaviors (Viau, 2002; Carré and Mehta, 2011; Mehta and Prasad, 2015). The dual-hormone hypothesis posits that testosterone positively correlates with status-seeking behavior (e.g., dominance, aggression, risk-taking, etc.) only when cortisol levels are low (see Mehta and Prasad, 2015 for a review). Consistent with the dual-hormone hypothesis, studies have demonstrated that testosterone's effect on anti-social behaviors such as aggression, dominance, and violent crime in both women and men are present only when cortisol levels are low (Dabbs et al., 1991; Popma et al., 2007; Mehta and Josephs, 2010). Furthermore, in a mixed sex sample of adolescents with high levels of emotional instability and disagreeableness, testosterone has been associated with externalizing problems solely when cortisol concentrations are low (Tackett et al., 2014). However, some studies have failed to garner support for the dual hormone hypothesis with outcome measures such as aggression (Geniole et al., 2013) and anti-social deviance (Mazur and Booth, 2014). Additionally, testosterone was found to be associated with reactive aggression only in those with high cortisol concentrations, demonstrating a reversal to the dual-hormone hypothesis (Denson et al., 2013). Recently, this dual-hormone relationship was examined with psychopathic traits and testosterone was found to be positively associated with men's psychopathy only when cortisol levels were relatively high. These results have been interpreted in conjunction with other findings suggesting a less common reversal of the dual-hormone hypothesis (see Mehta and Prasad, 2015 for a review). While these results are intriguing, they necessitate replication due to the paucity of dual-hormone reversals.

1.2. Inconsistencies with hormone assays

Much of the research on testosterone and behavior has relied on enzyme-linked immunoassays (ELISAs) to assess salivary testosterone concentrations. Although ELISAs provide a relatively affordable and easy means to assess hormones, they are not without limitations. Although results from different commercially-available ELISA kits tend to be strongly correlated with each other (Welker et al., 2016; Andersson et al., 2017), immunoassays overestimate testosterone concentrations when compared to mass spectrometry assays (Taieb et al., 2003; Baecher et al., 2013; Welker et al., 2016). Testosterone ELISAs have weaker correlations with liquid chromatography tandem mass spectrometry (LC–MS/MS) assays compared to other ELISAs such as cortisol (Welker et al., 2016). Thus, using a substantially more precise measurement tool to assess salivary testosterone (e.g., LC–MS/MS) may be ideal for studying testosterone and psychopathic traits.

1.3. Issues with replicability

Replicability in psychological science has been a growing concern with a substantial amount of studies having insufficient power and failing to replicate (e.g., Simons, 2014; Bohannon, 2015; Open Science Collaboration, 2015). Work related to the dual-hormone hypothesis has largely consisted of conceptual replications of the theory using different variables (e.g., Tackett et al., 2014; Mehta et al., 2015; Zilioli et al., 2015). While a variety of different outcomes and contextual moderators have been the focus of such research, there is need for direct replication to provide robust support for these findings. To help with the replication of previous findings, we attempted to replicate the findings of Welker et al., 2014 within two independent non-clinical samples using both ELISA and LC–MS/MS methods to assess testosterone.

1.4. The value of examining non-clinical samples

Naturally, a non-clinical sample will likely include less extreme levels of psychopathic behavior. However, past research has demonstrated that the general population may show diverse expressions of psychopathic traits (see Skeem et al., 2003). In addition, a non-clinical sample may have a greater range of psychopathic severity, from non-existent/mild forms of psychopathic behaviors to more severe forms of behaviors. Thus, investigating psychopathic traits in non-clinical samples may prove valuable in understanding how hormones influence a greater range of psychopathic behavior.

2. Materials and methods

2.1. Participants

Sample 1 consisted of 165 male undergraduate students from a large Midwestern urban university who participated for partial course credit as compensation. The sample was relatively young (M_{age} = 20.64, SD = 2.99) and diverse (full demographic data is provided in the supplemental online materials). Four participants were excluded from analysis for not providing baseline cortisol and/or testosterone samples. The current study was a part of a larger protocol examining testosterone and risk-taking behavior. Although data collected from this sample have been used in other publications (Mehta et al., 2015; Welker et al., 2015), the data from the measures assessed in this manuscript do not overlap with previously published work. All procedures and measures were approved by the University Institutional Review Board.

Sample 2 consisted of 213 participants (55.9% female). Participants were recruited through the psychology department's human subjects pool consisting of Psychology 101 students from an urban research university in New England, from flyers posted around campus, and from Craigslist advertisements targeting people in the community. Participants in the human subject pool were compensated with four research credit hours towards their class requirement. Other participants were compensated with a $30 Amazon.com gift card in lieu of class credit. Sample 2 was older and had a greater age range than Sample 1 (M_{age} = 23.37, SD = 6.28). Participants were also ethnically and socioeconomically diverse (see supplemental materials). Sixteen participants were excluded from the analysis because they failed to pass one or both of the attention checks in the survey. The current study was a part of a larger protocol examining hormones and interpersonal interactions. These data do not overlap with previously published work. All procedures and measures were approved by the University Institutional Review Board.

2.2. Materials and procedure

To ensure uncontaminated saliva, participants in Samples 1 and 2 were asked not to eat, drink, or brush their teeth during the hour prior to the study. Additionally, participants were asked not to exercise during the day of the study for a better measure of naturalistic baseline hormone levels. Upon arrival to the lab (Sample 1 at the Midwestern Campus, and Sample 2 at the New England Campus), participants completed the Self Report Psychopathy – short form (SRP-SF; Paulhus et al., 2016) among a series of other questionnaires not used in this study. The SRP-SF is an established shortened version of the SRP-III with 29 items assessing general psychopathy along with four factors of psychopathic traits: Interpersonal, Affective, Lifestyle, and Antisocial. The SRP-SF is shown to be a reliable measure with good internal consistency (Gordts et al., 2017). The average score on the SRP-SF was 52.50 (SD = 11.81) in a community sample of 1501 participants (Gordts et al., 2017). In the same sample, males on average had higher scores (M = 56.50, SD = 12.31) than females (M = 48.82, SD = 9.99). The SRP-SF scores in our samples were similar. Sample 1 participants averaged a score of 59.67 (SD = 15.75), and Sample 2 participants

Table 1
Correlations and Descriptive Statistics for Variables from Study 1 & 2.

