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Salivary Cortisol Lower in Posttraumatic Stress Disorder

Helané Wahbeh and

Department of Neurology, Oregon Health & Science University

Barry S. Oken

Department of Neurology, Oregon Health & Science University

Abstract

Altered cortisol has been demonstrated to be lower in posttraumatic stress disorder (PTSD) in most studies. This cross-sectional study evaluated salivary cortisol at waking, 30 minutes after, and bedtime in 51 combat veterans with PTSD compared to 20 veterans without PTSD. It also examined the relationship of cortisol to PTSD symptoms using two classifications: DSM-IV and the more recent four-factor classification proposed for DSM-V. The PTSD group had lower cortisol values than the control group ($F(6, 69) = 3.35, p = .006$). This significance did not change when adding age, body mass index, smoking, medications affecting cortisol, awakening time, sleep duration, season, depression, perceived stress, service era, combat exposure, and lifetime trauma as covariates. Post-hoc analyses revealed that the PTSD group had lower area under the curve ground and waking, 30min, and bedtime values while the cortisol awakening response and area under the curve increase were not different between groups. The four-factor avoidance PTSD symptom cluster was associated with cortisol but not the other symptom clusters. This study supports the finding that cortisol is lower in people with PTSD.

Keywords

Posttraumatic Stress Disorder; veteran; cortisol; cortisol awakening response

Cortisol is a hormone secreted by the adrenal gland and regulated by the hypothalamic-pituitary-adrenal (HPA) axis. In the normal acute stress response, cortisol increases as an adaptation to the stressor and then decreases to pre-stressor levels. Cortisol has two distinct secretion patterns: 1) a diurnal rhythm that peaks around 8 a.m. and declines thereafter until midnight, and 2) a cortisol awakening response (CAR) that includes a superimposed peak within one hour after waking (Clow, Thorn, Evans, & Hucklebridge, 2004; Fries, Dettenborn, & Kirschbaum, 2009). Morning cortisol levels or CAR peak within one hour after waking and may be determined by a specific awakening process (Clow, Hucklebridge, & Thorn, 2010); in particular, CAR magnitude may be regulated by the anticipation of the upcoming demands of the day (Fries, et al., 2009) or influenced by the person's experiences of the previous day (Adam, Hawkley, Kudielka, & Cacioppo, 2006).

HPA axis dysregulation as measured by lower cortisol values is associated with posttraumatic stress disorder (PTSD) in most but not all studies (Klaassens, Giltay, Cuijpers, van Veen, & Zitman, 2012; Morris, Compas, & Garber, 2012; Yehuda, 2002, 2006). Discrepancies in findings may result from methodological issues such as sample type (e.g. saliva, serum, or urine), or sample timing variability, or failure to account for important

confounding variable such as age, sex, and medication use. This latter problem is evidenced by a meta-analysis that showed no cortisol differences in people with PTSD compared to controls when important factors such as cortisol collection times, medical history, and childhood trauma were not taken into account (Klaassens, et al., 2012), while another larger meta-analysis clearly observed lower cortisol in people with PTSD compared to controls when accounting for collection timing and evaluating the role of potential moderators (e.g. age, gender, cortisol collection timing, time since trauma, developmental timing of trauma exposure) (Morris, et al., 2012). Chida et al. conducted a systematic review and meta-analysis examining CAR in PTSD studies and found that PTSD status was not associated with CAR. However, when including only the three high quality studies a reduced CAR was associated with PTSD (Chida & Steptoe, 2008). Another study since found no association with CAR and PTSD (Laudenslager et al., 2009). Small study size, methodological variability (e.g. not reporting cortisol-affecting medications), and uncontrolled, correlation study designs, while useful as preliminary findings, limit making definitive conclusions about cortisol, CAR and PTSD status. This highlights the importance of conducting larger studies that incorporate cortisol moderating factors into the analysis and their associations with PTSD status.

