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### Recommended Citation

Jayan, Devi; deRoon-Cassini, Terri A; Sauber, Garrett; Hillard, Cecilia J.; and Fitzgerald, Jacklynn M., "A Cluster Analytic Approach to Examining the Role of Cortisol in the Development of Post-Traumatic Stress and Dysphoria in Adult Traumatic Injury Survivors" (2022). *Psychology Faculty Research and Publications*. 532.

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*Psychoneuroendocrinology*, Vol. 135 (January 2022): 105450. [DOI](#). This article is © Elsevier and permission has been granted for this version to appear in [e-Publications@Marquette](#). Elsevier does not grant permission for this article to be further copied/distributed or hosted elsewhere without the express permission from Elsevier.

# A Cluster Analytic Approach to Examining the Role of Cortisol in the Development of Post-Traumatic Stress and Dysphoria in Adult Traumatic Injury Survivors

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## Abstract

Identification of specific risk factors for posttraumatic stress disorder (PTSD) versus depression after trauma has been challenging, in part due to the high comorbidity of these disorders. As exposure to trauma triggers activation of the hypothalamic-pituitary-adrenal (HPA)-axis, examining atypical stress responses via HPA-axis hormones, namely cortisol, may help in the delineation of these disorders. Indeed, extant research demonstrates that, following stress, individuals with chronic PTSD exhibit hypocortisolism (e.g., lower cortisol response than controls), while those with chronic depression exhibit hypercortisolism (e.g., higher response than controls). Less is known about the role of cortisol and these seemingly disparate profiles immediately following traumatic injury as well as whether cortisol can be used as a predictor of future development of PTSD versus depression symptoms. In this study cortisol was measured blood from 172 traumatic injury survivors during hospitalization (on average 2.5 days post-injury). PTSD and depression severity were assessed from Clinician Assessed PTSD Scale (CAPS-5) six-eight months later using a two-factor dimensional approach that measures trauma-specific symptoms of PTSD versus dysphoria (akin to depression). Cluster analysis was used to group individuals based on post-injury cortisol, PTSD, and dysphoria. Results demonstrated that trauma survivors who only developed symptoms of dysphoria at six months (with minimal symptoms of PTSD) were differentiated by high post-injury cortisol compared to other groups. By contrast, individuals who developed symptoms of both PTSD and dysphoria were differentiated by low post-injury cortisol and most severe symptoms of PTSD. Findings provide support for the presence of subgroups of trauma survivors defined, in part, by post-trauma cortisol.

## Keywords

Trauma, Traumatic injury, Cortisol, PTSD, Depression, Dysphoria, Cluster analyses

## 1. Introduction

Approximately 24% of survivors of a traumatic injury are diagnosed with PTSD within six months (Breslau et al., 1991), while upwards of 31% develop depression (Shih et al., 2010). In addition, ~50% of individuals with PTSD also exhibit comorbid depression (Rytwinski et al., 2013) demonstrating substantial co-occurrence of these disorders in this population. Clinically, comorbidity between PTSD and depression is associated with greater PTSD and depression severity, functional impairment, health care utilization, psychological distress, and suicidal ideation (reviewed in Sher, 2005). Considering the high negative clinical consequences of PTSD/depression comorbidity, isolating unique risk factors for individuals who are vulnerable for PTSD, depression, or its comorbidity is important to facilitate preventative interventions.

Traumatic injury is a serious stressor and causes changes in biological responding. In particular, the cortisol response mediated by the hypothalamic-pituitary-adrenal (HPA)-axis is a regulator of psychopathological trajectory in response to stressful life events (Oitzl et al., 2010). The secretion of cortisol is a productive response to stress; however, some individuals may exhibit HPA-axis dysregulation such as heightened (hypercortisolism) or blunted (hypocortisolism) secretion of cortisol after stress (Saxbe, 2008). Therefore, the evaluation of post-trauma cortisol in the development of unique or shared PTSD and depression symptoms is a logical area of investigation to determine how the dysregulation of cortisol is related to developing differential psychopathology. For instance, systematic reviews of cross-sectional research suggest that individuals diagnosed with PTSD have lower cortisol (e.g., hypocortisolism) measured in saliva, urine, and

blood compared to non-traumatized and/or traumatized controls (Morris et al., 2012, Pitman et al., 2012). In adults with PTSD, greater overall severity (Bonne et al., 2003), as well as specific severity of hyperarousal (Gill et al., 2008) and intrusions (Witteveen et al., 2010) negatively correlate with cortisol, highlighting its relationship with more than one symptom domain. Some inconsistencies in this effect do exist in adult trauma survivors, such as evidence of hypercortisolism in PTSD (Inslicht et al., 2006, Steudte et al., 2011) or no differences in cortisol in PTSD compared to controls when comorbidities were not considered (Klaassens et al., 2012, Young and Breslau, 2004). Zimmerman et al. (2020) found sex differences with respect to cortisol reactivity to stress in PTSD in adolescents (i.e., blunted cortisol reactivity in girls vs enhanced reactivity in boys). Despite these inconsistencies, the majority of studies suggest that hypocortisolism may be a biomarker of PTSD and related to severity of the disorder.