Study 1	1.	2.	3.	4.	5.	6.	7.
1. Total Psychopathy	—						
2. Affective	.85***	—					
3. Interpersonal	.86***	.69***	—				
4. Lifestyle	.85***	.65***	.61***	—			
5. Antisocial	.71***	.44***	.47***	.51***	—		
6. Cortisol (ln)	.03	.00	.02	.10	−.05	—	
7. Basal T (ln)	−.02	−.04	−.01	.03	−.03	.34***	—
M (SD)	59.67 (15.75)	15.52 (4.55)	15.70 (5.57)	16.79 (5.03)	10.53 (3.69)	2.97 (3.55)†	103.16 (43.23)††
Study 2	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**
1. Total Psychopathy	—						
2. Affective	.86***	—					
3. Interpersonal	.89***	.73***	—				
4. Lifestyle	.86***	.64***	.68***	—			
5. Antisocial	.63***	.38***	.40***	.45***	—		
6. Basal T (Z-scored)	.21**	.22**	.21**	.17*	.04	—	
7. Cortisol (ln)	.10	.04	.08	.14	.07	.00	—
M (SD) Combined	49.62 (15.49)	13.00 (5.01)	12.04 (5.33)	13.74 (4.90)	9.77 (3.29)	35.94 (37.50)†††	0.12 (0.10)††††
M (SD) Males	53.78 (15.15)	13.48 (5.53)	14.24 (4.64)	14.79 (4.71)	10.19 (3.80)	73.18 (26.10)†††	0.14 (0.13)††††
M (SD) Females	46.17 (14.78)	10.81 (4.76)	11.96 (5.01)	12.90 (4.91)	9.41 (2.80)	6.82 (4.17)†††	0.10 (0.05)††††

Note. Study 1: Cortisol (ln) - log transformed cortisol concentrations, Testosterone (ln) - log transformed testosterone concentrations. † Raw cortisol Means and SDs are presented in ng/mL. †† Raw testosterone Means and SDs are presented in pg/mL. Study 2: Cortisol (ln) - log transformed cortisol concentrations, Testosterone (z-scores) - standardized testosterone concentrations across males and females. ††† Raw testosterone Means and SDs are presented in pg/mL. †††† Raw cortisol Means and SDs are presented in ug/dL. One transgender case was removed for the gender-separated descriptive analysis.

* $p < .05$.
** $p < .01$.
*** $p < .001$.

averaged a score of 49.62 (SD = 15.49). Gender differences in average scores from Sample 2 were also in line with previous work, with men averaging slightly higher scores (M = 53.78, SD = 15.15) than women (M = 46.17, SD = 14.78). For more information on SRP-SF correlations and descriptive statistics, please see Table 1. The Cronbach's alphas of the SRP-SF in Sample 1 were, for the most part, acceptable (Total Psychopathy: α = .88; Interpersonal: α = .80; Affective: α = .66; Lifestyle: α = .74; Antisocial: α = .42). The Cronbach's alpha for the SRP-SF in Sample 2 were similar to Sample 1 (Total Psychopathy: α = .88; Interpersonal: α = .81; Affective: α = .74; Lifestyle: α = .74; Antisocial: α = .55).

Two attention check questions were embedded within the questionnaires provided to participants in Sample 2. One read, "Select 'Very often or always true' for this question." Failing to select this option was scored as a failure to pass the attention check. The other read, "I have all ten of my fingers." All participants had all ten of their fingers, thus a disagreement with this statement was scored as a failure to pass the attention check.

2.3. Saliva samples

After completing the SRP-SF (and the other self-report questionnaires not used for this study), participants in Samples 1 and 2 provided approximately 3–5 mL of saliva via unstimulated passive drool into polypropylene centrifuge tubes. All samples were taken between 11:00am and 5:00pm to control for diurnal variation in testosterone and cortisol concentrations. The samples were immediately frozen after collection and assayed at a later date. Saliva samples from Sample 1 were assayed for testosterone and cortisol using commercially-available ELISA kits (DRG International). Mean intra-assay and inter-assay coefficients of variation for testosterone were 6.2% and 8.9% respectively. Mean intra-assay and inter-assay coefficients of variation for cortisol were 5.7% and 6.3% respectively (Fig. 1).

As mentioned above, recent research has highlighted some inconsistencies between ELISAs of different manufacturers and LC–MS/MS in testing testosterone concentrations. To increase the precision and accuracy of testosterone measurements, saliva samples from Sample 2 were assayed for testosterone using LC–MS/MS (Brigham Research Assay Core, Boston, MA). Testosterone in saliva was extracted by solid phase extraction, then eluted by high performance liquid chromatography, and determined by mass spectrometry with electrospray ionization. LC–MS/MS has a dynamic range of 1–1000 pg/mL and a lowest reportable value of 1.0 pg/mL. The sensitivity and low detection limit of LC–MS/MS is ideal in measuring low-levels of testosterone, which is necessary in assessing samples that consist of female participants. Cortisol was assayed using Salimetrics ELISA kits at Brigham Research Assay Core. Mean intra-assay and inter-assay coefficients of variation for cortisol were 9.2% and 5.7% respectively (Fig. 2).