The relationship between cortisol levels and PTSD diagnosis status has been and will continue to be evaluated. However, the assessment of PTSD symptoms rather than just PTSD diagnosis status has been less studied. According to the currently used Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV), PTSD diagnosis requires the presence of three symptom clusters: re-experiencing, numbing/avoiding, and hyper-arousal (American Psychiatric Association, 1994). These clusters consist of diverse symptoms, and each person with PTSD will have a different combination of them. The mechanisms underlying the three symptom clusters are considered different and thus, may require different treatment approaches (Asmundson et al., 2000; O'Donnell, Hegadoren, & Coupland, 2004). Thus, understanding the symptom clusters biological mechanisms may improve future treatments. Others have begun to examine the relationship between PTSD symptoms and cortisol. Yehuda found the relationship between PTSD status in Holocaust survivors mean 24-hour urinary cortisol excretion was due to a substantial association with avoidance subscale scores ($r=-0.49$ $p<.001$). Another study evaluated salivary cortisol's relationship to PTSD symptoms in eight Persian Gulf veterans with PTSD ($r=-0.67$ $p<.05$). The other three symptoms clusters were not significant; however, it is unlikely that the study was adequately powered to detect these relationships with only eight participants (Kellner, Baker, & Yehuda, 1997). De Kloet assessed salivary cortisol's relationship to PTSD in 28 veterans with PTSD finding that morning levels (area under the curve with respect to ground) was negatively correlated with re-experiencing and hyper-arousal and the area under the response curve was negative correlated with re-experiencing, numbing/avoiding and hyper-arousal (de Kloet et al., 2007). Additionally, in another study of 28 trauma-exposed subjects the overall PTSD and hyper-arousal symptom scores were significantly negatively correlated with the morning area under the curve with respect to ground whereas the avoidance and re-experiencing correlations were not (Wessa, Rohleder, Kirschbaum, & Flor, 2006). These studies used correlational analyses to assess the relationship between cortisol, had small subject numbers, and implemented varying methodology to assess cortisol (e.g. urinary total output, single salivary sample, cortisol awakening response with multiple time-point sampling). While attempts have been made to assess the relationship of cortisol to PTSD symptoms clusters, the relationships are still unclear and additional studies evaluating these relationships are warranted.

More recently, strong evidence supports the use of a four-factor symptom cluster model of PTSD proposed for DSM-V rather than the currently used three-factor model (Friedman,

Resick, Bryant, & Brewin, 2011). The four-factor model proposed for DSM-V includes four symptom clusters:

1. *The presence of one or more intrusion symptoms such as:* spontaneous or cued recurrent, involuntary, and intrusive distressing memories, dreams or dissociative reactions related to the traumatic event(s); intense or prolonged psychological distress or marked physiological reactions at exposure to cues that symbolize or resemble the traumatic event(s).
2. *Persistent avoidance or efforts to avoid one or more stimuli associated with the traumatic event(s):* internal processes or external reminders that arouse distressing memories, thoughts, or feelings about or closely associated with the traumatic event(s).
3. *Two or more negative alterations in cognitions and mood associated with the traumatic event(s):* inability to remember an important aspect of the traumatic event(s); persistent and exaggerated negative beliefs or expectations about oneself, others, or the world; persistent, distorted blame of self or others about the cause or consequences of the traumatic event(s); persistent negative emotional state (e.g., fear, horror, anger, guilt, or shame); markedly diminished interest or participation in significant activities; feelings of detachment or estrangement from others; persistent inability to experience positive emotions;
4. *Marked alterations in arousal and reactivity associated with the traumatic event(s) as evidenced by two or more of the following:* irritable or aggressive behavior; reckless or self-destructive behavior; hyper-vigilance; exaggerated startle response; problems with concentration; sleep disturbance (e.g., difficulty falling or staying asleep or restless sleep).

Cortisol's relationship to the current PTSD symptom clusters has been examined in a limited way but not its relationship to new model clusters. Thus, cortisol's relationships to both PTSD symptom classifications are assessed in this study.