In contrast, hypercortisolism is predominantly seen in those with depression (reviewed in Pariante and Lightman, 2008). High comorbidity of depression occurs in a prominent form of hypercortisolism, Cushing's disease, suggesting that elevated cortisol may be mechanistically involved in depression (Pivonello et al., 2015). In fact, treatment is less effective for patients with depression who exhibit elevated cortisol (Fischer et al., 2017) and remission has been linked to reversal of the impairments in the cortisol feedback system (Vythilingam et al., 2004). Duval et al. (2001) found a positive relationship between cortisol levels and depression severity, whereas another study found no relationship between cortisol and depression severity but did find that those with a diagnosis of depression exhibited higher cortisol compared to healthy controls (Oquendo et al., 2003). Review of decades of research suggest that altered cortisol and/or hyperactivity of the HPA-axis is a robust factor predicting biological vulnerability for depression (Rothe et al., 2020).

Despite this, comparatively few studies have investigated cortisol as a *predictor* in the development of PTSD or depression severity separately after trauma. With regard to PTSD, polymorphisms of the glucocorticoid receptor genes that result in low blood cortisol levels have been identified as a significant risk factor for development of PTSD symptoms six months after a traumatic surgery (Hauer et al., 2011). Other studies demonstrate that low urine and blood cortisol within hours of civilian traumatic exposure predicted greater PTSD severity four weeks, six weeks, and six months later (Delahanty et al., 2000, Mouthaan et al., 2014). Ehring et al. (2008) found that lower levels of salivary cortisol measured in the emergency room after motor vehicle accidents (MVA) predicted higher PTSD severity six months later. Some studies have also found a correlation between low urinary cortisol following trauma and development of specific PTSD symptoms such as intrusion and avoidance one month post accident in MVA survivors (Delahanty et al., 2000, Delahanty et al., 2003). One study found a negative correlation between post-trauma salivary cortisol and PTSD severity at six months in adults when saliva was collected in the morning and a positive correlation when saliva was collected in the evening, suggesting that specific mechanisms of cortisol regulation are affected (McFarlane et al., 2011). Alternatively, high post-trauma urinary cortisol has been associated with increased risk of PTSD symptoms in children (Delahanty et al., 2005, Ostrowski et al., 2007). There were no differences in post-trauma cortisol from blood samples between adult civilian trauma survivors who did and did not develop PTSD 5–6 months after trauma (Shalev et al., 2008, Bonne et al., 2003).

Only a few of these studies investigated both PTSD and depression development concurrently. First, despite a negative correlation between post-trauma morning cortisol with developing PTSD, McFarlane et al. (2011) did not find a significant relationship between cortisol and depression. In contrast, Walsh et al. (2013) found a positive relationship between cortisol during a post-sexual assault medical exam and independent symptoms of PTSD and depression six weeks later, such that greater cortisol predicted more symptoms of PTSD *and* depression. Finally, Ehring et al. (2008) found that low cortisol predicted higher symptoms of PTSD six months post-injury, a relationship that was also true for depression. However, none of these studies accounted

for comorbidity between PTSD and depression; thus, discrepant findings (e.g., hypocortisolism associated with depression) may be driven by high comorbid symptoms of PTSD.

More studies are needed that examine cortisol with respect to comorbid PTSD and depression. Of those that have been completed, results suggest that PTSD/depression comorbidity may be characterized by hypercortisolism compared to healthy controls (Pinna et al., 2014, Young and Breslau, 2004). A meta-analysis by Morris et al. (2012) found a time-dependent pattern of cortisol dysregulation in PTSD/MDD comorbidity, such that hypercortisolism was evident in the short-term response to trauma, which switches into hypocortisolism, although the precise number of months for this transition was not reported. Notably, this meta-analysis involved studies with chronic conditions, but highlighted the importance of considering subsyndromal symptom presentations. Further, Morris et al. (2012) found that daily cortisol output was lower in PTSD individuals compared to healthy individuals, but that it did not differ when compared to trauma exposed controls, who themselves may have subsyndromal symptoms of psychopathology. Consequently, more research is needed to examine the relationship between cortisol and symptom severity in trauma-exposed groups using continuous measures.

The current study utilizes a longitudinal, prospective design to investigate the utility of acute blood cortisol measured on an average 2.5 days after a traumatic injury (e.g., “acute post-injury cortisol”) in predicting unique severity of PTSD, depression, and PTSD-depression comorbidity. There are various methods to assay cortisol such as from blood, saliva, urine, and hair samples. While hair sampling may provide a measure of the extended cortisol response following trauma (Sauvé et al., 2007), our goal in the present study was to examine acute HPA axis functioning. Variations in urine output in hospitalized trauma survivors can influence cortisol measurements (Russell et al., 2012) and salivary cortisol measurements may be impacted by blood contamination (Malamud and Tabak, 1993) in traumatically injured patients. Therefore, blood cortisol measurements were deemed most appropriate for this study design. Considering the heterogeneity of PTSD and depression highlighted in scientific literature (Galatzer-Levy and Bryant, 2013) a categorical diagnosis of psychopathology may constitute a barrier to understanding neuroendocrine underpinnings of these conditions given the complex interaction between biological mechanisms and the multifarious presentations of these conditions. Therefore, we assessed the severity of PTSD and depression symptoms dimensionally six-eight months after the traumatic injury. For capturing symptoms, we utilized evidence that the gold standard assessment tool for PTSD, the Clinician-Administered PTSD Scale; CAPS-5 (Weathers et al., 2013) measures two distinct post-traumatic factors: PTSD and general post-traumatic dysphoria, akin to depression (Hunt et al., 2018). Although the CAPS-5 has been validated only using veterans from a limited geographic location (Weathers et al., 2018),

this 2-factor model captured from the single CAPS-5 measure appeared to be the best approach for this study because if we utilized separate self-report measures for PTSD (e.g., PCL-5) and depression (e.g., BDI), then the overlapping mood symptoms in these measures would make it difficult to assess the differential impact of cortisol on PTSD and depression.