2.4. Analytic strategy

After adding a constant of 1, cortisol concentrations were natural-log transformed for all analyses to adjust for skewness in both Sample 1 and Sample 2. In Sample 1, testosterone concentrations were natural-log transformed to adjust for skewness for all analyses. A histogram of testosterone concentrations in Sample 2 demonstrated a seemingly normal distribution, therefore Sample 2 testosterone concentrations were not transformed. Sample 2 consisted of both men and women. Although normally distributed, testosterone levels differed between men (M = 73.18 pg/mL, SD = 26.10) and women (M = 6.82 pg/mL, SD = 4.17) (Cohen's d = 3.55), thus testosterone was standardized separately for both sexes[1] (e.g., Welker et al., 2017). Testosterone and cortisol descriptive statistics are presented in Table 1. Outliers that were 3 SDs away from the mean for cortisol and testosterone were winsorized in Sample 1 (testosterone: n = 1) and in Sample 2 (cortisol: n = 4; testosterone: n = 3). Statistical moderation analyses were tested with PROCESS, an SPSS add-in (Hayes, 2013) and interaction figures were created using the *visreg* package in R (Development and Team, 2009; Breheny and Burchett, 2017).

[1] One participant identified as transgender. The testosterone concentration for this case was typical of male samples, thus it was transformed with the men.

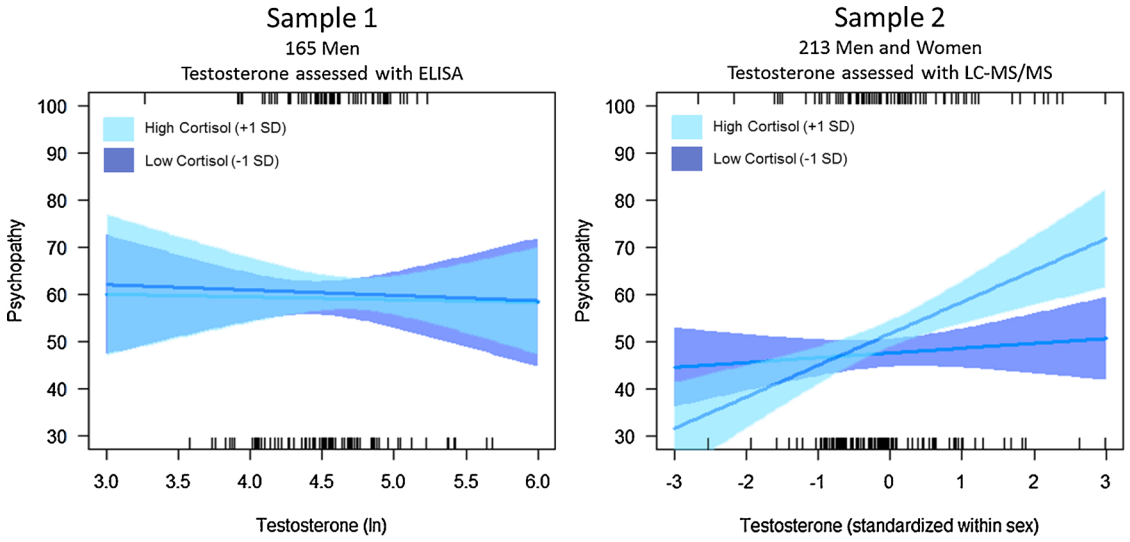

Fig. 1. Simple Slopes of Testosterone and Psychopathy for Samples 1 and 2.

3. Results

3.1. Correlation and descriptive statistics

A preliminary analysis was conducted by running bivariate correlations with testosterone and cortisol concentrations, and SRP-SF scores. For Study 1, no significant correlations were found between hormones and SRP-SF scores. In Study 2, testosterone positively correlated with total psychopathy, as well as every factor of psychopathic traits with the exception of the anti-social factor. There were no significant correlations between cortisol and psychopathy scores. We present the full correlations for Sample 1 and Sample 2, as well as the descriptive statistics for testosterone, cortisol, and SRP-SF scores in Table 1.

3.2. Bivariate associations

Total psychopathy scores from Sample 1 were regressed with testosterone concentrations and cortisol concentrations separately. In addition, each of the four factors of psychopathic traits (interpersonal, affective, lifestyle, and antisocial traits) were regressed separately with testosterone concentrations and then with cortisol concentrations. Results from the bivariate analysis of testosterone with total psychopathy and each of the four factors of psychopathic traits in Sample 1 were non-significant (*ps* = .640–.928). Additionally, the bivariate associations of cortisol and total psychopathy and each of the four factors of psychopathic traits were non-significant (*ps* = .258–.830). See Table 2 for the full regression results.

Bivariate associations were tested in the same method in Sample 2. Scatterplots of cortisol and testosterone concentrations with total psychopathy are presented in Fig. 3. Results revealed a significant positive association between testosterone and total psychopathy ($b = 3.86$, $t(193) = 3.46$, $r_p = .24$, $p = .001$). In addition, testosterone was positively correlated with the affective ($b = 1.25$, $t(193) = 3.43$, $r_p = .24$, $p = .001$), interpersonal ($b = 1.35$, $t(193) = 3.48$, $r_p = .24$, $p = .001$), and lifestyle ($b = 1.04$, $t(193) = 2.94$, $r_p = .21$, $p = .004$) facets of psychopathy but not the antisocial facet of psychopathy ($b = 0.25$, $t(193) = 1.03$, $r_p = .07$, $p = .306$). When cortisol was regressed with SRP-SF scores, a significant positive correlation was found with the lifestyle dimension exclusively ($b = 12.99$, $t(193) = 2.41$, $r_p = .17$, $p = .017$). See Table 3 for the full regression results.

