PTSD symptoms and cortisol stress reactivity in adolescence: findings from a high adversity cohort in South Africa

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Abstract

Background: Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is implicated in the pathophysiology of post-traumatic stress disorder (PTSD). However, there has been little study of HPA stress reactivity in association with PTSD symptoms (PTSS) in children; and there is limited research on PTSD in low and middle-income countries, where trauma exposure is more common and co-occurring stressors more likely.

Method: We assessed the relationship between PTSS and cortisol stress reactivity in children aged 13 years ($N = 291$) from an impoverished South African community. HPA axis stress reactivity was indexed by salivary cortisol during the Trier Social Stress Test.

Results: PTSS were associated with alterations in cortisol reactivity that were moderated by sex. In girls, PTSS were associated with reduced linear slope [$B = -0.07$, $p = 0.043$, 95% CI (-0.13, -0.00)] but larger quadratic slopes [$B = 0.01$, $p = 0.017$, 95% CI (0.002, 0.02)], whereas the opposite pattern was found in boys [linear $B = 0.07$, $p = 0.038$, 95% CI (0.01, 0.14); quadratic $B = -0.01$, $p = 0.016$, 95% CI (-0.02, -0.002)]. Thus, elevated PTSS were associated with overall blunted cortisol stress reactivity in girls, but with enhanced recovery in boys. There was no relationship between trauma exposure (with or without PTSS) and cortisol reactivity.

Conclusion: In children from a high adversity low-income country, sex specific associations were found between PTSS and cortisol responses to psychosocial stress. Further research should probe HPA axis functioning more comprehensively in such populations to understand the biological associations of PTSS.

Keywords: PTSD; stress; cortisol reactivity; trauma; adolescents; low and middle income country

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis plays a major role in the central nervous system response to stress (Teicher, Andersen, Polcari, Anderson, & Navalta, 2002), and has been implicated in the development of stress related psychological disorders, including posttraumatic stress disorder (PTSD) (De Bellis & Zisk, 2014; Pan, Wang, Wu, Wen, & Liu, 2018). Research in adults has found evidence of lower basal cortisol secretion in association with PTSD, though mixed findings have been reported (De Bellis & Zisk, 2014; Pan et al., 2018). In contrast, studies of paediatric PTSD have more consistently demonstrated basal cortisol elevations (Carrion et al., 2002; De Bellis & Zisk, 2014; Delahanty, Raimonde, & Spoonster, 2000; Fries, Hesse, Hellhammer, & Hellhammer, 2005). It has been argued that such discrepancies may reflect long term biological adaptation to stress, with prolonged periods of HPA hyperactivation following trauma ultimately being followed by "attenuation," due to reduced biosynthesis of hormones, downregulation of pituitary glucocorticoid receptors, and/or increased receptor negative feedback sensitivity (Fries et al., 2005). The resultant corticosteroid hyposecretion is proposed to prevent potentially deleterious effects of prolonged cortisol elevation on neural and cardiometabolic functioning (Fries et al., 2005; McEwen, 1998). In support of this hypothesis, a meta-analysis has found that basal cortisol is inversely correlated with time since trauma in adults with PTSD (Carrion et al., 2002; Miller, Chen, & Zhou, 2007), and there is preliminary cross-sectional evidence that equivalent effects may be present in child and young people (Weems & Carrion, 2007).

In contrast to the relatively comprehensive study of basal cortisol, there has been much less attention to cortisol stress reactivity in the PTSD field. Cortisol reactivity to stress is argued to better reflect psychopathological processes than baseline cortisol levels and is particularly relevant in the context of stress related disorders (Zorn, 2017). Studies that measure cortisol responses to a stress challenge in relation to adult PTSD are limited and have yielded inconsistent findings (De Bellis & Zisk, 2014; Li & Seng, 2018; Schalinski, Elbert, Steudte-Schmiedgen, & Kirschbaum, 2015). Importantly, there has been little investigation of HPA

axis reactivity in children and adolescents with PTSD. One study of 124 violence-exposed children (Peckins, 2012) found that while violence exposure was associated with lower cortisol reactivity in response to the Trier Social Stress Test (TSST) in boys (but not girls), there was no evidence of associations between PTSS and cortisol response. However, levels of PTSS were extremely low in this USA sample. A second study found evidence of blunted cortisol reactivity in maltreated adolescent girls (n = 67) compared to a control group (n = 25), but no effect of the presence of current PTSD symptoms in this association (MacMillan et al., 2009a). However, only 19% of the sample reported any current PTSD symptoms. Given the potential importance of childhood stressors in the development of the HPA axis, further examination of whether childhood PTSD is linked to cortisol reactivity is warranted, particularly in contexts of heightened trauma exposure or in clinical populations with paediatric PTSD.

In addition to the general lack of research focus on child cortisol stress reactivity in association with PTSD, it is striking that the available evidence derives almost exclusively from high-income country populations. In low and middle income countries (LMICs) childhood trauma exposure is higher (Atwoli, 2015), and occurs against a backdrop of prevailing adversity, which may modulate stress-related biological systems (McDade, Hoke, Borja, Adair, & Kuzawa, 2013), including HPA axis activity (Flinn & England, 1997). Consequently, findings to date may not generalise to contexts with a high prevalence of trauma and stress (Atwoli, 2015).