A cluster analytic data-driven approach was used to identify unique phenotypes based on post-injury cortisol, severity of PTSD at six-eight months, and severity of dysphoria at six-eight months. To our knowledge, no study to-date has used cluster analyses to examine profiles of post-injury cortisol in conjunction with PTSD and dysphoria severity in traumatic injury survivors.

## 2. Materials and methods

### 2.1. Sample

Traumatic injury survivors were recruited to participate in a prospective longitudinal cohort study at a Midwestern Level 1 trauma center. Traumatic experiences were varied and included forms of non-assaultive

(i.e., motor vehicle crashes) and assaultive traumas (i.e., gunshot, stab wounds). The sample and data are a part of a larger study (Study on Trauma and Resilience, STAR) that investigated PTSD symptom development in trauma survivors. Participants were recruited using a daily trauma census, a real time list of all trauma patients admitted to the trauma service. Subjects were excluded if they: 1) were younger than 18 years of age, 2) experienced traumatic brain injury resulting in > 30 min of peritraumatic amnesia, 3) incurred injuries that resulted in an inability to communicate, 4) had a Glasgow coma score of less than 13 on admission, or 5) were non-English speaking. Consent and enrollment in the study were carried out by trained research associates. Study procedures were approved by the Medical College of Wisconsin Institutional Review Board and participants were monetarily compensated for their time. At the time of hospitalization, information on mechanism of injury (MOI) and injury severity scores (ISS) were derived from the patient's chart and the institution's trauma registry. The ISS (Baker et al., 1974) utilizes a standardized anatomical scoring system to assess trauma severity. Scores range from 0 (no injury) to 75 (fatal injury) with a score of 16 and higher indicating severe injury.

## 2.2. Post-injury self-report measures

At the time of hospitalization, participants completed several self-report measures including the collection of sociodemographic information. Participants also completed the 20-item PTSD Checklist for DSM-5 (PCL-5; Weathers, Litz et al., 2013), which measured the participant's symptoms of PTSD post-injury using self-report severity rating that ranges from 0 (not at all) to 4 (extremely). When used with a cut-score of 30, the PCL-5 has high diagnostic accuracy (94.02%) for patients with CAPS-5 diagnosis of PTSD (sensitivity = 94.37 and specificity = 93.89; Geier et al., 2019). History of psychiatric conditions was assessed as a dichotomous variable; participants responded yes or no to receiving formal diagnosis of various mental health conditions before the trauma exposure (e.g., MDD, Generalized anxiety disorder, PTSD, Personality disorders, Schizophrenia etc.).

## 2.3. Acute post-injury cortisol

Blood samples during hospitalization were collected as early in the patient's hospitalization as possible for cortisol assay (e.g., acute post-injury cortisol). Time of cortisol collection could not be standardized due to the acute nature of injuries in most participants (range of cortisol collection times = 7 am to 4.30 pm,  $M = 11.56$  am). Standardizing time for cortisol collection may have significantly reduced the number of eligible participants due to ongoing complex medical procedures and varied sleep-wake cycles in most participants. After collection, blood samples were immediately transferred to the laboratory where they were centrifuged to separate serum and cells. Serum was immediately frozen and stored at  $-80^{\circ}\text{C}$  until cortisol measurement assay. Plasma cortisol levels were determined using commercially available radioimmunoassay (RIA) kits. The sensitivity for cortisol assay was 57.5 pg/mL and no data lower than the minimum detection level were found. Based on manufacturer's reporting, intra-assay precision varies from 7.3 to 10.5 and inter-assay precision varies from 8.6 to 13.4 for high-to-low cortisol levels.

## 2.4. Measures of PTSD and depression at six months

Six months following the trauma exposure participants completed the CAPS-5 with respect to the index trauma that resulted in the participant's hospitalization. The CAPS-5 was administered by graduate- and postdoctoral-level mental health professionals with interrater reliability ( $\kappa = 1.00$ ) in perfect agreement at the level of diagnosis for approximately 10% of the interviews (for continuous measures). In previous studies, CAPS-5 total severity score has demonstrated high internal consistency ( $\alpha = 0.88$ ) and interrater reliability (ICC = 0.91) as well as good test-retest reliability (ICC = 0.78; Weathers et al., 2018). The CAPS-5 includes 30-item questionnaires that corresponds to DSM-5 symptoms of PTSD and yields a PTSD symptom severity continuous score (based on symptom frequency and intensity) and a categorical rating of PTSD. The severity rating for each item assessed ranges from 0 (no symptoms) to 4 (extremely high symptom endorsement). The common approach is to

calculate symptom severity scores by adding individual severity scores for the 20 DSM-5 PTSD symptoms with a possible total score of 80. In the current study, based on the 2-factor model for PTSD, CAPS items were categorized into PTSD symptoms and dysphoria symptoms following standard, published guidelines (Hunt et al., 2018).