3.3. Testosterone and cortisol interactions

A testosterone x cortisol interaction term was added to the regression model to examine a potential dual-hormone interaction predicting total psychopathy. In Sample 1, there was no significant interaction between testosterone and cortisol predicting total psychopathy scores

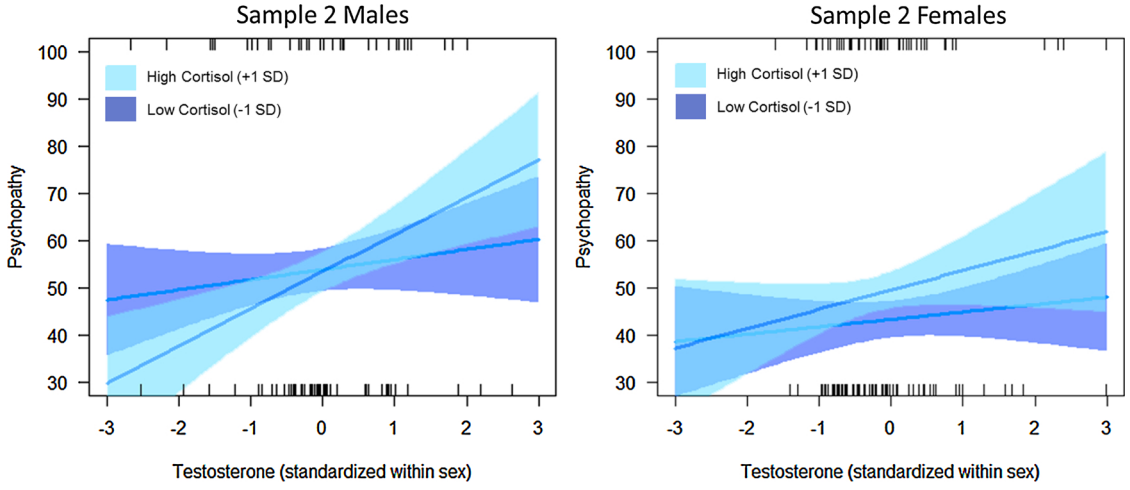

Fig. 2. Simple Slopes of Testosterone, Cortisol, and Psychopathy, Split by Biological Sex.

Table 2
Sample 1: Multiple Regression Analyses with Testosterone and Cortisol Predicting SRP-SF Scores.

Outcome	Predictor	B	$t(157)$	p	partial r	95% CI LB (r_p)	95% CI UB (r_p)
Total Psychopathy	Cortisol (ln)	1.13	0.43	0.666	0.03	−0.13	0.18
	Testosterone (ln)	−0.86	−0.26	0.795	−0.02	−0.18	0.14
	T x C Interaction	−0.55	−0.10	0.919	−0.01	−0.17	0.15
Affective	Cortisol (ln)	0.16	0.22	0.830	0.02	−0.14	0.18
	Testosterone (ln)	−0.44	−0.47	0.640	−0.04	−0.19	0.12
	T x C Interaction	−0.16	−0.10	0.920	−0.01	−0.17	0.15
Interpersonal	Cortisol (ln)	0.38	0.41	0.680	0.03	−0.13	0.18
	Testosterone (ln)	−0.14	−0.12	0.902	−0.01	−0.17	0.15
	T x C Interaction	−2.50	−1.33	0.187	−0.11	−0.26	0.05
Lifestyle	Cortisol (ln)	0.95	1.14	0.258	0.09	−0.07	0.24
	Testosterone (ln)	−0.12	−0.12	0.908	−0.01	−0.17	0.15
	T x C Interaction	1.10	0.64	0.526	0.05	−0.11	0.20
Antisocial	Cortisol (ln)	−0.41	−0.67	0.505	−0.05	−0.20	0.11
	Testosterone (ln)	−0.07	−0.09	0.928	−0.01	−0.17	0.15
	T x C Interaction	0.92	0.73	0.469	0.06	−0.10	0.21

Note. Cortisol (ln) - log transformed cortisol concentrations, Testosterone (ln) - log transformed testosterone concentrations, T x C Interaction - testosterone and cortisol interaction term.

(p = .919). Furthermore, there were no significant testosterone x cortisol interactions for any of the four factors of psychopathic traits (ps = .187–.920). For the full regression results, see Table 2. The simple slopes of the non-significant interaction between testosterone, cortisol, and total psychopathy for Sample 1 are presented in Fig. 1, left panel.

In Sample 2, results revealed a significant testosterone x cortisol interaction predicting total psychopathy across all participants, which included men and women (b = 44.54, $t(193)$ = 2.76, r_p = .19, p = .006). Moreover, testosterone and cortisol jointly interacted to predict the interpersonal (b = 13.06, $t(193)$ = 2.34, r_p = .17, p = .020), lifestyle (b = 13.27, $t(193)$ = 2.59, r_p = .18, p = .010), and antisocial (b = 8.33, $t(193)$ = 2.36, r_p = .17, p = .019) facets of psychopathy.