We examined associations between PTSD symptoms (PTSS) and HPA cortisol reactivity in young people (13-years) living in the township of Khayelitsha, an impoverished peri-urban settlement on the outskirts of Cape Town, South Africa, where rates of child trauma exposure are markedly high (Gregorowski, 2013). We tested the hypothesis that PTSS would be associated with cortisol reactivity using a controlled social stressor—the TSST – at 13 years old. Based on the limited available evidence, blunted cortisol stress reactivity in children with higher PTSS was predicted. Sex was included as a potential moderator of this

relationship, given preliminary evidence of sex differences in the cortisol response in the context of trauma (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009; Takai et al., 2007), and meta-analytic evidence that depression/anxiety disorder related alterations in cortisol reactivity are moderated by sex, with women showing blunted but men elevated reactivity compared to controls (Zorn, 2017). We also examined whether the extent of trauma exposure could explain any associations between PTSS and cortisol reactivity.

2 Methods and Materials

2.1 Participants

From 1999-2003 a randomized controlled trial was conducted in a socio-economically disadvantaged South African peri-urban settlement near Cape Town (Khayelitsha). A total of 449 pregnant women were randomised to a 16-session perinatal psychosocial intervention or control group. The intervention included home visits to mothers by lay community workers, which aimed to support their parenting. The intervention improved maternal sensitivity and caregiver child attachment(Cooper et al., 2009). At child age 13 years, study dyads from both control and intervention groups were re-invited to attend an assessment clinic. Twenty-four children had died since the original randomisation process. Of the remaining 425, 333 were able to travel to the assessment centre, which was necessary to provide cortisol measures. Wherever possible, the team arranged for these child and mother participants to travel to Cape Town for their study assessments Of these, 15 were excluded due to asthma steroid pump use, which can interfere with cortisol measurements, 14 were lost due to issues with labelling and storing the samples, 3 were missing PTSS data and 10 had missing data on covariates, resulting in a final sample of 291 adolescents for the current study.

2.2 Measures

2.2.1 PTSD

The Child PTSD checklist (Amaya-Jackson, 1995) was completed at the 13-year research assessment. This is a 28-item self-report scale assessing the presence of 17 DSM-IV PTSD symptoms over the past month with regard to the most upsetting traumatic experience a child has had. Each item was scored based on a 3-point Likert-scale, 0 = Never, 1 = Some days, 2 = Every Day. The PTSS is scored continuously. This scale has been used widely in the South-African context, and with native isiXhosa speakers such as those living in Khayelitsha(Cluver, Fincham, & Seedat, 2009; Suliman et al., 2009) and has good internal validity, reliability and discriminant abilities(Boyes, Cluver, & Gardner, 2012). As standard for the scale, adolescents were asked to think of the most frightening event that had happened to them and keep this in mind while responding to the questions.

2.2.2 Cortisol Reactivity: TSST

Adolescents completed the TSST in line with standard protocols (Kirschbaum, Pirke, & Hellhammer, 1993). Following a baseline saliva sample, participants were given 3 minutes to prepare a 5-minute speech on anything about themselves, to be delivered to an audience. They were then led to a second room where they gave their 5-minute speech to two 'examiners' in white coats in front of a camera, with a 24-inch monitor displaying the image of the participants that was captured. Following the speech, examiners administered a serial sevens subtraction task for 4 minutes. Upon completion, the participant returned to the first room where a post-stressor saliva sample was collected. Subsequent saliva samples were collected at +10, +20, +30, +40 and +50 minutes, while participants completed a structured, neutral interview unrelated to the task. The seven pre-labelled salicaps were stored in a deep freeze ($-4°C$) until they were shipped on dry ice to a lab in Germany for cortisol assay. Five individual observations (not whole cases) were excluded as biologically implausible due to extremely high readings, which typically occurs due to contamination with blood in the saliva.

We calculated area under the curve with respect to ground (AUCg) of cortisol concentrations. AUCg is associated with is associated with measures of total hormonal secretion. The trapezoid methods was used to calculated AUCg across the six repeated measures using the trapezoid method, following standard formula (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003):

$$AUCg = \sum_{i=1}^{n-1}(m_{(i+1)}+m_i).\frac{t_i}{2}$$

with t_i denoting the individual time distance between measurements, m_i the individual measurement, and n the total amount of measures.

2.2.3 Trauma Exposure

Trauma exposure was assessed using the child exposure to community violence checklist (CECV). Adolescents reported exposure to different types of community and household violence on an adapted version of the CECV with 23-items (Cluver, Gardner, & Operario, 2007). The frequency of exposure was rated on a 3-point Likert scale: 0 = Never, 1= A few times, 2 = Many times. This version of the measure has previously been used to index extent of trauma exposure among adolescents in South African townships, and total scores capture overall exposure to particularly common traumatic experiences in those communities, including robbery, assault, stabbings or shootings in the community, or domestic violence and abuse at home (Boyes et al., 2012; Cluver et al., 2009). The measure has previously been found to be reliable and valid in a South African context (Martin, Revington, & Seedat, 2013); showing good internal consistency ($\alpha = 0.89$) and it is associated ($r = 0.50$) with adolescent PTSS (Boyes et al., 2012).