## 2.5. Statistical analyses

All statistical analyses were carried out using the SPSS statistical software package, version 26 (IBM, 2019). A 2-step cluster analysis can be used to separate data with unknown features even when the appropriate number of groups into which the data can be categorized is unclear (Romesburg, 2004). Using a cluster analysis, a large dataset can be divided into sub-groups with homogeneous data points clustered within a subgroup while ensuring heterogeneity between subgroups. With the option to choose different algorithms for clustering the subgroups, cluster analysis is a useful data analytical tool for examining the structural organization of a dataset. Clustering can be carried out using hierarchical or non-hierarchical methods (e.g., K-means cluster analysis; Landau and Chis Ster, 2010; Romesburg, 2004). We followed a two-step sequential cluster analytic approach (i.e., a hierarchical cluster analysis followed by K-means analysis), which is a common approach that has been described and used in other studies assessing psychiatric symptom profiles of PTSD (Runyon et al., 2014). The K-means cluster analytic approach in SPSS requires the number of clusters to be specified. The 2-step approach is useful since using the hierarchical clustering helps to determine the number of clusters that is appropriate for the data. First, z-transformed post-injury cortisol, six-month PTSD severity, and six-month dysphoria severity were entered into the cluster model. An agglomerative, hierarchical cluster analysis was initially carried out to determine the number of clusters with Ward's clustering method (criterion for choosing the clusters) and squared Euclidean distances for the proximities matrix (i.e., quantitative measure for distance between cases); studies comparing different clustering algorithms have identified Ward's method to be superior to others methods (Overall et al., 1993). The Elbow method was used as a selection algorithm for the number of clusters. While there is no rule of thumb for sample size in cluster analyses (Siddiqui, 2013), some researchers recommend a sample size that is 70 times the number of variables in the cluster model (Dolnicar et al., 2014). Our sample size ( $N = 169$ ) is lower than this recommendation ( $n = 70 * 3 = 210$ ). In post-hoc analyses, we explored between-subgroup differences in demographic and additional clinical factors using univariate ANOVAs and  $\chi^2$  analyses, where appropriate. Main effects were followed up with Bonferroni corrected post-hoc comparisons. As previous studies have insufficiently assessed predictive utility of cortisol for the development of *comorbid* PTSD and depression, no directional hypotheses were formed. Statistical significance was examined using an alpha value of 0.05.

## 3. Results

### 3.1. Participants

Two-hundred and seventy-eight participants consented to participate and completed post-injury measures on average 2.6 days (range = 0–15 days) after trauma exposure. Of these, 172 participants completed follow up measures on average 6.3 months (range = 5.1–9.4 months) after injury reflecting a retention rate (e.g., 62%) that is comparable to similar studies (Martin-Herz et al., 2012). For the retained participants, the average time elapsed between trauma exposure and collection of post-injury measures was 2.5 days (range = 0–10). Individuals who were lost to follow-up did not differ in race, gender, MOI, ISS or post-injury symptoms of PTSD; however, these individuals were younger in age ( $M = 35.1$ ,  $SD = 12.78$ ) compared to those who were retained ( $M = 42.79$ ,  $SD = 16.54$ ;  $t(262.13) = -4.32$ ,  $p < 0.001$ ). In addition, retained participants exhibited higher post-injury cortisol ( $M = 3.44$ ,  $SD = 1.23$ ) compared to those lost to follow-up ( $M = 3.12$ ,  $SD = 1.13$ ;  $t(271) = -2.11$ ,  $p < 0.05$ ). Of the participants who were retained for follow-up analyses, cortisol at post-injury was not available for two participants. Additionally, for one of the participants, the cortisol measurement was

abnormally high (70.98 µg/dL), qualified by 6 standard deviations above the mean suggesting a possible measurement error and was therefore subsequently removed from the analyses. This left a final  $N = 169$  of available participants for data analysis.

The mean total CAPS-5 PTSD score at six-eight months ( $N = 169$ ) was  $M = 13.6$  ( $SD = 15.31$ ; range: 0–62), indicating that, on average, participants exhibited minimal symptoms of PTSD but that there was adequate distribution ranging from absence of symptoms to clinically relevant severity. Mean PCL-5 score at six months was  $M = 20.33$  ( $SD = 20.24$ ; range 0–70), indicating that self-reported severity, on average, was subthreshold (below 30; Geier et al., 2019), but exhibited a diverse range of clinical presentation. With respect to PTSD vs. dysphoria symptoms assessed via CAPS-5 using the 2-factor model, a total score of 40 was possible for each domain. Mean PTSD symptom severity score at six months was  $M = 8.2$  ( $SD = 9.1$ ; range: 0–37), whereas the mean dysphoria symptom severity score was  $M = 5.3$  ( $SD = 6.9$ ; range: 0–29), indicating that on an average, participants exhibited minimal symptoms of PTSD and dysphoria but that there was considerable variability in this manifestation. This distribution is logical and expected considering that majority of trauma survivors exhibit resiliency to post-trauma psychopathology (deRoos-Cassini et al., 2010). Demographics and trauma characteristics for the final sample are presented in Table 1.

Table 1. Demographics, Trauma Characteristics and Psychiatric History ( $N = 169$ ).

Factor		N	%
<b>Gender</b>			
	Male	118	69.82
	Female	51	30.18
<b>Race/Ethnicity</b>			
	White	81	47.92
	Black	73	43.20
	Hispanic or Latino	13	7.69
	American Indian	2	1.18
<b>Mechanisms of Injury</b>			
	Non-Assaultive	121	71.60
	Assaultive	48	28.40
<b>Trauma Exposure type</b>			
	Motor vehicle crash	55	32.54
	Fall	30	17.75
	Gunshot wound	28	16.57
	Stab	17	10.06
	Motorcycle crash	14	8.28
	Pedestrian struck	10	5.92
	Crush Injury	8	4.73
	Recreational injury	3	1.78
	Assault	3	1.78
	Other	1	0.59
<b>Psychiatric history</b>			
	Major Depressive Disorder	20	11.83
	Generalized Anxiety Disorder	7	4.14
	Posttraumatic Stress Disorder	4	2.37
	Bipolar Disorder	9	5.33
	Schizophrenia	6	3.55
	Other	5	3.0



Note: Mechanism of injury was dichotomized as assaultive vs non-assaultive injury and also classified based on different trauma exposure type.