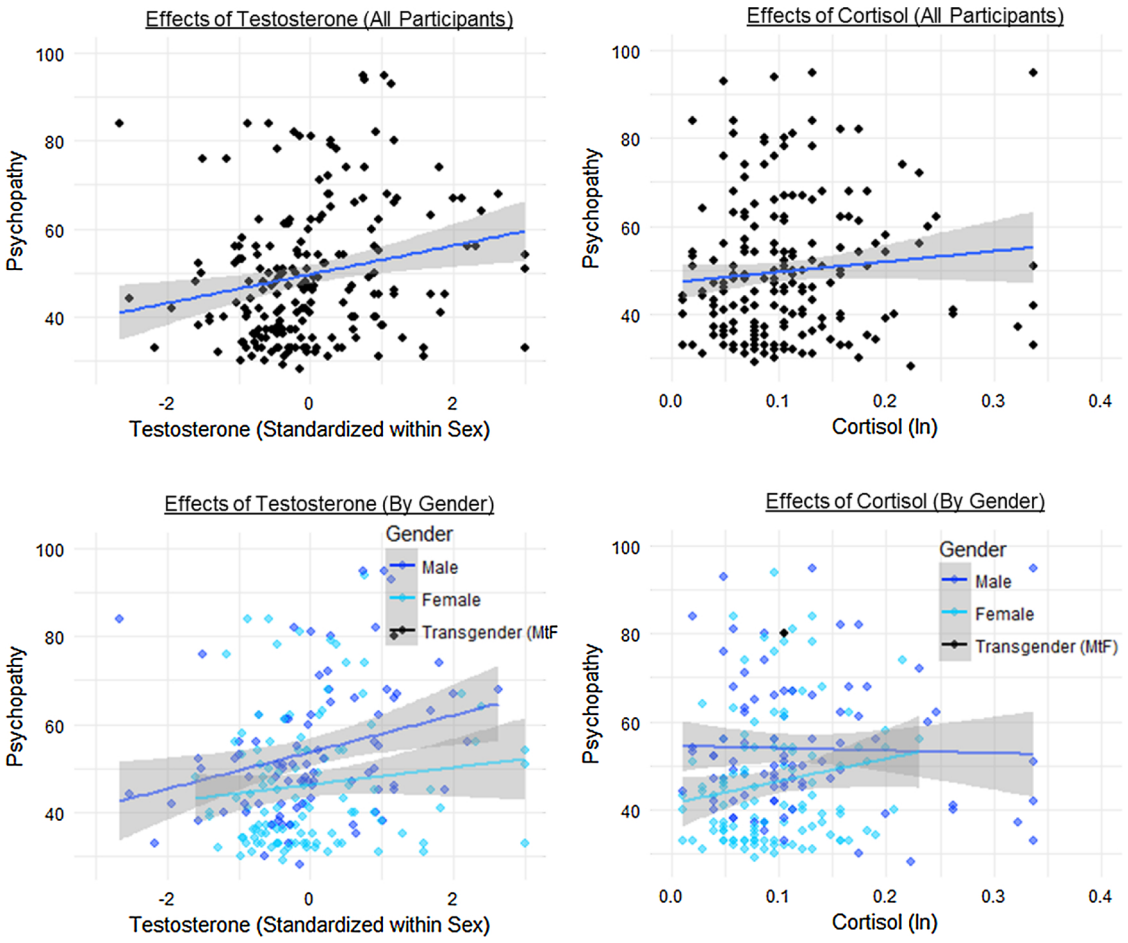

Fig. 3. Sample 2: Testosterone Concentrations and Total Psychopathy Scatterplot.

Table 3
Sample 2: Multiple Regression Analyses with Testosterone and Cortisol Predicting SRP-SF Scores.

Outcome	Predictor	B	$t(193)$	p	partial r	95% CI LB (r_p)	95% CI UB (r_p)
Total Psychopathy	Cortisol (ln)	32.31	1.91	0.058	0.14	0.00	0.28
	Testosterone (z-scores)	3.86	3.46	0.001	0.24	0.10	0.37
	T x C Interaction	44.54	2.76	0.006	0.19	0.05	0.32
Affective	Cortisol (ln)	4.58	0.82	0.411	0.06	−0.08	0.20
	Testosterone (z-scores)	1.25	3.43	0.001	0.24	0.10	0.37
	T x C Interaction	8.90	1.68	0.094	0.12	−0.02	0.26
Interpersonal	Cortisol (ln)	9.37	1.60	0.112	0.11	−0.03	0.25
	Testosterone (z-scores)	1.35	3.48	0.001	0.24	0.10	0.37
	T x C Interaction	13.06	2.34	0.020	0.17	0.03	0.30
Lifestyle	Cortisol (ln)	12.99	2.41	0.017	0.17	0.03	0.30
	Testosterone (z-scores)	1.04	2.94	0.004	0.21	0.07	0.34
	T x C Interaction	13.27	2.59	0.010	0.18	0.04	0.31
Antisocial	Cortisol (ln)	4.97	1.34	0.181	0.10	−0.04	0.24
	Testosterone (z-scores)	0.25	1.03	0.306	0.07	−0.07	0.210
	T x C Interaction	8.33	2.36	0.019	0.17	0.03	0.30

Note. Cortisol (ln) - log transformed cortisol concentrations, Testosterone (z-scores) - standardized testosterone concentrations across males and females, T x C Interaction - testosterone and cortisol interaction term.

Although testosterone x cortisol results were non-significant for the affective subscale, the pattern of findings was consistent with that observed for the other subscales (b = 8.90, $t(193)$ = 1.68, r_p = .12, p = .094). For the full regression results, see Table 3. Simple slopes analysis (see Fig. 1, right panel) indicated that testosterone was positively correlated with psychopathy when cortisol levels were high (1 *SD* above the mean; b = 6.71, $t(193)$ = 4.05, p < .001, r_p = .28) but not when cortisol levels were low (1 *SD* below the mean; b = 1.02, $t(193)$ = 0.74, p = .459, r_p = .05).

3.4. Gender moderation

Since Welker et al., 2014 found a significant gender moderation of the dual-hormone effects on psychopathy, a sex x cortisol x testosterone interaction term was entered and regressed with psychopathy, along with all other lower order main effects and two-way interactions.[2] We found no three-way interaction with total psychopathy (b = -11.22, t (188) = -.30, p = .766, r_p = -.02) or with any of the four facets of psychopathy (p = .661–.803). However, as a caution to readers, this sample is likely underpowered for testing three-way interactions (Aguinis et al., 2005). Although gender was not a significant moderator, it is important to note that the conditional effect was most pronounced in men (Men: b = 37.51, $t(188)$ = 2.01, p = .046, r_p = .14; Women: b = 26.29, $t(188)$ = 0.80, p = .423, r_p = .06). For simple slopes analysis of testosterone, cortisol, and psychopathy split by sex, please see Fig. 2.

4. Discussion

The objective of this study was to examine whether testosterone and cortisol jointly predicted psychopathic traits. As Welker et al. (2014) had found a reversal of the dual-hormone hypothesis in predicting psychopathy previously, we expected to see similar results. We stress the importance of replicability, and while this is not an exact replication of Welker et al. (2014), it uses the SRP-SF to assess psychopathic traits in a nonclinical sample.