2.2.4 Measurement of key covariates

During the 13-year clinic visit, participants' weight and height were measured to calculate BMI (kg/m^2). A categorical measure of cumulative contextual adversity was used to reflect

socioeconomic status. Using data completed by the child's carer and the child at 13 years old, the following variables were coded as 0=Present or 1=Absent: overcrowding (number of people dwelling in the household reported by carer, above or below highest quintile), primary caregiver unemployed, primary caregiver only has primary-level education, house lacks running water/toilet/electricity, family members have gone without food for a whole day in the last week, relationship breakup with partner or husband, partner has been violent toward mother/caregiver. The average of these indicators was taken for each child, resulting in a score from 0-1 for each child, where 1 reflects lowest level of adversity.

2.3 Data analysis

Multiple regression analyses were used to investigate associations between cortisol AUCg and PTSS, with PTSS as the independent variables and cortisol AUCg as the dependent variable. We controlled for sex, intervention group status (coded as 0 = no intervention and 1 = perinatal intervention), and cumulative contextual adversity. We also assessed the relationship between cortisol AUCg and PTSS when trauma exposure was controlled for in the analysis. Due to missing data for BMI we did not include this in the final model, however in preliminary analysis results were identical when BMI was included/excluded.

Subsequent analyses were conducted using the MIXED procedure in STATA version 15. Cortisol reactivity over the TSST was analysed using two-level hierarchical linear modelling (HLM). First, we estimated individual variance in baseline levels and the trajectory of cortisol concentration over time (level 1) using linear, quadratic and cubic terms, as reported previously (Fearon et al., 2017). Random effects for both constant and slopes were included in all models.

Hypothesis testing was then carried out by including between-subjects (level 2) predictors in the model. In an analysis to test for the effect of PTSS scores on cortisol reactivity to stress, we ran a HLM model examining PTSS, sex, and the sex × PTSS interaction in relation to the cortisol intercept and linear and quadratic slopes, and we additionally ran an equivalent

model to test for potential effects of trauma exposure, with CECV scores replacing the PTSS term. We tested for effect modification by sex in all analyses. Where interaction effects were identified, we conducted further exploration using tests of simple slopes, and illustrated findings via plots of estimated marginal means (i.e., model-based predictions). Intervention group status (coded as 0 = no intervention and 1 = perinatal intervention), and cumulative contextual adversity and pubertal status in girls (age at first menstruation) were included as covariates in all analyses. Due to missing data for BMI we did not include this in the final model, however in preliminary analysis results were identical when BMI was included/excluded.

3. Results

3.1 Participant information

The final sample of 291 adolescents comprised 146 girls and 144 boys aged 13 years with an average BMI of 2.32±4.9 kg/m^2. Adolescents reported, on average, being exposed to 4.9 different types of trauma on the CECV and PTSS were lower (M = 9.2±8.3) compared to a similar study (M = 18.7±13.9) taken using the same PTSS measure in a community sample of South African children and adolescences who experienced a traumatic event . PTSS were positively correlated with trauma exposure (r = 0.41, p < 0.001) and contextual adversity (r = 0.17, p = 0.01).

3.2 Univariate associations of PTSS and trauma exposure with cortisol AUCg

The regression analysis showed little evidence for an association between cortisol AUCg and PTSS (B = 0.37, p = 0.46; 95% CI [-0.61, 1.34]. There was also little evidence for an association with cortisol AUCg and trauma exposure (B = -0.83, p = 0.26; 95% CI [-2.30, 0.63]. There was no evidence for sex differences in the associations between AUCg and PTSS (P > 0.05).

3.3 HLM: Cortisol trends

First, HLM analysis was used to establish linear, quadratic and cubic functions of time cortisol reactivity over time, as previously reported for this sample (Boyes et al., 2012). Summary statistics are shown in Table 1. As expected, the change in cortisol over time was curvilinear, with a peak at +10 to +20 minutes post-stressor (see Figure 1); and there was evidence for linear, quadratic and cubic slopes: linear $B = 0.97$, $p < 0.001$; 95% CI [0.75, 1.18]; quadratic $B = -0.22$, $p < 0.001$; 95% CI [−0.27, −0.18]; cubic $B = 0.02$, $p < 0.001$; 95% CI [0.01,0.02].

3.4 HLM: Effects of PTSS on cortisol

Next, PTSS, sex and PTSS x sex interactions were included in the model, to examine associations with cortisol intercept and change over time. Results are presented in Table 2, Model 1. PTSS were not significant predictor of the cortisol intercept (B = -0.42, p = 0.060, 95% CI [-0.85,0.02]). There was evidence for effects of PTSS on the linear and quadratic slopes of the cortisol curves, and these were moderated by sex × PTSS interactions (Table 2, Model 1). In girls, PTSS were weakly negatively associated with the linear (B = -0.07, p = 0.043, 95% CI [−0.13, −0.002]) cortisol slope, and positively associated with the quadratic (B = 0.01, p = 0.017, 95% CI [0.002, 0.02]) cortisol slope for reactivity over time. In boys, PTSS were positively associated with the linear slope (B= 0.07, p = 0.038, 95% CI [0.01, 0.14]) and negatively associated with the quadratic slope (B = -0.01, p = 0.016, 95% CI [−0.02, -0.002]) for cortisol change over time. Figure 2 presents the model-based predicted cortisol concentration for boys and girls separately, with cortisol concentration estimated at 1 SD above and below the mean of the PTSS scores, for visual purposes. As illustrated in the Figure, in girls, higher levels of PTSS were associated with a reduced peak in the cortisol reactivity curve in response to the TSST. The positive coefficient for the quadratic term reflects this shallower cortisol response curve with higher PTSS. In boys, the association with PTSS was in the opposite direction, with higher PTSS associated with a steeper cortisol response curve. Note that in males with higher PTSS the baseline tended to be lower, and

so the peak response is not greater than those males with low PTSS; however, the increase from baseline is larger.