### 3.2. Cluster analyses

A cluster analysis with z-transformed post-injury cortisol, six-month PTSD and six-month dysphoria symptom severity scores was conducted. Ward’s hierarchical algorithms supported a four-cluster model which was further validated by good (average Silhouette = 0.5) silhouette measure of cohesion and separation. On follow-up with K-mean cluster analysis choosing four clusters, the sample was characterized as follows: 57% ( $n = 97$ ) in a Resilient subgroup (mean PTSD and dysphoria values 0.6 SD below the sample mean), 15% ( $n = 25$ ) in a Dysphoria subgroup (dysphoria values 0.08 SD above sample mean and PTSD symptoms 0.05 SD below the mean), 21% ( $n = 36$ ) in a High comorbid subgroup (mean PTSD values 1.0 SD above and mean dysphoria 0.7 SD above the sample mean), and 7% ( $n = 11$ ) in a Severe comorbid subgroup (mean PTSD and dysphoria values 2.0 SD above the sample mean). Table 2 provides the mean symptom severity raw scores for PTSD and dysphoria along with cortisol measurements for each subgroup. The clusters are represented in the Fig. 1.

Table 2. Mean and SD for Cortisol and Symptom Severity for Subgroups Based on Cluster Analysis with PTSD, Dysphoria and Post-injury Cortisol.

	<b>Subgroups</b>			
	<b>Resilient (<math>N = 97</math>)</b>	<b>Dysphoria (<math>N = 25</math>)</b>	<b>High Comorbid. (<math>N = 36</math>)</b>	<b>Severe Comorbid. (<math>N = 11</math>)</b>
<b>Variable</b>	<b>M (SD)</b>	<b>M (SD)</b>	<b>M (SD)</b>	<b>M (SD)</b>
<b>PCL – 5 (Post-injury)</b>	12.96 (12.75)	16.96 (15.01)	28.14 (17.36)	41.18 (22.61)
<b>CAPS PTSD Severity (6 months)</b>	2.85 (3.41)	7.72 (7.80)	17.42 (5.93)	26.91 (7.35)
<b>CAPS Dysphoria Severity (6 Months)</b>	1.36 (2.19)	5.88 (6.25)	10.42 (4.07)	23.0 (4.62)
<b>Cortisol (Post-injury)</b>	11.47 (5.87)	30.48 (6.74)	9.08 (5.25)	6.68 (4.82)
<b>PCL-5 (6-months)</b>	8.27 (9.16)	19.16 (18.03)	41.83 (12.59)	58.04 (10.52)

Note: Dysphoria and PTSD symptom severity scores were calculated from CAPS-5 using 2-factor model; PCL-5 = PTSD Checklist-DSM-5 version; post-injury cortisol = cortisol during hospitalization.

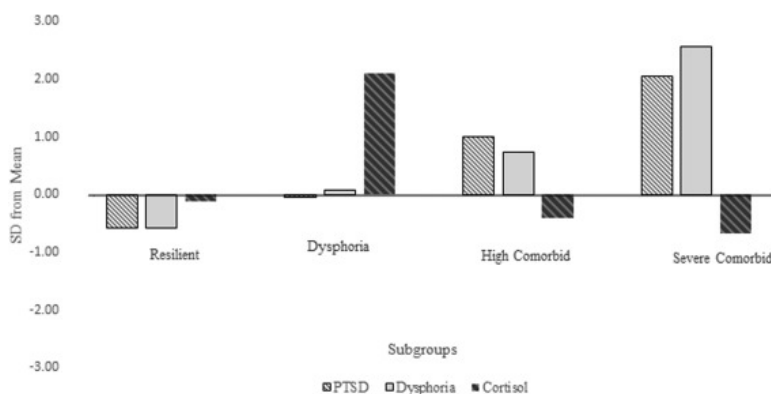


Fig. 1. Graphical representation of the four clusters with respect to SD from the mean. The variations in SDs of six-month PTSD and dysphoria symptom severity and post-injury cortisol are represented. Note: In this figure, 0 represents the mean of the sample.

#### 3.2.1. Between cluster comparisons of cortisol

Prior to analysis, the distribution of cortisol data was assessed for normality and it was found to be positively skewed; thus, it was subsequently square-root transformed. To examine differences between sub-groups based on post-injury cortisol values, a univariate ANOVA was carried out which identified a significant main effect of

subgroup membership ( $F(3, 165) = 52.65, p < 0.001, \eta_p^2 = 0.49$ ). The significant main effect of subgroup membership was retained after controlling for both the number of days between trauma exposure and post-injury blood sample collection and the time day of the blood collection using an ANCOVA by entering these factors as covariates into the previous model ( $F(3, 161) = 50.45, p < 0.001, \eta_p^2 = 0.49$ ).

