

Montoya et al., 2012; Terburg et al., 2009; Van Honk & Schutter, 2006). Testosterone and cortisol are the end products of the hypothalamic-pituitary-gonadal (HPG) and the hypothalamic-pituitary-adrenal (HPA) axes, respectively. High levels of testosterone independently are merely weakly associated with aggression in humans, whereas in animal studies this relation is well established (meta-analysis by Archer, Graham-Kevan, and Davies (2005). Similarly, low levels of cortisol have been reported to correlate with aggressive and antisocial behavior (e.g., De Vries-Bouw et al., 2012; McBurnett, Lahey, Rathouz, & Loeber, 2000; Platje et al., 2013; Popma, Doreleijers, et al., 2007; Shoal, Giancola, & Kirillova, 2003), but not consistently (see meta-analysis by Alink et al., 2008). However, the HPG and HPA axes mutually inhibit one another (Viau, 2002); testosterone can inhibit HPA axis function at the level of the hypothalamus (Viau & Meaney, 1996), as well as downstream pathways (Carsia, Reisch, Fennell, & Weber, 1987; Colby, Gaskin, & Kitay, 1973; McCormick, Linkroum, Sallinen, & Miller, 2002; Nowak, Neri, Nussdorfer, & Malendowicz, 1995), and cortisol inhibits HPG axis activity at all levels (Johnson, Kamilaris, Chrousos, & Gold, 1992; Tilbrook, Turner, & Clarke, 2000; Viau, 2002). On a psychological level, HPG axis activity is related to approach in rewarding situations, whereas HPA axis activity is related to withdrawal from fearful situations (Schulkin, 2003). Together they thus maintain a healthy balance between reward and fear. As such, it has been suggested that when this balance is disturbed, with high levels of testosterone and concurrent low levels of cortisol, an individual may attend to the more rewarding aspects of aggression, rather than the more negative aspects of aggression (Denson et al., 2012; Montoya et al., 2012; Terburg et al., 2009; Van Honk & Schutter, 2006).

This theory has largely been based on the finding from a study by Dabbs et al. (1991), who reported that cortisol moderated the correlation between testosterone and violent crime in 113 late-adolescent male offenders. Specifically, basal testosterone was positively correlated with violent behavior, but only among individuals with relatively low basal cortisol concentrations. In the two decades that followed, relatively few studies actually investigated this relation, with inconsistent results. In a similar sample of 103 delinquent male adolescents, Popma, Vermeiren, et al. (2007) also found a positive relationship between basal testosterone and overt aggression in adolescents with low basal cortisol levels, but not in those with high cortisol levels. Recently, Tackett et al. (2014) also reported that in 106 community boys and girls high basal testosterone was associated with self-reported externalizing in those with low basal cortisol. Similarly, in 94 students enrolled in a university

course basal testosterone was positively related to dominance, but only in those with low basal cortisol levels (Mehta & Josephs, 2010). Other studies reported less consistent results. In 40 clinic-referred disruptive children no interaction between testosterone and cortisol was found in relation to a general aggression questionnaire (Scerbo & Kolko, 1994). In 53 young women reactive aggression was associated with a different direction of the interaction, that is, concurrent high levels of basal testosterone and cortisol (Denson et al., 2012). A similar interaction was found in a relatively large sample of 237 undergraduate students with psychopathic traits, which are associated with aggression and a lack of fear (Welker et al., 2014). Recently, in a very large sample of 4462 male army veterans testosterone was associated with deviance, but this was not moderated by cortisol levels (Mazur & Booth, 2014).