There were no effects of additional covariates, intervention group status (B = -0.03, p = 0.34, 95% CI [−0.20, 0.14]), cumulative contextual adversity (B = -0.23, p = 0.75, 95% CI [−0.71, 0.24]) or puberty status in girls (B = 0.10, p = 0.37, 95% CI [−0.12, 0.32]) on associations between PTSS and cortisol reactivity.

3.5 Trauma exposure

Next, we re-ran our models, replacing PTSS scores with extent of trauma exposure (CECV scores), including sex × trauma exposure interactions. These analyses revealed no effects of trauma exposure (as main effect or in interaction with gender) on the intercept, linear or quadratic slopes. Findings are presented in Table 2, Model 2. There were no effects of additional covariates, intervention group status (B = -0.04, p = 0.68, 95% CI [−0.21, 0.13]) or cumulative contextual adversity (B = -0.12, p = 0.64, 95% CI [−0.61, 0.38]) or puberty status in girls (B = 0.09, p = 0.44, 95% CI [−0.13, 0.31]) on associations between PTSS and cortisol reactivity.

4. Discussion

In adolescents from a deprived LMIC context, we found associations between symptoms of PTSD and cortisol reactivity to psychosocial stress that were moderated by sex. Specifically, girls with high levels of PTSS showed a shallower cortisol response to the TSST compared those with fewer symptoms, whereas for boys, posttraumatic distress was associated with a stronger post-stressor cortisol response. In contrast, we found no evidence that extent of trauma exposure was associated with cortisol reactivity in our sample. While no association was found between PTSS and AUC_g of cortisol concentrations, the mixed model teases apart differences in the shape of the curve, whereas AUCg does not.

In adults, PTSD has been associated with both hyper (Elzinga, Schmahl, Vermetten, van Dyck, & Bremner, 2003; Zaba et al., 2015) and hypo-reactivity cortisol to stress (Wichmann, Kirschbaum, Bohme, & Petrowski, 2017; Zaba et al., 2015), while others have found no association (Bremner et al., 2003; Roelofs et al., 2009; Simeon et al., 2007). There has been extremely limited prior investigation of cortisol stress reactivity in association with child PTSD. Our observations of enhanced cortisol reactivity and recovery in association with PTSS in boys, versus a blunted stress response in girls, provide important new evidence of sex specific effects in the relationship between PTSS and HPA axis activity in children. These findings are consistent with observations of equivalent sex differences in cortisol secretion in the context of anxiety/depression, where males have been found to show increased reactivity and females blunted reactivity compared to their control group counterparts (Zorn, 2017). Nonetheless, the current evidence, deriving from a group of young people experiencing high levels of co-occurring adversity, contrasts with previous studies that have failed to identify PTSD effects on HPA reactivity in adolescents (MacMillan et al., 2009a; Peckins, 2012). Previous null findings have derived from samples in which the levels of PTSD symptoms are extremely low, which may explain the discrepancy. However, further investigation of high-adversity populations of young people is warranted, particular considering that that the HPA axis activity may be sensitive to context levels of threat (Giudice, Ellis, & Shirtcliff, 2011).

The neurobiological underpinnings of sex differences in HPA axis reactivity remain poorly understood (Zorn, 2017), as do the reasons for increased vulnerability to PTSD in girls versus boys that emerges during adolescence (Haag, 2019). Previous work with South African youth has highlighted problems with aggression and externalising symptoms in trauma exposed boys, whereas girls maybe vulnerable to depression (du Plessis, Kaminer, Hardy, & Benjamin, 2015). This is consistent with some evidence of sex differences in the adult presentation of PTSD, where higher levels of irritability and anger in males have particularly been reported (Green, 2003). Exploration of potentially varied clinical

presentations of PTSD in relation to cortisol reactivity could further our understanding of sex differences. Importantly, our findings highlight the critical importance of taking account of sex differences in analyses in this context.

We did not find evidence of an effect of trauma exposure on cortisol reactivity in our sample, suggesting that the HPA axis alterations observed are specific to young people with PTSS, versus being a consequence of exposure to trauma per se. This is inconsistent with previous observations that trauma exposure is associated with either blunted (MacMillan et al., 2009b) or elevated (Rao, Hammen, Ortiz, Chen, & Poland, 2008) cortisol reactivity in adolescents. Discrepant findings may be explained by differences across studies in the type of trauma measured, whether the trauma is a single-event or repeated, and the time between trauma exposure and measurement of cortisol reactivity. Future studies should look to tease apart the effects of PTSS and trauma exposure on cortisol and HPA dysregulation. Importantly, more research is required in LMIC contexts, where trauma exposure is extremely prevalent and may be more normative (Atwoli, 2015).

Findings should be considered in the light of several limitations. Our data are cross-sectional and correlational, so causal relationships cannot be inferred. Plausibly, altered cortisol responses may be linked with the expression of PTSS, or may represent a psychobiological vulnerability, with some evidence suggesting that that traumatised individuals with a blunted cortisol response to stress could be more susceptible to developing PTSD (Delahanty et al., 2000; McFarlane, Atchison, & Yehuda, 1997). Cross-lagged longitudinal studies and experimental trials are required to understand the causal direction of this association. In addition, the stress task was artificial, and thus the cortisol response may not be generalizable to real world stressful situations, or non-social stress.