This study's goal was to build upon previous research with a larger participant pool accounting for important covariates and a more complete assessment of salivary cortisol's relationship to PTSD symptom clusters. The rationale for these study goals is based on: 1) most but not all studies report lower cortisol in people with PTSD and there are only a few, small-sample CAR studies with contradictory findings, and 2) cortisol's relationship to the new PTSD cluster model has yet to be examined. The primary objectives of this cross-sectional study were to: 1) evaluate salivary cortisol in people with PTSD compared to controls, and 2) examine the relationship of salivary cortisol to the DSM-IV and four-factor model PTSD symptom clusters. We hypothesized that the PTSD group would have decreased CAR and total cortisol based on the most recent meta-analysis with a larger number of studies assessed (Morris, et al., 2012). Because of the small number of studies assessing the cortisol's relationship to PTSD symptom clusters and their heterogeneity, we hypothesized that cortisol would be negatively correlated with PTSD clusters but not which clusters would be correlated.

Method

Participants

Potential participants were recruited through flyers at the Portland Veterans Administration Medical Center, Portland Veterans Center, and other veterans groups throughout the Portland Metropolitan area. The participants were 86 veterans, 58 with PTSD and 28 without PTSD. Participant data are from a convenience sample that was collected during a

cross-sectional and an intervention study and pooled for this report to maximize subject number (Table 1). Other cross-sectional study data have been published elsewhere (Wahbeh & Oken, 2011). Saliva collection from intervention study participants occurred at baseline prior to intervention onset. Both studies had the same inclusion/exclusion criteria and recruitment sources. Veterans were excluded if they had a current significant chronic medical illness; bipolar, schizoaffective, or psychotic disorders; any DSM-IV cognitive disorder; a substance dependence disorder within 3 months of the study or current substance use other than 2 drinks or less of alcohol per day; or if sexual assault was a primary PTSD event. Volunteers over 65 years old were also excluded to reduce heterogeneity in cortisol and other physiological outcomes used in the cross-sectional and intervention studies that are highly affected by age. The participants needed to be in good general health (defined as with no acute life-limiting illness) and on stable doses of medications for at least 4 weeks prior to study onset. Participants on corticosteroid medications were excluded from the analyses. The study was approved by the institutional review boards of Portland Veterans Administration Medical Center and Oregon Health & Science University. Written informed consent was obtained from all subjects.

Study Procedures

Participants underwent a telephone screening, a screening visit, and a collection visit.

Screening visit—Study procedures, inclusion/exclusion criteria, and the risks and benefits of participating were explained during a telephone screening. The screening visit included informed consent and collection of diagnostic, demographic, medical, health history, lifestyle, and military service information including sex, age, body mass index (BMI), smoking status, cigarettes smoked, awakening time, time awake, sleep duration, season, depression, perceived stress, lifetime traumatic events which are important in cortisol analyses (Chida & Steptoe, 2008; Clow, et al., 2004; Fries, et al., 2009; Huber, Issa, Schik, & Wolf, 2006) (Table 1). Era served (Vietnam, Operation Enduring Freedom/Operation Iraqi Freedom, Other) and years of service were also collected. Volunteers were interviewed by trained clinicians using the Clinician-Administered PTSD Scale for DSM-IV (CAPS), which includes a lifetime traumatic events checklist (LEC) (Blake et al., 1995). Participants met DSM-IV criteria A-F; criteria B, C, and D when the frequency score was 1 or more and the intensity score was 2 or more. The no-PTSD status for control participants was confirmed with the same interview and criteria. DSM-IV cluster scores include these CAPS criteria items: re-experiencing = all B items; numbing/avoiding = all C items; and hyper-arousal = all D items. The four-factor model symptom clusters include these CAPS criteria items: 4F-Intrusions = all B items; 4F-Avoidance = C1, C2; 4F-Dysphoria = C3–7, D1–3; 4F-Hyper-arousal = D4–5. The DSM-IV re-experiencing and 4F-Intrusions have the same criteria and thus the same value. The Structured Clinical Interview from the DSM-IV was performed to screen for excluded DSM-IV disorders (First, Spitzer, Gibbon, & Williams, 2002). Combat exposure was determined with the self-report Combat Exposure Scale (CES) (Keane et al., 1989). The Beck Depression Inventory (BDI) (Beck, Steer, & Brown, 1996) and the Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983) assessed depression and perceived stress symptoms. Participant medications were classified into five categories based on their potential influence on cortisol: 1) Corticosteroids, 2) Nonspecific neurotransmitter effects (NSTE) (e.g. selective-serotonin reuptake inhibitors, tricyclic antidepressants), 3) Medications that may influence the cortisol synthesis biochemical pathway (BP) (e.g. hypolipidemic statins, niacin fibrates), 4) Pain, and 5) Hormonal. A dichotomous covariate was used to indicate medication status for each class other than corticosteroids since these participants were excluded from the analysis (Almeida, Piazza, & Stawski, 2009; Granger, Hibel, Fortunato, & Kapelewski, 2009).