The above-mentioned studies investigated the dual-hormone hypothesis by examining the interaction between testosterone and cortisol. Besides an interaction, the interconnectedness of the HPG and HPA axes can also be described by the ratio between testosterone and cortisol (Glenn et al., 2011; Romero-Martinez et al., 2013; Terburg et al., 2009). The ratio reflects the relative level of testosterone to cortisol within each individual, whereas the interaction treats testosterone and cortisol as two distinct variables. Indeed, a positive relation between the ratio of baseline testosterone to cortisol reactivity and psychopathic traits was found in 178 general population adults (Glenn et al., 2011), and intimate partner violence perpetrators had higher testosterone to cortisol ratios than controls in preparation for and after acute stress (Romero-Martinez et al., 2013). Comparable results were found for girls with conduct disorder (CD), where aggressive CD symptoms were related to a lower ratio of cortisol to dehydroepiandrosterone (DHEA), a testosterone precursor (Pajer et al., 2006). Although the direction of the relation between the two hormones and aggression was similar to the findings of Dabbs et al. (1991) and Popma, Vermeiren, et al. (2007) in (late) adolescents, Glenn et al. (2011) found no interactions between testosterone and cortisol, while Romero-Martinez et al. (2013) and Pajer et al. (2006) did not examine interactions. In students enrolled in a university course, however, the interaction between testosterone and cortisol was related to dominance, whereas the testosterone/cortisol ratio was only marginally associated with dominance in a mixed-gender sample (Study 1, Mehta & Josephs, 2010), and unrelated in a male sample (Study 2, Mehta & Josephs, 2010).

In females the relation between testosterone, cortisol and aggression may be different compared to males. Testosterone is not only lower in women compared to men, it is also produced differently. Whereas in men testosterone is synthesized by the Leydig cells of the

testes, part of the HPG axis, in women approximately half of testosterone is produced by the adrenal cortex, which is part of the HPA axis (Burger, 2002). To date, only three studies investigated the dual-hormone hypothesis in mixed-gender samples studying respectively dominance, externalizing behavior and psychopathic traits (Study 1, Mehta & Josephs, 2010; Tackett et al., 2014; Welker et al., 2014). Dominance was associated with an interaction indicating high testosterone and low cortisol levels in both men and women, as well as a marginally significant association with the ratio (Study 1, Mehta & Josephs, 2010). Tackett et al. (2014) did not specifically mention testosterone–cortisol interactions for boys and girls separately, but reported that these analyses confirmed the overall pattern of high testosterone and low cortisol in relation to externalizing behavior. Psychopathic traits, however, were associated with a testosterone-cortisol interaction in men only, and indicated a positive relation with testosterone in individuals with high cortisol levels (Welker et al., 2014). The two studies using female samples (Denson et al., 2012; Pajer et al., 2006) yielded contrasting results as well. Although Glenn et al. (2011) and Scerbo and Kolko (1994) investigated mixed-gender samples, the large majority in these studies (88% and 92% respectively) was male, and gender comparisons could not be made. Gender differences have been investigated for the ratio of testosterone to cortisol only once, and regarding aggressive behavior, men and women have not been compared on the joint relation of testosterone and cortisol.

As such, although the proposed interplay between testosterone and cortisol in relation to aggression is likely to aid in understanding its underlying mechanisms, more research is required before further inferences can be drawn. More specifically, the generalizability of the finding of concurrent high testosterone and low cortisol in relation to aggression in non-clinical and female samples needs further elucidation. Therefore, in the current study testosterone and cortisol in relation to aggression were studied in a larger non-clinical sample of both boys and girls. In order to investigate the severity of aggressive behavior more comprehensively, both linear and categorical analyses were performed. As different researchers have taken different statistical approaches to investigate the joint relation of testosterone and cortisol, the current study further implemented both the interaction between testosterone and cortisol, as well as the ratio.

METHODS

Participants

Participants were 259 adolescents (144 boys and 115 girls), with a mean age of 16.98 years ($SD = 0.42$). They

were recruited from the Research on Adolescent Development And Relationships (RADAR) study. RADAR is a Dutch population-based cohort study, with oversampling (50%) of boys and girls with a borderline-clinical score on the externalizing scale of the Teacher's Report Form (TRF, Achenbach) at age 11. Behavioral assessments were subsequently assessed annually at the participants' home, and between January 2010 and January 2011 all participants were invited to participate in a lab session. This paper deals with the behavioral assessment performed at age 17 in February and March of 2010, and the lab session, which included assessment of cortisol and testosterone levels. On average aggression assessment took place 116 days before the lab session ($SD = 75$ days).

All participants and their parents provided written informed consent and received a reimbursement for their participation. The RADAR study was approved by the responsible medical ethics committee, and was conducted in accordance with the Declaration of Helsinki.