4.1 Conclusion

Few previous studies have assessed cortisol reactivity in children with PTSS, and none have done so in LMIC contexts. We provide evidence that the cortisol response to stress is altered

in children with PTSS, and that such changes are sex specific. The findings require replication to establish the causality of the relationship, though they suggest that PTSD impairs cortisol reactivity, which can impact the stress response system in later life.

Conflict of interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

References

Amaya-Jackson, L., McCarthy, G., Cherney, M. S., & Newman, E. (1995). Child PTSD checklist. *Durham, NC: Duke University Medical Center.* Retrieved from https://scholar.google.co.uk/scholar?hl=en&as_sdt=0%2C5&q=Child+PTSD+checklist+Amaya-Jackson%2C+McCarthy%2C+Cherney%2C+%26+Newman%2C+1995&btnG=

Atwoli, L., Stein, D. J., Koenen, K. C., & McLaughlin, K. A. (2015). Epidemiology of posttraumatic stress disorder: prevalence, correlates and consequences. *Current opinion in psychiatry, 28*(4), 307.

Bouma, E. M., Riese, H., Ormel, J., Verhulst, F. C., & Oldehinkel, A. J. (2009). Adolescents' cortisol responses to awakening and social stress; effects of gender, menstrual phase and oral contraceptives. The TRAILS study. *Psychoneuroendocrinology, 34*(6), 884-893. doi:10.1016/j.psyneuen.2009.01.003

Boyes, M. E., Cluver, L., & Gardner, F. (2012). Psychometric Properties of the Child PTSD Checklist in a Community Sample of South African Children and Adolescents. *PLoS One, 7*, e46905. doi:10.1371/journal.pone.0046905

Bremner, J. D., Vythilingam, M., Vermetten, E., Adil, J., Khan, S., Nazeer, A., . . . Charney, D. S. (2003). Cortisol response to a cognitive stress challenge in posttraumatic stress disorder (PTSD) related to childhood abuse. *Psychoneuroendocrinology, 28*(6), 733-750. Retrieved from http://dx.doi.org/

Carrion, V. G., Weems, C. F., Ray, R. D., Glaser, B., Hessl, D., & Reiss, A. L. (2002). Diurnal salivary cortisol in pediatric posttraumatic stress disorder. *Biological Psychiatry, 51*(7), 575-582. Retrieved from http://dx.doi.org/

Cluver, L., Fincham, D. S., & Seedat, S. (2009). Posttraumatic stress in AIDS-orphaned children exposed to high levels of trauma: the protective role of perceived social support. *Journal of Trauma Stress, 22*(2), 106-112. doi:10.1002/jts.20396

Cluver, L., Gardner, F., & Operario, D. (2007). Psychological distress amongst AIDS-orphaned children in urban South Africa. *Journal of Child Psychology and Psychiatry, and allied disciplines, 48*(8), 755-763. doi:10.1111/j.1469-7610.2007.01757.x

Cooper, P. J., Tomlinson, M., Swartz, L., Landman, M., Molteno, C., Stein, A., . . . Murray, L. (2009). Improving quality of mother-infant relationship and infant attachment in socioeconomically deprived community in South Africa: randomised controlled trial. *BMJ, 338*, b974. doi:10.1136/bmj.b974

De Bellis, M. D., & Zisk, A. (2014). ‚ÄúThe Biological Effects of Childhood Trauma‚Äù. *Child and Adolescent Psychiatric Clinics of North America, 23*(2), 185-222. doi:10.1016/j.chc.2014.01.002

Delahanty, D. L., Raimonde, A. J., & Spoonster, E. (2000). Initial posttraumatic urinary cortisol levels predict subsequent PTSD symptoms in motor vehicle accident victims. *Biological Psychiatry, 48*(9), 940-947. Retrieved from http://dx.doi.org/

du Plessis, B., Kaminer, D., Hardy, A., & Benjamin, A. (2015). The contribution of different forms of violence exposure to internalizing and externalizing symptoms among young South African adolescents. *Child Abuse and Neglect, 45*, 80-89. doi:10.1016/j.chiabu.2015.02.021

Elzinga, B. M., Schmahl, C. G., Vermetten, E., van Dyck, R., & Bremner, J. D. (2003). Higher cortisol levels following exposure to traumatic reminders in abuse-related PTSD. *Neuropsychopharmacology, 28*(9), 1656-1665. doi:10.1038/sj.npp.1300226

Fearon, R. P., Tomlinson, M., Kumsta, R., Skeen, S., Murray, L., Cooper, P. J., & Morgan, B. (2017). Poverty, early care and stress reactivity in adolescence: Findings from a prospective, longitudinal study in South Africa. *Developmental Psychopathology, 29*(2), 449-464. doi:10.1017/s0954579417000104

Flinn, M. V., & England, B. G. (1997). Social economics of childhood glucocorticoid stress response and health. *American Journal of Physical Anthropology, 102*(1), 33-53. doi:10.1002/(sici)1096-8644(199701)102:1<33::aid-ajpa4>3.0.co;2-e