The current findings offer mixed evidence of replication of previous work. In Sample 2, which consisted of men and women, there was a positive relationship between testosterone and psychopathy scores when cortisol levels were high, but not low. This was true for total psychopathy and the facets of psychopathic traits with the exception of the affective trait. Perhaps Welker et al. (2014) were unable to extend these results to women in their sample because of their use of ELISAs, which have been shown to be unreliable in measuring low-levels of testosterone (Taieb et al., 2003; Baecher et al., 2013; Welker et al., 2016). The findings of Welker et al. (2014) in men were not replicated in Sample 1. Although the non-significant results in Sample 1 may be caused by sampling error, the prominent difference between the two studies is the assay method. The use of ELISAs in Sample 1, being less precise than LC–MS/MS, may have added additional noise that led to non-significant results.

Clinical samples with psychopathic traits have been found to have lower cortisol than non-clinical samples (Cima et al., 2008). Higher cortisol in non-clinical samples could be related to reactive aggression. For instance, being provoked could elicit reactive aggression in women when cortisol levels are high (Van Bokhoven et al., 2005; Denson et al., 2013). In addition, reactive aggression tends to be higher than instrumental aggression in undergraduate samples, indicating they may have secondary psychopathic traits (Falkenbach et al., 2008). Although we found cortisol to be positively associated with psychopathy, it was only associated with the lifestyle facet of psychopathy. It could be that cortisol is associated with secondary psychopathic traits (i.e. psychopathic traits that develop in response to adverse experiences). Neither hormone was associated with the antisocial facet alone, which could be due to the lower internal consistency of this measure (alphas < .60). However, testosterone and cortisol still interacted to predict psychopathy overall.

It is important to investigate psychopathic traits in non-clinical samples. Non-clinical samples could be associated with both primary and secondary psychopathic traits (Lee and Salekin, 2010; Prado et al., 2015). Studies that focus on the affective (e.g., Colins et al., 2017) and cognitive (e.g., Newman et al., 2010) aspects of psychopathic traits in the general population can help further the understanding of the functioning of psychopathy. Furthermore, most clinical studies have been focused around the antisocial trait, while there is research suggesting that studying subclinical affective traits such as callous-unemotional traits with or without the presence of antisociality can be informative to a broader understanding of antisocial personality disorder and conduct disorder (Viding and McCroy, 2012).

[2] For this analysis, an additional case was removed on account of a participant identifying as transgender. Including this participant in the analyses and coding this participant by either her gender identity or sex at birth did not change the significance of any reported results.

5. Conclusion

Cortisol can have inhibitory effects on androgen receptors and the HPG axis (Chen et al., 1997; Tilbrook et al., 2000). The HPG and HPA axes may also have mutually inhibitory effects on each other through heterodimer formation of the androgen and glucocorticoid receptors (Chen et al., 1997; Viau, 2002). Because of these physiological reasons, cortisol in theory could moderate testosterone's link to psychopathy, only when cortisol is low. However, there have been well-powered failures to support the dual-hormone hypothesis (Mazur and Booth, 2014), "reversals" of the dual-hormone effect (e.g., Denson et al., 2013; Welker et al., 2014), and different approaches to understanding HPA and HPG crosstalk (e.g., Edwards and Casto, 2015; Ruttle et al., 2015; Welker et al., 2017). Overall, more research is needed to discern how the HPA and HPG axes interact with personality traits and social behaviors. It is essential that this work is unbiased by the "file-drawer effect," uses accurate measures of hormones, and contributes cumulatively and comprehensively to research in psychoneuroendocrinology.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations of interest

None.

Acknowledgements

We thank Brigham Research Assay Core in Boston, MA for conducting analyses of salivary hormones. We additionally thank Jordan Liphardt, Shyneth Galicia, Nicholas Jones, Eliana Lozoya and Brian Tyminsky for their assistance with data collection in Sample 1, and Erinda Morina, Olivia Keaveny, Animan Randhawa, Adriana Rodriguez, and Kaosisochukwu Onochie for assistance with data collection in Sample 2.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.09.006.

References

Aguinis, H., Beaty, J.C., Boik, R.J., Pierce, C.A., 2005. Effect size and power in assessing moderating effects of categorical variables using multiple regression: a 30-year review. J. Appl. Psychol. 90 (1), 94. https://doi.org/10.1037/0021-9010.90.1.94.

Andersson, C.R., Bergquist, J., Theodorsson, E., Ström, J.O., 2017. Comparisons between commercial salivary testosterone enzyme-linked immunosorbent assay kits. Scand. J. Clin. Lab. Invest. 1–5. https://doi.org/10.1080/00365513.2017.1339231.

Archer, J., Graham-Kevan, N., Davies, M., 2005. Testosterone and aggression: a reanalysis of Book, Starzyk, and Quinsey's (2001) study. Aggress. Violent Behav. 10 (2), 241–261. https://doi.org/10.1016/j.avb.2004.01.001.

Baecher, S., Azad, S.C., Vogeser, M., 2013. Inter-method comparison of salivary cortisol measurement. Laboratoriumsmedizin 37 (5), 269–273. https://doi.org/10.1515/labmed-2013-0008.

Bohannon, J., 2015. Many psychology papers fail replication test. Science 349 (6251), 910–911. https://doi.org/10.1126/science.349.6251.910.

Breheny, P., Burchett, 2017. Visualizing regression models using visreg. R J. 9, 56–71.

Carré, J.M., Mehta, P.H., 2011. Importance of considering testosterone–cortisol interactions in predicting human aggression and dominance. Aggress. Behav. 37 (6), 489–491. https://doi.org/10.1002/ab.20407.

Chen, S., Wang, J., Yu, G., Liu, W., Pierce, D., 1997. Androgen and glucocorticoid receptor heterodimer formation. A possible mechanism for mutual inhibition of transcriptional activity. J. Biol. Chem. 272, 14087–14092. https://doi.org/10.1074/jbc.272.22.14087.

Cima, M., Smeets, T., Jelicic, M., 2008. Self-reported trauma, cortisol levels, and aggression in psychopathic and non-psychopathic prison inmates. Biol. Psychol. 78 (1), 75–86. https://doi.org/10.1016/j.biopsycho.2007.12.011.