Of the 418 adolescents who participated in the RADAR study in 2010, 303 participated in the lab session. The 303 participants did not differ from those not participating in the lab session on age, gender or level of aggressive behavior (all $P > .10$). Forty participants were unable to produce sufficient saliva to assay for testosterone and cortisol, they did not differ from the 263 adolescents for whom sufficient saliva was sampled on age, gender or level of aggressive behavior (all $P > .10$). Of these 263 adolescents, four participants did not provide information on alcohol or nicotine use, resulting in 259 adolescents included in the final analyses (see Table I).

Aggressive Behavior

Aggressive behavior was assessed by means of the Youth Self Report (YSR; Achenbach, 1991), administered to the adolescents. The aggression subscale consisted of 19 items assessing physical acts against persons or things (i.e., fighting, being cruel to others) and verbal aggression (e.g., screaming, arguing), which are scored on a three-point scale (0 = not true, 1 = somewhat true, 2 = very true or often true). Cut-off scores distinguish scores in the borderline and clinical range from those in the normal range (Achenbach, 1991; Verhulst, Van der Ende, & Koot, 1997). Good reliability and validity have been reported for the Dutch version (Verhulst et al., 1997). The reliability of the aggression subscale of the YSR in the current study is high (Cronbach's $\alpha = .82$).

Testosterone and Cortisol

Testosterone and cortisol were assessed during resting conditions of the lab session. Before the saliva sample

TABLE I. Descriptive Statistics

	All Participants		Boys		Girls		<i>t</i> -test/ χ^2	<i>P</i>
	Mean/ <i>n</i>	<i>SD</i> / <i>%</i>	Mean/ <i>n</i>	<i>SD</i> / <i>%</i>	Mean/ <i>n</i>	<i>SD</i> / <i>%</i>		
N	259		144	56%	115	44%		
Age	16.98	0.42	17.00	0.43	16.95	0.40	0.77	.445
Aggressive behavior	6.31	5.17	6.58	5.65	5.97	4.51	0.93	.353
Cortisol (nmol/L)	6.31	3.26	6.91	3.46	5.52	3.46	3.47	.001
Testosterone (pmol/L)	182.78	114.43	266.87	80.71	77.50	36.75	23.30	<.001
Alcohol use	44	17%	33	23%	11	9%	8.08	.005
Nicotine use	64	25%	35	24%	29	25%	0.03	.886

Note. Untransformed levels of testosterone and cortisol are presented, whereas in the analyses these were standardized. Alcohol and nicotine used in the 24 hr before sampling.

was taken, participants were instructed on the protocol of the lab session, watched empathy inducing film clips (30 min) and performed two neurocognitive tasks (30 min). Next, a 20-min break was introduced and they filled in a few questionnaires. One of the questions concerned their stress levels; on average they scored 20.94 ($SD = 18.81$) on a scale from 0 to 100 (with 0 indicating no stress). At the end of this break the saliva sample was collected. Testosterone and cortisol were assessed in one saliva sample obtained by passive drool. The average time of collection was 3:58 p.m. ($SD = 26$ min). After this sample a stress task started.

Saliva was stored uncentrifuged at -20°C until analysis. Testosterone was analyzed in duplicate using an in-house competitive radio-immunoassay employing a polyclonal anti-testosterone-antibody (Dr. Pratt AZG 3290). $[1,2,6,7\text{-}^3\text{H}]$ -Testosterone (NET370250UC, PerkinElmer) was used as a tracer following chromatographic verification of its purity. The lower limit of detection was 20 pmol/L. The mean and intra- and inter-assay coefficients of variation were 5% and 11% respectively. Cortisol was analyzed using electrochemiluminescence immunoassay (ECLIA). The lower detection limit was .5 nmol/L, with mean intra-assay and inter-assay coefficients of variation of 3.4% and 12.2%.

Control Variables

Control variables were assessed at the lab session. During the lab session temperature and humidity of the test room were recorded, and the season of saliva sampling was taken into account. Intake of food, drinks, alcohol, nicotine and drugs, as well as physical exercise, within the last 24 hr, were assessed. Stressful situations in the past year, such as sexual assault, physical assault, and being threatened with violence, were assessed with a questionnaire based on the International Crime Victims Survey (ICVS; Nieuwbeerta, 2002). Medication use, diseases, physical and dental conditions (including allergies and oral bleeding), body mass index (BMI), as well as menstrual phase and contra-conceptive (OC)

use for females were assessed as well.¹ Pubertal status was assessed a year prior to the other assessments, at age 16 by means of a modification of the Pubertal Development Scale² (PDS; Petersen, Crockett, Richards, & Boxer, 1988). The time between the assessment of aggression and the lab session in which testosterone and cortisol were assessed was calculated as a control variable as well.