Fries, E., Hesse, J., Hellhammer, J., & Hellhammer, D. H. (2005). A new view on hypocortisolism. *Psychoneuroendocrinology, 30*(10), 1010-1016. doi:10.1016/j.psyneuen.2005.04.006

Giudice, M. D., Ellis, B. J., & Shirtcliff, E. A. (2011). The Adaptive Calibration Model of stress responsivity. *Neuroscience and Biobehavior Reviews, 35*(7), 1562-1592. doi:10.1016/j.neubiorev.2010.11.007

Green, B. (2003). Post-traumatic stress disorder: symptom profiles in men and women. *Current Medical Research and Opinions, 19*(3), 200-204. doi:10.1185/030079903125001604

Gregorowski, C., & Seedat, S. (2013). Addressing childhood trauma in a developmental context. *Journal of Child & Adolescent Mental Health, 25*(2), 105-118.

Haag, K., Fraser, A., Hiller, R., Seedat, S., Zimmerman, A., & Halligan, S. (2019). The emergence of sex differences in PTSD symptoms across development: Evidence from the ALSPAC cohort. *Psychological Medicine*. Retrieved from https://researchportal.bath.ac.uk/en/publications/the-emergence-of-sex-differences-in-ptsd-symptoms-across-developm

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*(1-2), 76-81. doi:10.1159/000119004

Li, Y., & Seng, J. S. (2018). Child Maltreatment Trauma, Posttraumatic Stress Disorder, and Cortisol Levels in Women: A Literature Review. *Journal of the American Psychiatric Nurses Association, 24*(1), 35-44. doi:10.1177/1078390317710313

MacMillan, H. L., Georgiades, K., Duku, E. K., Shea, A., Steiner, M., Niec, A., . . . Schmidt, L. A. (2009a). Cortisol Response to Stress in Female Youths Exposed to Childhood Maltreatment: Results of the Youth Mood Project. *Biological Psychiatry, 66*(1). doi:10.1016/j.biopsych.2008.12.014

MacMillan, H. L., Georgiades, K., Duku, E. K., Shea, A., Steiner, M., Niec, A., . . . Schmidt, L. A. (2009b). Cortisol response to stress in female youths exposed to childhood maltreatment: results of the youth mood project. *Biological Psychiatry, 66*(1), 62-68. doi:10.1016/j.biopsych.2008.12.014

Martin, L., Revington, N., & Seedat, S. (2013). The 39-item Child Exposure to Community Violence (CECV) scale: exploratory factor analysis and relationship to PTSD symptomatology in trauma-exposed children and adolescents. *Internal Journal of Behavioural Medicine, 20*(4), 599-608. doi:10.1007/s12529-012-9269-7

McDade, T. W., Hoke, M., Borja, J. B., Adair, L. S., & Kuzawa, C. (2013). Do environments in infancy moderate the association between stress and inflammation in adulthood? Initial evidence from a birth cohort in the Philippines. *Brain Behaviour, and Immunity, 31*, 23-30. doi:10.1016/j.bbi.2012.08.010

McEwen, B. S. (1998). Stress, adaptation, and disease. Allostasis and allostatic load. *Annals of New York Academy of Sciences, 840*, 33-44. doi:10.1111/j.1749-6632.1998.tb09546.x

McFarlane, A. C., Atchison, M., & Yehuda, R. (1997). The acute stress response following motor vehicle accidents and its relation to PTSD. *Annals of New York Academy of Sciences, 821*, 437-441. Retrieved from http://dx.doi.org/

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin, 133*(1), 25-45. doi:10.1037/0033-2909.133.1.25

Pan, X., Wang, Z., Wu, X., Wen, S. W., & Liu, A. (2018). Salivary cortisol in post-traumatic stress disorder: a systematic review and meta-analysis. In *BMC Psychiatry* (Vol. 18).

Peckins, M. K., Dockray, S., Eckenrode, J. L., Heaton, J., & Susman, E. J. (2012). The longitudinal impact of exposure to violence on cortisol reactivity in adolescents. *Journal of Adolescent Health, 51*(4), 366-372.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28*(7), 916-931.

Rao, U., Hammen, C., Ortiz, L. R., Chen, L. A., & Poland, R. E. (2008). Effects of early and recent adverse experiences on adrenal response to psychosocial stress in depressed adolescents. *Biological Psychiatry, 64*(6), 521-526. doi:10.1016/j.biopsych.2008.05.012

Roelofs, K., van Peer, J., Berretty, E., Jong, P., Spinhoven, P., & Elzinga, B. M. (2009). Hypothalamus-pituitary-adrenal axis hyperresponsiveness is associated with increased social avoidance behavior in social phobia. *Biological Psychiatry, 65*(4), 336-343. doi:10.1016/j.biopsych.2008.08.022

Schalinski, I., Elbert, T., Steudte-Schmiedgen, S., & Kirschbaum, C. (2015). The Cortisol Paradox of Trauma-Related Disorders: Lower Phasic Responses but Higher Tonic Levels of Cortisol Are Associated with Sexual Abuse in Childhood. *PLoS One, 10*(8). doi:10.1371/journal.pone.0136921

Simeon, D., Knutelska, M., Yehuda, R., Putnam, F., Schmeidler, J., & Smith, L. M. (2007). Hypothalamic-Pituitary-Adrenal Axis Function in Dissociative Disorders, PTSD, and Healthy Volunteers. *Biological Psychiatry, 61*(8), 966-973. doi:10.1016/j.biopsych.2006.07.030