Cortisol collection—Salivary cortisol was collected at waking, 30 minutes after (30min), and bedtime in Sarstedt Salivettes® (Sarstedt, Germany) on two consecutive weekdays (Clow, et al., 2004; Hellhammer et al., 2007) as previously described (Wahbeh, Kishiyama, Zajdel, & Oken, 2008). Participants fasted for the waking and 30min sample and the importance of collecting the sample immediately upon waking and exactly 30 minutes after was stressed to the participants. Participants recorded their saliva collection times on a data sheet. Cortisol values were quantified by the Oregon Clinical and Translational Research Institute lab in duplicate with enzyme-linked immunoassay (Salimetrics of State College, PA). Two controls were run in every assay (Inter-assay coefficient of variation- 4.74% at 0.11 and 3.03% at 1.03 respectively).

The Day 1 and Day 2 cortisol values from each time-point (waking, 30 min, and bedtime) were averaged resulting in one value that was used for the analysis. If Day 1 or Day 2 values were missing at any time-point, the remaining value was used rather than the average. CAR was calculated by taking the 30min value and subtracting the waking value as previously described (Clow, et al., 2004) and used in our and other studies observing CAR effects (Inslicht et al., 2011; Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004; Wahbeh, et al., 2008). Area under the curve with respect to increase (AUCi) and area under the curve with respect to the ground (AUCg-including waking, 30min, and bedtime) were also calculated (Clow, et al., 2004; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Statistical Analyses

Means, medians, and standard deviations were calculated for each variable and values examined for outliers and normality of distribution. Non-normally distributed variables were transformed with natural log or box-cox transforms, and nonparametric tests used when transforms did not normalize the data. Participant data that precluded AUCi and AUCg calculations were excluded from analyses. A False Discovery Rate (FDR) correction for multiple comparisons was used (Benjamini & Hochberg, 1995). The number of hypotheses tested for each FDR correction is listed after the analysis description. Group differences in participant characteristics were assessed with the chi-square test for discrete variables, an analysis of variance for normally distributed variables, and the two-sample Kruskal-Wallis test for non-normally distributed data (FDR-13). Pearson correlation coefficients were calculated to assess test/re-test reliability between Day 1 and Day 2 samples and paired *t* tests evaluated whether significantly increased or decreased across days (FDR-4 each).

Group cortisol differences were calculated with a nonparametric multivariate analysis of variance (MANOVA)(Finch, 2005) with waking, 30min, bedtime, and AUCg, and AUCi as the dependent variables and PTSD status as the fixed variable. All covariates were then included in the model (age, BMI, smoking, medications affecting cortisol, awakening time, sleep duration, season, depression (BDI), perceived stress (PSS), service era, combat exposure (CES), and lifetime trauma (LEC)) in a non-parametric multivariate analysis of covariance analysis (FDR-2). Post-hoc analyses were then conducted for each dependent variable with the Kruskal-Wallis test (post-hoc comparisons $p < .05$ considered significant).

Multiple linear regression analyses assessed the relationship between DSM-IV and four-factor model symptoms and AUCg in the PTSD group in two models. Normality, linearity, and outliers were evaluated through normal probability plots and scatter plots of the standardized residuals. None of these assumptions were violated. Zero-order Pearson product-moment correlation coefficients between measures were calculated. For model 1, AUCg was the dependent variable and DSM-IV symptoms were independent variables. For model 2, AUCg was the dependent variable and four-factor symptoms were the independent variables. Statistical analyses were performed with SPSS 19.0 (IBM, USA) and STATA 10.0 (Statacorp, LP, Texas, USA).