Statistical Analyses

Outliers in testosterone and cortisol were winsorized at 3 SD above the mean ($n = 6$). As the distribution of cortisol values was positively skewed, a square root transformation was performed, after which values were normally distributed.

Testosterone and cortisol values were standardized separately for boys and girls by means of a Z-transformation (Study 1, Mehta & Josephs, 2010). For the testosterone to cortisol ratio a T-transformation (mean 50; SD 10) was performed separately for boys and girls. Individual testosterone/cortisol ratio scores were calculated, as in Hermans, Ramsey, and van Honk (2008). The testosterone/cortisol ratio scores were normally distributed and outliers were not present.

Control variables associated with aggression as well as testosterone and/or cortisol were controlled for in the final analyses. Four linear regression analyses were performed with aggression as the dependent variable. First, main effects of standardized cortisol and testosterone respectively were examined in two separate analyses. In a third model, standardized cortisol and testosterone

¹ Four female participants (3%) had not yet menstruated, of those who had, 82 (73%) experienced a regular cycle with an average of 27.41 days ($SD = 2.55$). Sixty-one female participants (52%) used hormonal birth control. Of those who did not ($n = 36$), the first day of the last menstruation was equally distributed; $n = 11$: 0–7 days ago; $n = 11$: 8–14 days ago; $n = 11$: 15–21 days ago, and $n = 3$: 22–28 days ago.

² At age 16, 96% of the participants reported experiencing pubertal development in the last 0.5–1 year, and 34% reported full maturation.

were entered simultaneously in Step 1. In Step 2, the interaction term between testosterone and cortisol was added. In the fourth model, only the ratio of testosterone to cortisol was entered as a predictor. Gender by joint testosterone-cortisol effects were tested in each model. If interaction effects with gender were not found, gender was controlled for in the analyses that included both boys and girls.

Post hoc, categorical analyses on associations of testosterone and cortisol with aggression were further examined with logistic regression analyses, to assess whether borderline-clinical aggressive behavior can be distinguished from aggressive behavior in the normal range. The same control variables as in the linear regression analyses, and gender, were controlled. Four logistic regression analyses were performed, with the likelihood of scoring in the borderline-clinical range of aggression as dependent variable. As predictors, the same four models were tested as in the linear regression analyses.

RESULTS

The average score of all participants on aggressive behavior fell within the normal range of aggressive behavior, 242 (90%) of the participants scored within the normal range, and 25 (10%) in the borderline-clinical range.

For all control variables differences in or associations with aggression as well as testosterone and/or cortisol were examined. Only for nicotine (higher aggression scores $t(257) = 3.48, P = .001$, and higher cortisol $t(257) = 1.52, P < .001$) and alcohol used in the 24 hr before saliva sampling (higher aggression scores $t(257) = 2.90, P = .004$, and slightly lower testosterone $t(257) = 1.62, P = .107$) differences were found, and these were therefore controlled in the analyses.³

In Table I descriptive statistics are shown. It can be seen that boys showed higher levels of cortisol, testosterone, and more often reported to have used alcohol in the 24 hr before sampling. In Table II it can be seen that cortisol and testosterone are positively correlated for the entire sample, but not for boys or girls separately. Also, the ratio of testosterone to cortisol is negatively correlated to cortisol and positively to testosterone for the entire sample, as well as for boys and girls separately.

Table III shows the results of the linear regression analyses. A negative association of cortisol with aggressive behavior was found for all participants, indicating that those with lower cortisol levels reported higher levels of aggression (see Fig. 1). This effect remained present after controlling for testosterone. No main effect of testosterone was found in association with aggressive behavior.

The interaction variable testosterone \times cortisol was also not significantly related to aggressive behavior. However, a positive ratio of testosterone to cortisol was found to be associated with aggressive behavior for all participants, explaining 7% of the variance in aggressive behavior (see Fig. 2).⁴

Gender differences were examined by adding a gender \times testosterone \times cortisol interaction, and a gender \times testosterone/cortisol ratio. The interaction terms were not significant for gender \times testosterone \times cortisol ($B = .30 [-1.09-1.69], P = .671$) nor for the gender \times testosterone/cortisol ratio ($B = -.00 [-.00-.00], P = .253$) in association with aggressive behavior. This indicates that the association between aggressive behavior and (1) the testosterone \times cortisol interaction, and (2) the testosterone/cortisol ratio, are statistically equivalent in boys and girls.