Suliman, S., Mkabile, S. G., Fincham, D. S., Ahmed, R., Stein, D. J., & Seedat, S. (2009). Cumulative effect of multiple trauma on symptoms of posttraumatic stress disorder, anxiety, and depression in adolescents. *Comprehensive Psychiatry, 50*(2), 121-127. doi:10.1016/j.comppsych.2008.06.006

Takai, N., Yamaguchi, M., Aragaki, T., Eto, K., Uchihashi, K., & Nishikawa, Y. (2007). Gender-specific differences in salivary biomarker responses to acute psychological stress. *Annals of the New York Academy of Science, 1098*, 510-515. doi:10.1196/annals.1384.014

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., & Navalta, C. P. (2002). Developmental neurobiology of childhood stress and trauma. *Psychiatrics Clinics of North America, 25*(2), 397-426, vii-viii. Retrieved from http://dx.doi.org/

Weems, C. F., & Carrion, V. G. (2007). The association between PTSD symptoms and salivary cortisol in youth: the role of time since the trauma. *Journal of Trauma Stress, 20*(5), 903-907. doi:10.1002/jts.20251

Wichmann, S., Kirschbaum, C., Bohme, C., & Petrowski, K. (2017). Cortisol stress response in post-traumatic stress disorder, panic disorder, and major depressive disorder patients. *Psychoneuroendocrinology, 83*, 135-141. doi:10.1016/j.psyneuen.2017.06.005

Zaba, M., Kirmeier, T., Ionescu, I. A., Wollweber, B., Buell, D. R., Gall-Kleebach, D. J., . . . Schmidt, U. (2015). Identification and characterization of HPA-axis reactivity endophenotypes in a cohort of female PTSD patients. *Psychoneuroendocrinology, 55*, 102-115. doi:10.1016/j.psyneuen.2015.02.005

Zorn, J. V., Schür, R. R., Boks, M. P., Kahn, R. S., Joëls, M., & Vinkers, C. H. (2017). Cortisol stress reactivity across psychiatric disorders: a systematic review and meta-analysis. *Psychoneuroendocrinology, 77*, 25-36.

Legends for tables and figures

Table 1. Summary statistics for salivary cortisol concentration by sex

Table 2. HLM growth curve analysis of cortisol response in relation to PTSS, by sex (Model 1) and trauma exposure, by sex (Model 2). Male sex is reference category (N = 291)

Figure 1. Estimated cortisol concentrations as a function of PTSS and sex

Figure 2. Estimated cortisol concentrations as a function of PTSS and sex

Tables and Figures

Table 1. Summary statistics for salivary cortisol concentration by sex

Time Point	Boys		Girls	
	Mean (*μg/dL*) ±SD	N	Mean (*μg/dL*) ±SD	N
Baseline <mark>(no stress)</mark>	2.89±0.91	149	2.50±0.86	152
Post-test <mark>(stress)</mark>	3.36±0.83	148	3.38±0.83	152
+10 mins	3.67±0.79	147	3.70±0.77	153
+20 mins	3.67±0.85	148	3.55±0.79	153
+30 mins	3.50±0.77	149	3.43±0.80	153
+40 mins	3.27±0.78	149	3.34±0.79	153
+ 50 mins	3.23±0.83	148	3.18±0.78	151

Table 2. HLM growth curve analysis of cortisol response in relation to PTSS, by sex (Model 1) and trauma exposure, by sex (Model 2). Male sex is reference category (N = 291)