Bonferroni corrected post-hoc analyses revealed significant differences in post-injury cortisol between multiple clusters. The Severe Comorbid subgroup exhibited significantly lower cortisol than the Resilient ( $MD = -5.31, SE = 1.84, p < 0.05$ ) and Dysphoria ( $MD = -23.72, SE = 2.07, p < 0.001$ ) groups. Notably, the Dysphoria group exhibited significantly higher cortisol than the Resilient ( $MD = 18.41, SE = 1.31, p < 0.001$ ), High Comorbid ( $MD = 20.83, SE = 1.51, p < 0.001$ ) and Severe Comorbid subgroups (see Fig. 2). The High Comorbid subgroup was not significantly different from the Severe Comorbid ( $p = 0.56$ ) or the Resilient subgroups ( $p = 0.23$ ). There were no significant differences in time of the day cortisol was collected among the different clusters ( $p = 0.46$ ).

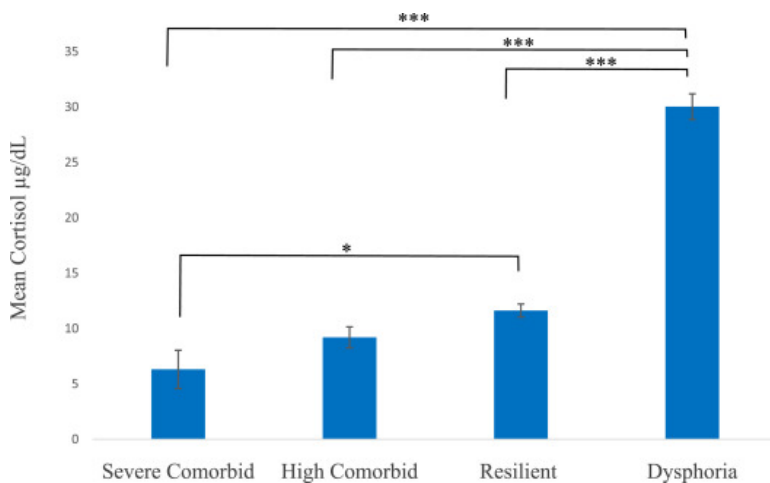


Fig. 2. Differences in cortisol among the four subgroups based on cluster analysis. The Dysphoria subgroup exhibited significantly higher cortisol compared to all the other groups. The Severe comorbid subgroup exhibited significantly lower cortisol compared to the Resilient and Dysphoria subgroups; \* $p < 0.05$ , \*\*\* $p < 0.001$ . Error bars represent 95% confidence interval.

### 3.2.2. Between cluster comparisons of PTSD and dysphoria symptom severity

Symptom severity scores for PTSD and Dysphoria were positively skewed and subsequently log transformed. A Multivariate Analysis of Covariance (MANOVA) examined differences in PTSD and Dysphoria symptom severity across the subgroups and identified a significant main effect of subgroup membership ( $F(6, 328) = 66.72, p < 0.001, \eta_p^2 = 0.55$ ). Bonferroni corrected post-hoc analyses revealed significant differences in symptom severity scores between all the clusters for symptoms of Dysphoria. The Severe Comorbid subgroup exhibited significantly more severe symptoms of dysphoria than the Resilient, Dysphoria, and High Comorbid subgroups. The High Comorbid subgroup had significantly more severe symptoms of dysphoria than the Resilient and Dysphoria subgroups. Finally, the Dysphoria subgroup had significantly more severe symptoms of dysphoria than the Resilient subgroup.

For PTSD symptoms severity, the Severe Comorbid subgroup exhibited significantly more severe symptoms of PTSD than the Resilient and Dysphoria subgroups, but not from High Comorbid group. The High comorbid subgroup showed significantly more severe symptoms of PTSD than the Resilient and Dysphoria subgroups. The Dysphoria subgroup indicated more symptom severity for PTSD than the Resilient subgroup. Mean differences and standard errors for PTSD and dysphoria symptom severity between the subgroups are listed in Table 3.

Table 3. Mean Differences between the subgroups.

	Subgroups	Resilient MD (SE)	Dysphoria MD (SE)	High comorbid MD (SE)
<b>PTSD Symptom Severity</b>	Severe Comorbid	24.06 (1.63)***	19.19 (1.86)***	9.49 (1.77)
	Dysphoria	4.87 (1.15)**		-9.70 (1.34)***
	High Comorbid	14.57 (1.0)***	9.70 (1.34)***	
<b>Dysphoria Symptom Severity</b>	Severe comorbid	21.64 (1.16)***	17.12 (1.32)***	12.58 (1.26)*
	Dysphoria	4.52 (0.82)***		-4.54 (0.95)***
	High Comorbid	9.06 (0.71)***	4.54 (0.95)***	

Note: Dysphoria and PTSD symptom severity scores were calculated from CAPS-5 using 2-factor model; MD = Mean Difference, SE = Standard Error; \*  $p = 0.01$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

### 3.3. Post-hoc analyses

Differences between subgroups in demographics were further tested with parametric analyses (ANOVA) for continuous variables and nonparametric analyses ( $\chi^2$ ) for categorical variables. Results demonstrated that subgroups differed in psychiatric history ( $\chi^2 (3) = 9.55, p = 0.02$ ). Specifically, the Severe Comorbid group (where 64% of individuals reported positive psychiatric history) differed from all other groups (25% in Resilient group [ $\chi^2 (1) = 7.3, p = 0.01$ ]; 16% in the Dysphoria group [ $\chi^2 (1) = 8.17, p = 0.004$ ]; and 28% in the High Comorbid group [ $\chi^2 (1) = 4.69, p = 0.03$ ]). There were also significant differences in subgroup membership based on gender ( $\chi^2 (3) = 9.1, p = 0.03$ ), such that the High Comorbid subgroup comprised of 50% females, which was significantly higher compared to 20% in the Dysphoria group ( $\chi^2 (1) = 5.65, p = 0.02$ ), and 25% in the Resilient group ( $\chi^2 (1) = 7.75, p = 0.01$ ), but not from the 36% in the Severe Comorbid group ( $\chi^2 (1) = 6.29, p = 0.25$ ). No other significant differences in demographics or trauma characteristics were found based on subgroup membership. MOI indicated a trending relationship with subgroup membership ( $p = 0.06$ ) with greater number of assaultive trauma survivors in the Severe Comorbid subgroup (55%) compared to Resilient (23%), Dysphoria (24%), and High Comorbid (39%) subgroup. Race was not included in the analysis due to very small number of counts for various races in the subgroups.