Analyses for boys and girls separately showed a trend towards a negative association between cortisol and aggressive behavior for boys, which remained present after controlling for testosterone. Cortisol was not associated with aggressive behavior in girls. For girls a trend towards a positive association between testosterone and aggressive behavior appeared, when controlling for cortisol. Analyses for boys and girls separately confirmed that for neither boys nor girls the testosterone \times cortisol interaction was associated with aggressive behavior. The direction of the relation between the testosterone/cortisol ratio was the same for boys and girls, confirming the non-significant interaction between the ratio and gender, although for girls only a positive ratio was significantly associated with aggressive behavior.

Post hoc, we examined whether the likelihood that participants show aggression in the borderline-clinical range ($n = 25$, of whom 5 were girls) versus aggression in the normal range, is related to testosterone and cortisol. Logistic regression analyses controlling for gender,

³ Analyses were also run without controlling for alcohol and nicotine use, results can be found in Table IV in the additional material. All effects of cortisol and testosterone are no longer significant, and a trend is observed for the ratio in association with aggression for all participants. Hence, for participants of whom 17–25% has used these substances, alcohol and nicotine use confound the relation between testosterone, cortisol, and aggressive behavior.

⁴ Because this concerns a population-based sample with over-sampling, the analyses were repeated excluding those with aggressive behavior in the clinical range. It appeared that all effects are also present in this actual non-clinical sample. Analyses were also repeated including the outliers, results remained similar, although the trend of cortisol for aggressive behavior in boys when corrected for testosterone, and the trend for the ratio predicting aggression in the borderline-clinical range versus normal range aggression were no longer significant.

TABLE II. Correlations

	All Participants			Separate Per Gender			
	2	3	4	1	2	3	4
1. Aggressive behavior	-.05	.08	.11	—	-.04	.14	.14
2. Cortisol ^a		.26***	-.62***	.09	—	.16	-.65***
3. Testosterone ^a			.33***	.03	.14	—	.61***
4. Testosterone/cortisol ratio ^a				.09	-.62***	.65***	—

Note. ^aStandardized values. Correlations per gender: Boys in upper half and girls in lower half. *** $P < .001$.

TABLE III. Results of the Four Linear Regression Analyses Predicting Aggression

		All Participants				Boys				Girls				
		B	95%CI	P	Adj. R ²	B	95%CI	P	Adj. R ²	B	95%CI	P	Adj. R ²	
Model 1	<i>Main effects</i>													
	Cortisol	-.64	-1.27 to -.00	.050	.06	-.83	-1.75 to .09	.077	.09	-.40	-1.26 to .46	.362	.02	
Model 2														
	Testosterone	.23	-.041 to .87	.477	.05	-.05	-1.00 to .90	.915	.07	.65	-.20 to 1.50	.131	.03	
Model 3	<i>Combined effects</i>													
	Step 1	Cortisol	-.68	-1.32 to -.04	.038	.06	-.83	-1.76 to .10	.078	.09	-.51	-1.38 to .35	.242	.03
		Testosterone	.31	-.32 to .96	.325		.05	-.90 to -.99	.921		.73	-.13 to 1.58	.093	
	Step 2	Testosterone × cortisol interaction	.20	-.47 to .88	.551	.06	.26	-.72 to 1.23	.605	.08	-.01	-.95 to .94	.992	.03
		Testosterone/cortisol ratio	2.46	.13 to 4.79	.039	.07	2.13	-1.23 to 5.49	.212	.08	3.20	.10 to .38	.049	.04

Note. Results are controlled for gender (for analyses including all participants), alcohol and nicotine use in the last 24 hr. Cortisol and testosterone values are standardized; B's for main effects and interaction are based on Z-scores, B's for the ratio are based on T-scores.