Colins, O.F., Fanti, K.A., Salekin, R.T., Andershed, H., 2017. Psychopathic personality in the General population: differences and similarities across gender. J. Personal. Disord. 31 (1), 49–74. https://doi.org/10.1521/pedi_2016_30_237.

Dabbs, J.M., Jurkovic, G.J., Frady, R.L., 1991. Salivary testosterone and cortisol among late adolescent male offenders. J. Abnorm. Child Psychol. 19 (4), 469–478. https://doi.org/10.1007/BF00919089.

Denson, T.F., Mehta, P.H., Tan, D.H., 2013. Endogenous testosterone and cortisol jointly influence reactive aggression in women. Psychoneuroendocrinology 38 (3), 416–424. https://doi.org/10.1016/j.psyneuen.2012.07.003.

Development, R., Team, Core, 2009. R: A Language and Environment for Statistical Computing. http://www.R-project.org.

Edwards, D.A., Casto, K.V., 2015. Baseline cortisol moderates testosterone reactivity to women's intercollegiate athletic competition. Physiol. Behav. 142, 48–51. https://doi.org/10.1016/j.physbeh.2015.01.037.

Falkenbach, D., Poythress, N., Creevy, C., 2008. The exploration of subclinical psychopathic subtypes and the relationship with types of aggression. Personal. Individ. Diff. 44 (4), 821–832. https://doi.org/10.1016/j.paid.2007.10.012.

Geniole, S.N., Busseri, M.A., McCormick, C.M., 2013. Testosterone dynamics and psychopathic personality traits independently predict antagonistic behavior towards the perceived loser of a competitive interaction. Horm. Behav. 64 (5), 790–798.

Giese-Davis, J., Sephton, S.E., Abercrombie, H.C., Durán, R.E., Spiegel, D., 2004. Repression and high anxiety are associated with aberrant diurnal cortisol rhythms in women with metastatic breast cancer. Health Psychol. 23 (6), 645. https://doi.org/10.1037/0278-6133.23.6.645.

Glenn, A.L., Raine, A., Schug, R.A., Gao, Y., Granger, D.A., 2011. Increased testosterone-to-cortisol ratio in psychopathy. J. Abnorm. Psychol. 120 (2), 389. https://doi.org/10.1037/a0021407.

Gordts, S., Uzieblo, K., Neumann, C., Van den Bussche, E., Rossi, G., 2017. Validity of the self- report psychopathy scales (SRP-III full and short versions) in a community sample. Assessment 24 (3), 308–325. https://doi.org/10.1177/1073191115606205.

Hayes, A.F., 2013. Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach. Guilford Press, New York, NY.

Hermans, E.J., Putman, P., Van Honk, J., 2006. Testosterone administration reduces empathetic behavior: a facial mimicry study. Psychoneuroendocrinology 31 (7), 859–866. https://doi.org/10.1016/j.psyneuen.2006.04.002.

Holi, M., Auvinen-Lintunen, L., Lindberg, N., Tani, P., Virkkunen, M., 2006. Inverse correlation between severity of psychopathic traits and serum cortisol levels in young adult violent male offenders. Psychopathology 39 (2), 102–104. https://doi.org/10.1159/000091021.

Hunt, M.K., Hopko, D.R., Bare, R., Lejuez, C.W., Robinson, E.V., 2005. Construct validity of the balloon analog risk task (BART) associations with psychopathy and impulsivity. Assessment 12 (4), 416–428. https://doi.org/10.1177/1073191105278740.

Korte, S.M., 2001. Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci. Biobehav. Rev. 25 (2), 117–142. https://doi.org/10.1016/S0149-7634(01)00002-1.

Lee, Z., Salekin, R.T., 2010. Psychopathy in a noninstitutional sample: differences in primary and secondary subtypes. Personal. Disord.: Theory, Res. Treat. 1 (3), 153–169. https://doi.org/10.1037/a0019269.

Lykken, D.T., 1995. The Antisocial Personalities. Erlbaum, Hillsdale, NJ.

Mazur, A., Booth, A., 1998. Testosterone and dominance in men. Behav. Brain Sci. 21 (3), 353–363.

Mazur, A., Booth, A., 2014. Testosterone is related to deviance in male army veterans, but relationships are not moderated by cortisol. Biol. Psychol. 96, 72–76.

Mehta, P.H., Josephs, R.A., 2010. Testosterone and cortisol jointly regulate dominance: evidence for a dual-hormone hypothesis. Horm. Behav. 58 (5), 898–906. https://doi.org/10.1016/j.yhbeh.2010.08.020.

Mehta, P.H., Prasad, S., 2015. The dual-hormone hypothesis: a brief review and future research agenda. Curr. Opin. Behav. Sci. 3, 163–168. https://doi.org/10.1016/j.cobeha.2015.04.008.

Mehta, P.H., Welker, K.M., Zilioli, S., Carré, J.M., 2015. Testosterone and cortisol jointly modulate risk-taking. Psychoneuroendocrinology 56, 88–99. https://doi.org/10.1016/j.psyneuen.2015.02.023.

Newman, J.P., Curtin, J.J., Bertsch, J.D., Baskin-Sommers, A.R., 2010. Attention moderates the fearlessness of psychopathic offenders. Biol. Psychiatry 67 (1), 66–70. https://doi.org/10.1016/j.biopsych.2009.07.035.

Open Science Collaboration, 2015. Estimating the reproducibility of psychological science. Science 349 (6251), aac4716. https://doi.org/10.1126/science.aac4716.

Paulhus, D.L., Neumann, C.S., Hare, R.D., 2016. Toronto, Canada. Manual for the Self-Report Psychopathy Scale, 4th ed. Multi-health systems.

Popma, A., Vermeiren, R., Geluk, C.A.M.L., Rinne, T., van den Brink, W., Knol, D.L., Jansen, L.M.C., van Engeland, H., Doreleijers, T.A.H., 2007. Cortisol moderates the relationship between testosterone and aggression in delinquent male adolescents. Biol. Psychiatry 61, 405–411. https://doi.org/10.1016/j.biopsych.2006.06.006.