## 4. Discussion

The central aim of the current study was to extend earlier findings by assessing whether a heterogeneous group of traumatic injury survivors could be meaningfully differentiated by acute post-injury cortisol as well as the development of symptoms of PTSD and dysphoria. Using a cluster analytic model involving acute post-injury cortisol from blood and symptom severity scores of PTSD and dysphoria at six months, we found four distinct sub-groups. Results showed that 57% ( $n = 97$ ) of our sample were Resilient, based on very minimal symptoms of PTSD and dysphoria six months after injury, 15% ( $n = 25$ ) had a profile of mildly elevated dysphoria and low PTSD symptoms (Dysphoria), 21% ( $n = 36$ ) had a profile of high comorbid PTSD and dysphoric symptoms (High Comorbid), and 7% ( $n = 11$ ) had a profile of severe comorbid PTSD and dysphoric symptoms (Severe Comorbid). Subgroups differed in acute post-injury cortisol, such that the Dysphoria subgroup had significantly greater cortisol compared to all other groups. In contrast, the Severe Comorbid subgroup exhibited significantly lower cortisol compared to the Dysphoria and Resilient subgroups. The High Comorbid subgroup exhibited cortisol measurements that fell in between the Severe Comorbid and Resilient subgroup but was not significantly different from either. In post-hoc analyses it was also determined that sub-groups differed on demographic and clinical characteristics. Specifically, while the High and Severe Comorbid groups did not differ with respect to severity of PTSD symptoms, individuals in the Severe Comorbid group were more likely to have premorbid positive psychiatric histories. In addition, the comorbid groups were more likely to be comprised of female participants compared to the Dysphoric and Resilient subgroups.

Based on prior literature, we expected to observe reduced post-injury cortisol (i.e., hypocortisolism; Ehring et al., 2008) in PTSD and elevated post-injury cortisol (i.e., hypercortisolism; Wichers et al., 2008) in dysphoria. Our results demonstrating high acute post-injury cortisol in individuals from the Dysphoria subgroup confirms our hypothesis and extends prior literature. In particular, this result is in agreement with studies that found elevated cortisol associated with biological vulnerability for depression (Vreeburg et al., 2009, Wichers et al., 2008). Although we did not find evidence of a “pure” PTSD-only subgroup in this sample, we did find that the comorbid subgroups exhibited both the greatest PTSD severity and the lowest cortisol levels. This points to the possibility that severity of PTSD symptoms is associated with lower levels of post-injury cortisol. These findings align with research that associates low post-trauma cortisol with the development of PTSD (Delahanty et al., 2000, Ehring et al., 2008). To note, we observed this relationship specifically for PTSD/depression comorbid subgroups, while the comorbidity of these disorders is often disregarded in literature. Prior studies on cortisol’s role in the development of depression that have found null effects (McFarlane et al., 2011) or evidence of hypocortisolism in this population (Ehring et al., 2008) may benefit from re-examination of the presence of comorbid symptoms of PTSD in these samples.

It is notable that the Dysphoria subgroup exhibited mild symptoms of dysphoria whereas highest symptom severity of dysphoria was exhibited by the Severe Comorbid subgroup. In fact, both the Severe and High Comorbid subgroups presented with more severe symptoms of dysphoria than the Dysphoria subgroup. Yet, the Dysphoria subgroup was considered “depression-specific” owing to a few major considerations: a) this subgroup exhibited significantly lower symptoms of PTSD compared to both the comorbid subgroups, suggesting the presence of dysphoria in the absence of PTSD, and b) this subgroup exhibited significantly more symptoms of dysphoria compared to the Resilient subgroup. That the Dysphoric group exhibited high cortisol therefore suggests that high cortisol may be a feature of the development of dysphoric symptoms in the absence of the development of PTSD symptoms. Nevertheless, high cortisol may be a feature of depression and not necessarily driven by lack of PTSD symptomology per se, as the Resilient subgroup exhibited the lowest PTSD severity, while their cortisol values were significantly lower than the Dysphoric subgroup. That is, the lack of PTSD symptoms is not enough on its own to explain these results and suggests that here there may be a dysphoric-specific elevation in cortisol. It is also noteworthy that the Resilient and Dysphoria subgroups were only differentiated by acute post-injury cortisol and there were no differences in other demographic variables between these two groups. This highlights the importance of measuring post-injury hypercortisolism in the prospective development of dysphoria, and perhaps depression.