nicotine and alcohol use, showed no main effect for cortisol (OR = .68 [.42–1.10], $P = .114$, Nagelkerke $R^2 = .11$), or testosterone (OR = 1.14 [.74–1.75], $P = .567$, Nagelkerke $R^2 = .09$). When controlling for testosterone, a trend was found for lower cortisol levels for those in the borderline-clinical range than those with aggression in the normal range (OR = .66 [.41–1.08], $P = .095$, Nagelkerke $R^2 = .11$). The interaction variable testosterone × cortisol was not significantly related to aggressive behavior (OR = 1.10 [.70–1.74], $P = .670$,

Nagelkerke $R^2 = .11$) and a trend towards a higher ratio in the borderline-clinical range of aggressive behavior was found (OR = 3.80 [.84–17.32], $P = .084$, Nagelkerke $R^2 = .11$).

DISCUSSION

It has been proposed that, in adults and adolescents, aggression is related to high levels of testosterone in combination with low levels of cortisol. This hormonal

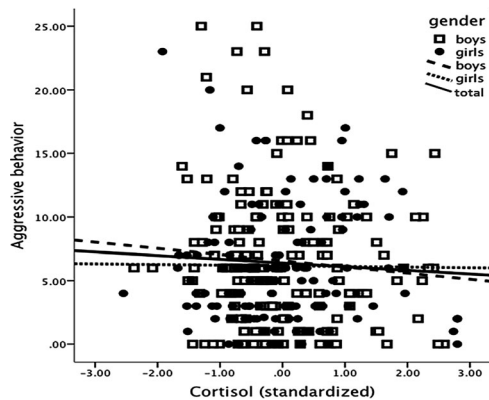


Fig. 1. Scatterplot of the association between aggressive behavior and cortisol levels, presented for boys, girls, and all participants.

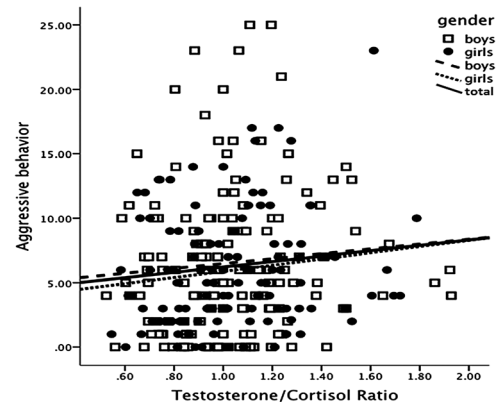


Fig. 2. Scatterplot of the association between aggressive behavior and testosterone/cortisol ratio, presented for boys, girls, and all participants.

TABLE IV. Results of the Four Linear Regression Analyses Predicting Aggression Without Correction for Alcohol and Nicotine Use

		All Participants				Boys				Girls			
		<i>B</i>	95%CI	Adj. <i>R</i> ²	<i>P</i>	<i>B</i>	95%CI	Adj. <i>R</i> ²	<i>P</i>	<i>B</i>	95%CI	Adj. <i>R</i> ²	<i>P</i>
		<i>Main effects</i>											
Model 1	Cortisol	-.33	-.97 to .30	.00	.303	-.47	-1.40 to .46	.00	.318	-.16	-1.00 to .68	.00	.709
Model 2	Testosterone	.40	-.24 to 1.04	.00	.223	.21	-.74 to 1.15	.00	.664	.63	-.21 to 1.47	.01	.140
		<i>Combined effects</i>											
Model 3	Step 1												
	Cortisol	-.40	-1.04 to .24	.00	.217	-.51	-1.45 to .43	.00	.283	-.27	-1.11 to .58	.01	.537
	Testosterone	.46	-.19 to 1.11		.163	.29	-.67 to 1.24		.553	.67	-.18 to 1.55		.121
	Step 2												
	Testosterone × cortisol interaction	.24	-.44 to .93	.00	.486	.38	-.61 to 1.38	.00	.451	-.01	-.97 to .95	.00	.984
Model 4	Testosterone/cortisol ratio	2.09	-.26 to 4.46	.01	.081	1.87	-1.56 to 5.30	.00	.283	2.39	-.76 to 5.55	.01	.136

Note. Results are controlled for gender (for analyses including all participants). *B*'s for main effects and interaction are based on *Z*-scores, *B*'s for the ratio are based on *T*-scores.

interrelation may aid in understanding individual differences in the occurrence of aggressive behavior. Until now, research mainly focused on specific (i.e., delinquent adolescents or clinic-referred disruptive children), small and/or male samples, and results are inconclusive. The present study aimed to examine the generalizability of the relation between aggression and high testosterone combined with low cortisol to a non-clinical sample of male and female adolescents. The current findings indicate that this relation is indeed present in non-clinical adolescents. The ratio of testosterone to cortisol explained 7% of the variance in aggressive behavior. The interaction between testosterone and cortisol was however not associated with aggression, and gender differences were not found in the relation of testosterone and cortisol with aggression.