Prado, C.E., Treeby, M.S., Crowe, S.F., 2015. Examining relationships between facial emotion recognition, self-control, and psychopathic traits in a non-clinical sample. Personal. Individ. Diff. 80, 22–27. https://doi.org/10.1016/j.paid.2015.02.013.

Ruttle, P.L., Shirtcliff, E.A., Armstrong, J.M., Klein, M.H., Essex, M.J., 2015. Neuroendocrine coupling across adolescence and the longitudinal influence of early life stress. Dev. Psychobiol. 57 (6), 688–704. https://doi.org/10.1002/dev.21138.

Shirtcliff, E.A., Vitacco, M.J., Graf, A.R., Gostisha, A.J., Merz, J.L., Zahn-Waxler, C., 2009. Neurobiology of empathy and callousness: implications for the development ofantisocial behavior. Behav. Sci. Law 27 (2), 137–171. https://doi.org/10.1002/bsl.862.

Simons, D.J., 2014. The value of direct replication. Perspect. Psychol. Sci. 9 (1), 76–80. https://doi.org/10.1177/1745691613514755.

Skeem, J.L., Poythress, N., Edens, J.F., Lilienfeld, S.O., Cale, E.M., 2003. Psychopathic personality or personalities? Exploring potential variants of psychopathy and their implications for risk assessment. Aggress. Violent Behav. 8 (5), 513–546. https://doi.org/10.1016/S1359-1789(02)00098-8.

Sollberger, S., Ehlert, U., 2016. How to use and interpret hormone ratios. Psychoneuroendocrinology 63, 385–397. https://doi.org/10.1016/j.psyneuen.2015.09.031.

Stålenheim, E.G., Eriksson, E., von Knorring, L., Wide, L., 1998. Testosterone as a biological marker in psychopathy and alcoholism. Psychiatry Res. 77 (2), 79–88. https://doi.org/10.1016/S0165-1781(97)00143-1.

Tackett, J.L., Herzhoff, K., Harden, K.P., Page-Gould, E., Josephs, R.A., 2014. Personality × hormone interactions in adolescent externalizing psychopathology. Personal. Disord.: Theory, Res., Treat. 5 (3), 235. https://doi.org/10.1037/per0000075.

Taieb, J., Mathian, B., Millot, F., Patricot, M.C., Mathieu, E., Queyrel, N., Lacroix, I., Somma- Delpero, C., Boudou, P., 2003. Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography–mass spectrometry in sera from 116 men, women, and children. Clin. Chem. 49 (8), 1381–1395. https://doi.org/10.1373/49.8.1381.

Terburg, D., Morgan, B., van Honk, J., 2009. The testosterone–cortisol ratio: a hormonal marker for proneness to social aggression. Int. J. Law Psychiatry 32 (4), 216–223. https://doi.org/10.1016/j.ijlp.2009.04.008.

Tilbrook, A.J., Turner, A.I., Clarke, I.J., 2000. Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. Rev. Reprod. 5 (2), 105–113. https://doi.org/10.1530/ror.0.0050105.

Van Bokhoven, I., Van Goozen, S.H.M., van Engeland, H., Schaal, B., Arseneault, L., Séguin, J.R., Nagin, D.S., Vitaro, F., Tremblay, R.E., 2005. Salivary cortisol and aggression in a population-based longitudinal study of adolescent males. J. Neural Transm. 112 (3), 1083–1096. https://doi.org/10.1007/s00702-004-0253-5.

Van den Bergh, B.R.H., Van Calster, B., Puissant, S.P., Van Huffel, S., 2008. Self-reported symptoms of depressed mood, trait anxiety and aggressive behavior in post-pubertal adolescents: associations with diurnal cortisol profiles. Horm. Behav. 54 (2), 253–257. https://doi.org/10.1016/j.yhbeh.2008.03.015.

Viau, V., 2002. Functional cross-talk between the hypothalamic-pituitary-gonadal and-adrenal axes. J. Neuroendocrinol. 14 (6), 506–513. https://doi.org/10.1046/j.1365-2826.2002.00798.x.

Viding, E., McCrory, E.J., 2012. Why should we care about measuring callous–unemotional traits in children? Br. J. Psychiatry 200 (03), 177–178. https://doi.org/10.1192/bjp.bp.111.099770.

Visser, B.A., Ashton, M.C., Pozzebon, J.A., 2012. Is low anxiety part of the psychopathy construct? J. Pers. 80 (3), 725–747. https://doi.org/10.1111/j.1467-6494.2011.00745.x.

Welker, K.M., Lozoya, E., Campbell, J.A., Neumann, C.S., Carré, J.M., 2014. Testosterone, cortisol, and psychopathic traits in men and women. Physiol. Behav. 129, 230–236. https://doi.org/10.1016/j.physbeh.2014.02.057.

Welker, K.M., Goetz, S.M., Carré, J.M., 2015. Perceived and experimentally manipulated status moderates the relationship between facial structure and risk-taking. Evol. Hum. Behav. 36 (6), 423–429. https://doi.org/10.1016/j.evolhumbehav.2015.03.006.

Welker, K.M., Lassetter, B., Brandes, C.M., Prasad, S., Koop, D.R., Mehta, P.H., 2016. A comparison of salivary testosterone measurement using immunoassays and tandem mass spectrometry. Psychoneuroendocrinology 71, 180–188. https://doi.org/10.1016/j.psyneuen.2016.05.022.

Welker, K.M., Prasad, S., Srivastava, S., Mehta, P.H., 2017. Basal cortisol's relation to testosterone changes may not be driven by social challenges. Psychoneuroendocrinology 85, 1–5. https://doi.org/10.1016/j.psyneuen.2017.07.493.

Zilioli, S., Ponzi, D., Henry, A., Maestripieri, D., 2015. Testosterone, cortisol and empathy: evidence for the dual-hormone hypothesis. Adapt. Hum. Behav. Physiol. 1, 421–433. https://doi.org/10.1007/s40750-014-0017-x.