Results

From the 89 total, five participants were excluded because of corticosteroid use, six because they did not record their collection times, and four because their collection times varied by 15 or more minutes from the protocol. Also, three control group participants were excluded because they had sub-threshold PTSD (defined as having significant distress and impairment and endorsing one re-experiencing symptom and either three avoidance or two arousal symptoms) (Blanchard, Hickling, Taylor, Loos, & Gerardi, 1994). Data from fifty-one combat veterans with PTSD and 20 veterans without PTSD (10 with combat exposure and 10 without) were analyzed. There were no group differences on age, race, education, marital status, BMI, smoking status or cigarettes-per-day, medication use, awakening time, sleep duration, time awake before bedtime collection, season of collection, military era, and years in service with multiple comparison corrections (Table 1). All participants were in good general health with no acute life-limiting illness. Participant reported chronic conditions showed no group differences except for chronic pain (No-PTSD, PTSD respectively: chronic pain-5%, 33%, $\chi^2(1) = 6.1, p = .01$; hypertension-10%, 27%, $\chi^2(1) = 2.5, p = .11$; gastrointestinal complaints-10%, 25%, $\chi^2(1) = 2.1, p = .20$; hypercholesterolemia-20%, 31%, $\chi^2(1) = 0.92, p = .34$; Type 2 Diabetes-5%, 4%, $\chi^2(1) = 0.04, p = .84$). Drug classes were similar for both groups except for NSTE which includes antidepressants (No-PTSD, PTSD respectively: NSTE-35%, 71%, $\chi^2(1) = 6.6, p = .01$; BP-25%, 29%, $\chi^2(1) = 0.14, p = .71$; pain-20%, 23%, $\chi^2(1) = 0.1, p = .75$; hormones-none).

Combat exposure, PTSD symptom, LEC, BDI, and PSS scores were significantly higher in the PTSD group (Table 2). The coefficient alpha's for the CAPS total and cluster scores, CES, BDI and PSS are: CAPS total (17 items, $\alpha = .92$), RE (5 items, $\alpha = .76$), NA (7 items, $\alpha = .84$), HA (5 items, $\alpha = .80$), 4F-Avoidance (2 items, $\alpha = .74$), 4F-Dysphoria (8 items, $\alpha = .82$), 4F-Hyper-arousal (2 items, $\alpha = .68$), CES (7 items, $\alpha = .90$), BDI (21 items, $\alpha = .94$), and PSS (10 items, $\alpha = .28$).

Waking, 30min, bedtime, and CAR values were correlated across days (waking $r = .77, p < .001$; 30 min $r = .77, p < .001$; bed $r = .54, p < .001$; CAR $r = .28, p = .03$). Paired t -tests at each time-point were not different (waking $t = 0.96, p = 0.34$; 30 min $t = 1.64, p = 0.10$; bedtime $t = 1.45, p = 0.15$; CAR $t = 0.63, p = 0.53$).

The PTSD group had lower cortisol values across all time-points in the MANOVA model ($F(6, 69) = 3.35, p = .006$). Including covariates did not change the overall significance ($F(6, 49) = 2.85, p = .01$). In post-hoc analyses, the PTSD group had lower AUCg and waking, 30min, and bedtime values (Table 2; Figure 1) while CAR and AUCi did not differ.

In the correlation analysis of the PTSD group only, AUCg was correlated with 4F-Avoidance ($r = 0.35, p < .05$); Re-experiencing/4F-Intrusions was correlated to Numbing/avoiding ($r = 0.46, p < .01$), 4F-Avoidance ($r = 0.52, p < .001$), 4F-Dysphoria ($r = 0.43, p < .01$), and Hyper-arousal ($r = 0.28, p < .05$) scores; Numbing/avoiding was correlated to 4F-Avoidance ($r = 0.70, p < .001$), and 4F-Dysphoria ($r = 0.81, p < .001$); Hyper-arousal was correlated with 4F-Dysphoria ($r = 0.38, p < .01$); and 4F-Avoidance was correlated to 4F-Dysphoria ($r = 0.38, p < .01$). All other correlations were not significant. Post-hoc Pearson correlations of LEC and CES to PTSD symptoms assessed the affect of lifetime trauma exposure and combat exposure on PTSD symptom clusters in the PTSD group. All correlations were not significant (all p 's $> .05$) except for the correlation between lifetime trauma exposure and Re-experiencing/4F-Intrusions ($r = 0.30, p = .03$). The only significant linear regression component was in model 2 where 4F-Avoidance was a significant independent variable although both models were not significant overall (Table 3).