The current finding that aggression is associated with high testosterone and low cortisol is in line with previous research (Dabbs et al., 1991; Glenn et al., 2011; Mehta & Josephs, 2010; Popma, Vermeiren, et al., 2007; Tackett et al., 2014), and extends these findings to a larger sample of non-clinical adolescents. These results suggest that also normative, non-pathological levels of aggression in a general population of adolescents, may be related to the same neurobiological risk factors as for example violent aggression in incarcerated youths (Dabbs et al., 1991), aggression in delinquent boys (Popma, Vermeiren, et al., 2007), and psychopathic traits in adults (Glenn et al., 2011). Others, however, found no joint relation between testosterone, cortisol and aggression (Mazur & Booth, 2014; Scerbo & Kolko, 1994), or reported concurrent high testosterone and cortisol (Denson et al., 2012; Welker et al., 2014).

It is however important to note that in the current study the ratio of testosterone to cortisol was associated with aggressive behavior, and that the interaction between

these hormones was not. With regard to previous studies, psychopathic traits were also found in relation to the ratio between testosterone and cortisol, but not the interaction (Glenn et al., 2011). However, an association was observed for the ratio of basal testosterone to cortisol reactivity only, whereas in the current study the ratio of basal testosterone to basal cortisol was associated with aggressive behavior. Romero-Martinez et al. (2013) investigated only the ratio surrounding a stress paradigm, and reported that intimate partner violence perpetrators had higher testosterone to cortisol ratios than controls, in preparation for, and after acute stress (Romero-Martinez et al., 2013). Mehta and Josephs (2010) reported an interaction indicating that high testosterone and low cortisol were associated with dominance in both a mixed-gender sample and a male sample, as well as a marginally significant association with the ratio in the mixed-gender sample only (Mehta & Josephs, 2010). Dabbs et al. (1991) and Popma, Vermeiren, et al. (2007) found an interaction indicating that high testosterone and low cortisol were associated with aggression in male offenders, although they did not examine the ratio between testosterone and cortisol. Several other studies also examined interactions only, but did not find associations with aggressive behavior (Mazur & Booth, 2014; Scerbo & Kolko, 1994), or found one in a different direction (Denson et al., 2012; Welker et al., 2014). In sum, to date, the ratio of testosterone to cortisol has been studied less often than the interaction, and results are inconsistent. The current results and those of Glenn et al. (2011) suggest that the ratio may better reflect the interconnectedness of the HPG and HPA axis than the interaction. Where the interaction describes the level of these hormones relative to the group, the ratio reflects the level of testosterone to the level of cortisol within an individual. As few studies examined both the ratio and

the interaction, more research comparing these two different statistical approaches to investigate the joint relation of testosterone and cortisol is warranted.

The ratio of testosterone to cortisol could be interpreted as an index of the imbalance between the HPA and HPG axes within a specific individual. At a receptor level, both testosterone and cortisol bind to steroid-responsive centers in the amygdala (Wood, 1996). The amygdala has a key role in emotional processing (LeDoux, 2000), and where higher levels of testosterone facilitate fight/ approach reactions, higher levels of cortisol have an opposing effect by facilitating flight/fright reactions (Schulkin, 2003). Moreover, a high testosterone/cortisol ratio has been found to be associated with greater amygdala reactivity to facial signs of threat (Hermans et al., 2008), which in turn, biases one's propensity towards aggressive behavior (Carré, McCormick, & Hariri, 2011; Coccaro, McCloskey, Fitzgerald, & Phan, 2007). On the behavioral level, a disturbed balance towards higher testosterone over lower cortisol is thus indicative of more approach and less fear, increasing the likelihood of aggressive behavior. The additional variance in aggression explained by the ratio of testosterone to cortisol, than by cortisol alone, or cortisol controlled for testosterone, is however very small. This suggests that the effect of the ratio may be primarily driven by cortisol (controlled for testosterone). As the ratio of testosterone to cortisol is better at explaining the variance in aggressive behavior than cortisol (controlled for testosterone), the current findings do suggest that testosterone and cortisol jointly regulate aggressive behavior.