|	Intercept		Linear slope		Quadratic slope	
	B (95% CI)	*p*	*B (95% CI)*	*p*	*B (95% CI)*	*p*
Model 1						
PTSS	-0.42 (-0.85, -0.02)	.060	0.22 (0.06, 0.37)	.007	-0.03 (-0.05, -0.01)	.001
Sex	-0.26 (-0.57, 0.05)	.119	0.18 (0.06, 0.30)	.003	-0.03 (-0.04, -0.01)	.000
Sex × PTSS	0.20 (-0.06, 0.48)	.135	-0.14 (-0.24, -0.05)	.004	0.02 (0.01, 0.03)	.001
Model 2						
Trauma exposure	-0.06 (-0.67, 0.56)	.855	0.05 (-0.17, 0.27)	.653	-0.01 (-0.03, 0.02)	.550
Sex	-0.03 (-0.37, 0.31)	.883	0.07 (-0.06, 0.19)	.286	-0.01 (-0.02, 0.01)	.178
Sex x exposure	-0.13 (-0.52, 0.27)	.529	-0.03 (-0.17, 0.12)	.719	0.01 (-0.01, 0.02)	.571
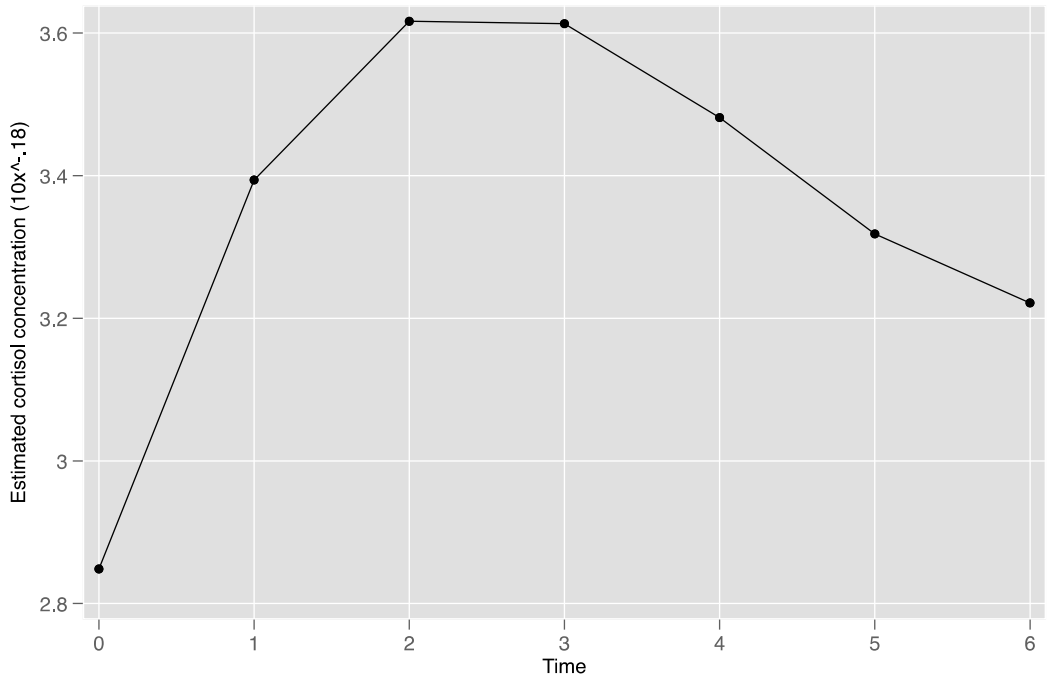
Figure 1. HLM growth curve estimates for transformed cortisol data
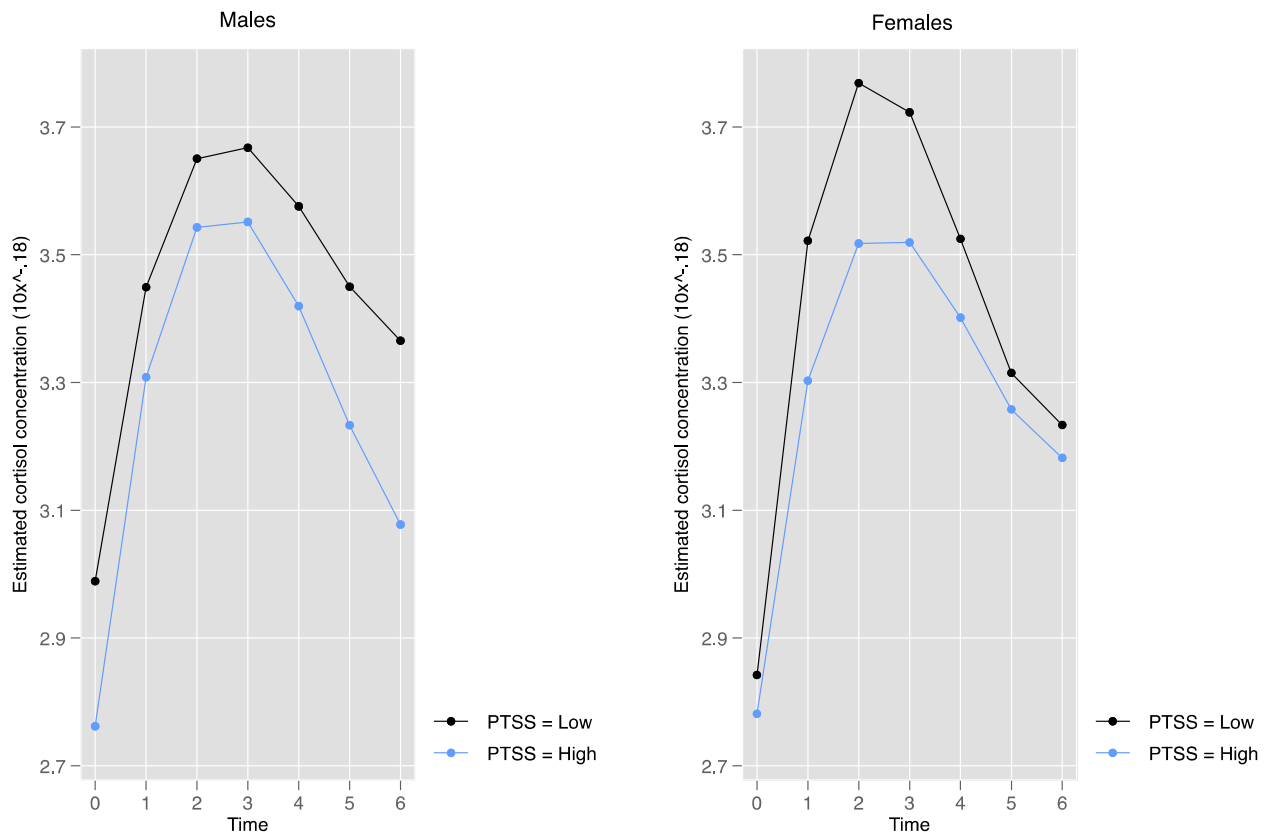
Figure 2. Estimated cortisol concentrations as a function of PTSS and sex