Discussion

In summary, this cross-sectional study of veterans with and without PTSD assessed waking, 30min, bedtime, AUCg, and AUCi cortisol values and the relationship between AUCg and PTSD symptoms. PTSD participants had lower cortisol values at all three time-points and the AUCg but not CAR or AUCi. The 4F-Avoidance symptom cluster was positively correlated with AUCg.

The two groups had similar important demographic characteristics with multiple comparison corrections. All these comparisons were greater than $p > .05$ except smoking status, medication use, and era. While the statistical insignificance of these comparisons is important, clinically, the group differences may influence our understanding of participant characteristics and cortisol. The fact that cortisol's relationship with PTSD status remained when covarying for these important variables is reassuring (i.e. these potentially important clinical group differences did not influence the results). Our study also collected data on known cortisol affecting variables not always reported in other cortisol studies (e.g. season, medication use, health conditions). The groups differed on PTSD symptoms supporting the appropriate PTSD/No-PTSD status classification. They also differed on combat exposure, depression symptoms, lifetime trauma, and perceived stress, variables included as covariates to ensure their potential effect on cortisol did not independently influence the results.

PTSD participants had a lower AUCg and cortisol values at all time-points. One meta-analysis found contrasting results (Klaassens, et al., 2012), while another more recent larger meta-analysis found similar results (Morris, et al., 2012). Our study also included covariates removing the argument that potential confounders may have influenced the results. Our study results added to the Morris meta-analysis data pool supports the concept that diurnal cortisol values are in fact lower in people with PTSD. While absolute cortisol values were lower in the PTSD group, we did not find CAR or AUCi group differences. One CAR meta-analysis reported no differences in CAR compared to controls overall but a reduced CAR in people with PTSD when only including the high quality papers (3 studies) (Chida & Steptoe, 2008). We did not assess or control for the quality of experiences the day before collection or anticipation of the upcoming demands of the day which may influence CAR (Adam, et al., 2006; Fries, et al., 2009). Important factors to be considered when analyzing CAR were accounted for in the analyses but not all other CAR studies and may contribute to contradictory findings. While evidence that people with PTSD have lower cortisol values in general is increasing, studies confirming lower CAR in PTSD are still small and few. Additional and larger studies examining CAR in people with PTSD compared to controls are warranted followed by meta-analyses.

We hypothesized that any total cortisol relationship with PTSD symptoms would be negatively correlated but did not predict which ones. Only 4F-Avoidance was associated with total cortisol but in the opposite direction. No other studies have observed this relationship. One study observed a negative correlation between 24-hour total urinary cortisol and DSM-IV avoidance (Yehuda, 1995). Another study found that hyper-arousal symptoms were associated with cortisol values in trauma-exposed individuals (Wessa, et al., 2006). However, their correlation analysis included all trauma-exposed participants rather than those with PTSD only. If we run a post-hoc correlational analysis including all participants regardless of PTSD status, then all PTSD symptom clusters are negatively correlated with the AUCg. Thus, the correlation of hyper-arousal and cortisol values may be related to PTSD status rather than from specific relationships between cortisol and PTSD symptom clusters. Another study found that CAR was negatively correlated with hyper-arousal and re-experiencing symptoms in a PTSD-only group using AUC of CAR but not other time-points beyond the awakening hour (de Kloet, et al., 2007) If we repeat a similar

correlational analysis using the AUC_i rather than the AUC_g, we find no correlations. It is unclear why we found a positive correlation of AUC_g with the 4F-avoidance. The 4F-avoidance criterion difference may influence the results; however the DSM-IV avoidance cluster did not have any relationship with cortisol in our data set either. It is premature in the study of cortisol's relationship to PTSD symptoms to make any conclusions based on these limited findings. Additional studies examining the relationships between PTSD symptom clusters, especially the new proposed four-factor model symptoms, and cortisol are warranted and may help elucidate the mechanism of behavioral aspects of PTSD in relation to biology.