Although generally large gender differences exist in the levels of direct, particularly physical, aggressive behavior (Archer, 2004; Card, Stucky, Sawalani, & Little, 2008), and testosterone levels are not only much lower in girls as compared to boys, but also produced differently (Burger, 2002), a lack of gender interactions were found in the current study. This is in line with previous research showing similar interactions for men and women in relation to dominance (Study 1, Mehta & Josephs, 2010) and externalizing behavior (Tackett et al., 2014). When we examined boys and girls separately, for girls only the ratio remained significantly associated with aggressive behavior. Moreover, previous research indicated that psychopathic traits were associated with concurrent high testosterone and cortisol in men only (Welker et al., 2014). To further investigate gender differences in the relation between testosterone, cortisol and aggression, larger samples of boys and girls should be included in future studies.

The current non-clinical sample consists of adolescents derived from the general population. As at age 11 oversampling took place, this is not a strict general population sample. However, at age 17 merely 10% showed

aggressive behavior in the borderline-clinical range, similar to 7% in the general population (Achenbach, 1991). Besides, the findings are similar when we examined the participants in the non-clinical range of aggressive behavior only. Moreover, the ratio between cortisol and testosterone only marginally distinguished severe aggression from normative levels of aggressive behavior. These results suggest that the testosterone/cortisol ratio is related to all levels of aggressive behavior.

In contrast to many previous studies, the current sample consisted of adolescents. Dabbs et al. (1991), Popma, Vermeiren, et al. (2007), and Tackett et al. (2014) studied adolescents as well and reported an interaction for higher testosterone and lower cortisol. The ratio has not been studied previously in adolescents, and therefore needs replication for this developmental stage, especially as hormone levels increase drastically in puberty and aggression may further decrease in adulthood. However, at age 17, at which age the current study was performed, testosterone and cortisol have generally reached adult levels (Root, 1973). Aggressive behavior generally peaks in toddlerhood, and by age 17 has usually decreased to adult levels (Bongers, Koot, van der Ende, & Verhulst, 2003; Nagin & Tremblay, 1999). As previous studies also reported an increased ratio in relation to psychopathic traits and violence (Glenn et al., 2011; Romero-Martinez et al., 2013), it could be expected that this relation also holds in adult samples. As not all studies found a relation with the ratio (Mehta & Josephs, 2010), the testosterone to cortisol ratio needs further examination in various age groups.

The results of the present study should be interpreted in the context of some limitations. First, testosterone and cortisol were analyzed from a single saliva sample, not enabling an average of several samples, which could have minimized the effect of pulsatility. These findings should be replicated in future studies assessing multiple samples on multiple days. Second, the sample size for boys appeared to be too small to detect an association between the testosterone/cortisol ratio and aggression. Although the current findings do point towards similar associations between the ratio of testosterone and cortisol in relation to aggression in both genders, more research in larger samples is necessary. Third, aggression was assessed with the Youth Self Report, this is a very broad measure of aggressive behavior, and does for instance not distinguish between reactive aggression and proactive aggression or verbal and physical aggression. Moreover, an experimental aggression paradigm would assess aggressive behavior more directly than a questionnaire. The advantage of the YSR, on the other hand, is that this is a highly valid, reliable and often used measure, and assesses aggressive behavior in various real-life

situations. Fourth, cortisol and testosterone were assessed in saliva obtained after the participants had performed several tasks (e.g., an empathy task), which may modulate testosterone and cortisol concentrations, and perhaps differently depending on the level of aggression of participants. Saliva was, however, sampled within a standardized protocol and at the same time of day for all participants. As many studies obtain saliva within a testing protocol, future studies should not only standardize the time of day of sampling, but also sample saliva at valid resting conditions.

In conclusion, the current findings extend previous research by showing that the relation between aggression and high testosterone combined with low cortisol appears to represent variation along a spectrum that includes normative levels of aggression. The ratio of testosterone to cortisol could serve as a useful basis for further theories and studies to increase our understanding of aggression.

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