There are limitations to this study. It was a cross-sectional study and did not allow for causal inference. Other prospective studies have assessed cortisol's and CAR's predictive value on the development of PTSD and its symptoms. A few studies looked at cortisol levels immediately following trauma and its predictive value on PTSD development, one finding no relationship between cortisol and PTSD (Shalev et al., 2008) while two others reported that lower cortisol levels acute post-trauma predicted PTSD development (Delahanty, Raimonde, & Spoonster, 2000; Resnick, Yehuda, & Acerno, 1997). Cortisol levels prior to trauma however were not related to PTSD development in firefighters with a 2 year follow-up (Heinrichs et al., 2005). Similarly, CAR assessed before deployment did not predict PTSD symptoms after deployment in 470 male soldiers (van Zuiden et al., 2011). Although CAR evaluated before training was associated with greater peritraumatic dissociation, acute stress disorder symptoms but not peritraumatic distress or PTSD symptoms in police recruits 3 years later (Inslicht, et al., 2011). More prospective trials are needed to evaluate the predictive value of pre-trauma cortisol levels on PTSD development. No female veterans were in the study although they were not excluded. The all male veteran population precludes result generalizability to women veterans and non-veteran populations. The influence of sex on cortisol in PTSD was studied in a meta-analysis that found no group cortisol differences compared to controls but when the PTSD groups included females cortisol was found to be lower (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007). Conversely, the percentage of males in PTSD study groups were negatively associated with daily cortisol output in a meta-analysis (Morris, et al., 2012). Ensuring balanced sex ratios in future studies will elucidate the sex effect in these findings. We used two time-points as a method for CAR calculation (Clow, et al., 2004) which has been used previously in other studies (Inslicht, et al., 2011; Kunz-Ebrecht, et al., 2004). Ideally, three or more time-points in the first hour after awakening may have better characterized the CAR as others have noted (Chida & Steptoe, 2008). The wide range of bedtime time-points may have affected the results also. Additionally, participants were on medications that could potentially affect cortisol and also had depression symptoms. These variables were included as covariates with no effect. Excluding these participants would have more clearly assessed the research question. However, using medication use and depression symptoms as exclusion criteria is unfeasible both from a recruitment and result generalizability perspective. Also, combining trauma-exposed and non-trauma exposed controls may have affected results. A sub-analysis comparing the PTSD group to trauma versus non-trauma controls was not conducted since each control group would only have 10 participants. We attempted to mitigate this potential confounding by using combat exposure and lifetime traumatic event scores as covariates. Post-hoc correlations between lifetime trauma exposure (an instrument which includes childhood trauma), combat exposure and total cortisol values were not significant. Finally, the regression analysis included clusters from two overlapping models. Four-factor model proponents argue its greater appropriateness and practicality over the current model, a theory that evidence supports (Friedman, et al., 2011). Regardless, results should be interpreted knowing of the inter-relationships of the DSM-IV and four-factor model symptom clusters.

In conclusion, our large study that controlled for important factors affecting cortisol supports the evidence that people with PTSD have lower diurnal salivary cortisol values but not a lower CAR. More extensive research is needed to confirm or refute that people with PTSD have lower CAR and cortisol's relationship to PTSD symptom clusters.

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Salivary Cortisol

Figure 1.
Salivary Cortisol in the PTSD and No-PTSD Groups.

Table 1

Characteristics for Veteran Groups With and Without PTSD

Characteristic	No-PTSD (<i>n</i> = 20)		PTSD (<i>n</i> = 51)	
	% or <i>M</i>	<i>SD</i>	% or <i>M</i>	<i>SD</i>
Sex	100% male		100% male	
Age	55.5	8.9	54.4	11.3
Caucasian	100%		82%	
Education (>14 years)	40%		47%	
Married	30%		52%	
Body Mass Index	26.1	2.6	29.1	5.5
Smokers	45%		19%	
On Medications	45%		78%	
Awakening Time	6:00am	1:45	6:45am	1:45
Sleep Duration (hours)	8.4	2.9	8.4	2.9
Time awake before bedtime	16.4	1.7	16.0	2.1
Bedtime	10:41pm	1:54	10:57pm	1:19
	Range (7:30pm–3:00am)		Range (8:30pm–3:00am)	
Season				
Winter	45%		27%	
Spring	20%		24%	
Summer	25%		27%	
Fall	10%		22%	
Era				
Vietnam	50%		67%	
OEF/OIF	5%		20%	
Other	45%		13%	
Years in service	3.3	1.4	5.8	5.6

Note: OEF/OIF = Operation Enduring Freedom/Operation Iraqi Freedom. Chi-square test was used for discrete variables, analysis of variance for normally distributed variables, and the two-sample Kruskal-Wallis test for non-normally distributed continuous variables.

Table 2
PTSD and related symptoms and Cortisol values for Veteran Groups With and Without PTSD

Assessment/Time-Point	No-PTSD (<i>n</i> = 20)		PTSD (<i>n</i> = 51)		Effect Size	95% CI
	% or <i>M</i>	<i>SD</i>	% or <i>M</i>	<i>SD</i>		
Combat Exposure Scale	10.30	12.39	26.88	9.73	1.58***	[1.00,2.15]
CAPS Total	8.65	10.26	68.20	14.13	4.52***	[3.62,5.43]
Re-experiencing/4F-Intrusions	2.50	3.93	16.94	6.16	2.56***	[1.89,3.23]
Numbing/Avoiding	3.60	4.59	26.94	8.89	2.94***	[2.23,3.65]
Hyper-arousal	3.70	5.17	24.13	5.13	3.97***	[3.14,4.81]
4F-Avoidance	0.60	1.35	8.92	3.51	2.71***	[2.03,3.39]
4F-Dysphoria	6.90	8.63	32.51	8.02	3.13***	[2.04,3.86]
4F-Hyper-arousal	2.00	2.85	9.90	2.58	2.98***	[2.26,3.76]
Lifetime trauma (LEC)	22.30	11.42	33.94	5.90	1.49***	[0.92,2.06]
Depression (BDI)	9.95	8.47	18.01	10.22	0.83**	[0.29,1.36]
Perceived Stress (PSS)	15.45	7.71	22.76	6.51	1.07***	[0.52,1.61]
Cortisol Waking	0.91	0.98	0.53	0.49	0.60*	[0.12,1.10]
Cortisol 30 minute after	1.17	0.73	0.70	0.66	0.90**	[0.39,1.41]
Cortisol Bedtime	0.33	0.37	0.16	0.19	0.52*	[0.04,1.01]
CAR (30 min – Awakening)	0.26	0.59	0.15	0.34	0.20	[-0.26,0.67]
AUCg (0-Bedtime)	719.55	411.81	401.58	332.03	1.01**	[0.48,1.55]
AUCi (0–30 minutes after)	-134.03	607.83	-113.96	369.92	0.02	[-0.46,0.49]

Note: One outlier bedtime cortisol value for the PTSD group was removed from the analysis that was 29 standard deviations away from the mean and two people did not have bedtime values for calculating AUCg and AUCi. Cortisol units are µg/dl. OEF/OIF = Operation Enduring Freedom/Operation Iraqi Freedom; BDI = Beck Depression Inventory; 4F = Four-factor model; LEC = Life Events Checklist (CAPS); PSS = Perceived Stress Scale. CAR = Cortisol awakening response; AUCg = Area under the curve in relation to the ground; AUCi = Area under the curve in relation to the increase. Analysis of variance was used for normally distributed variables, and the two-sample Kruskal-Wallis test for non-normally distributed variables. Standardized mean difference was used to calculate the effect size.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

Table 3

Relationship of PTSD symptoms to AUCg in the PTSD group

Variable	<i>B</i>	<i>SE B</i>	<i>R/ R</i> ²	
Model 1: Dependent Variable-AUCg				
Independent Variables:				
Re-experiencing/4F-Intrusions	0.002	0.007	0.05	0.07
Numbing/Avoiding	0.006	0.004	0.21	
Hyper-arousal	−0.002	0.008	−0.03	
Model 2: Dependent Variable-AUCg				
Independent Variables:				
4F-Avoidance	0.03	0.01	0.43*	0.15
4F-Dysphoria	−0.002	0.005	−0.05	
4F-Hyper-arousal	0.003	0.02	0.04	

Note: AUCg = area under the curve in relation to the ground. Multiple linear regression was used for each model in the PTSD group.