The exact pathway for the relationship between high cortisol and dysphoria is not clear from existing literature, but there are several theories. These existing theories do not necessarily specify the direction of relationship between cortisol and dysphoria, but they deepen our understanding of potential underlying mechanisms that establish a relationship between the two. Elevated cortisol-induced functional changes in neuronal regions (e.g., hippocampus and prefrontal cortex) in combination with alterations of cortisol-induced cognitive and emotional responses to stressful situations may predispose individuals to depression (Gold et al., 2002). According to the glucocorticoid cascade hypothesis (Sapolsky et al., 1986), excessive exposure of the hippocampus to cortisol can lead to hippocampal neuronal damage which further reduces the cortisol feedback inhibition in the HPA-axis resulting in hippocampal atrophy, which is commonly seen in depression (reviewed in Frodl and O’Keane, 2013). Some researchers suggest that elevated cortisol induced depression may be a result of impairments in the glucocorticoid receptors whereas hippocampal atrophy may be a result of chronic depression (Anacker et al., 2011). With evidence that HPA-mediated neuroendocrine hyperactivity is a vulnerability for depression in trauma survivors, future studies may continue to examine the neurological underpinnings for this relationship. Researchers have proposed that low cortisol influences cognitive processes such as traumatic memory consolidation and retrieval to increase risk for PTSD (Dominique, 2007, Yehuda et al., 1997). The relationship between low cortisol and increased susceptibility in PTSD to develop comorbid

depression may be also explained by psychosocial factors associated with low cortisol in trauma survivors. Mason et al. (2001) showed that low cortisol in PTSD is associated with maladaptive coping strategies (i.e., disengagement strategies) and post-trauma depression. Since the above-cited study was carried out in individuals with chronic PTSD, it is unclear if low cortisol plays a cause or effect role in the development of this unique presentation of depressive symptoms.

Contrary to DSM-5 categorical diagnosis of PTSD, this study assessed the severity of core PTSD symptoms versus dysphoria symptoms (as a marker of depression) dimensionally (Hunt et al., 2018), rather than predicting diagnoses. Since longstanding functional impairments are seen in trauma victims who do not fulfill all DSM symptom criteria (Cukor et al., 2010), we considered that subsyndromal PTSD and dysphoria symptoms may be correlated with biological mechanisms and overall psychological health and well-being. The 2-factor approach allowed for a trans-diagnostic assessment of symptoms of PTSD and dysphoria along a continuum and encourages the possibility that symptoms of PTSD such as re-experiencing, avoidance, and hyperarousal may be present in varying degrees in different individuals and can co-occur with depression. Using this approach, the present study is the first to assess the relationship between acute post-injury cortisol and prospective symptoms of the 2-factor model of PTSD and dysphoric symptoms.

The increased comorbidity between symptoms of PTSD and dysphoria in this study is in agreement with the shared relationship between PTSD and PTSD/depression comorbidity proposed by O'Donnell et al. (2004). Although the Dysphoria subgroup may not represent individuals with a depressive disorder, the recurring comorbidity observed in the subgroups may be attributed to shared risk factors between PTSD and PTSD-depression comorbidity (e.g., hypocortisolism) as opposed to the Dysphoria subgroup, which carries its own unique risk factors (e.g., hypercortisolism). Indeed, our results fit with that of Morris et al. (2012), who found hypocortisolism in those with both PTSD and depression. Demographic and psychiatric history differences among our subgroups also fit with prior literature. The Severe comorbid subgroup was more likely to have a prior psychiatric history (64%) compared to all other subgroups, including the high comorbid subgroup (28%). Although not examining comorbid presentations, previous studies have identified psychiatric history as a predisposing factor for the development of PTSD (Powers et al., 2014) and the present results suggest that greater severity of PTSD and dysphoria is uniquely predicted by premorbid history. The exact pathway by which psychiatric history increases severity of PTSD-depression comorbidity is unclear. Some possibilities are shared risk factors (McMillen et al., 2002) or enhanced susceptibility to increased severity for subsequent mental health conditions in individuals with psychiatric history due to social (e.g., low social support) and economic circumstances (Powers et al., 2014). The high incidence of PTSD/depression comorbidity for females in our study aligns with previous longitudinal research and may be a result of the bidirectional relationship shared between PTSD and depression specifically seen in females (Horesh et al., 2017).

Results from this study have several clinical implications. First, the study highlights the important role of measuring cortisol post-trauma for examining the development of symptoms of PTSD and dysphoria. Second, results from this study underscore that individuals with previous history of mental health conditions are at elevated risk for developing more severe symptoms of PTSD *and* depression. Psychiatric history has long been documented as a risk factor for PTSD (Ozer et al., 2003), yet understanding that in particular psychiatric history is related to more severe and comorbid pathology is an important clinical distinction. Considering that PTSD and depression comorbidity is associated with various psychological and functional impairments, early identification of risk factors for this population are especially useful. These individuals may require specialized treatments involving additional focus on the symptoms of depression above and beyond what is included in standard modules for PTSD treatments. Similarly, the relationship that high cortisol predicts even mild symptoms of dysphoria can provide scaffolding for future clinical research focusing on interventions that are channeled specifically to address post-trauma depression. Finally, this study shows that it is important to

consider which patients are labeled as “resilient” and to consider the potential impact of clustering individuals with low PTSD, but more severe depressive symptoms, in this group. In this study, the Dysphoria subgroup presented with low symptoms of PTSD on standard clinical PTSD symptom assessment measures at six months, which is indicative of individuals who are often grouped together with resilient controls in research and clinical work. Noting that these individuals exhibited significantly higher cortisol compared to the Resilient subgroup, such clustering may be problematic and potentially skew outcomes, particularly when examining cortisol’s relationship to developing psychopathology.

The present study has some noteworthy limitations. While the inclusion of several different trauma types increases the generalizability of our findings, such variability may obscure vulnerabilities that are unique to specific trauma types. Moreover, with the ongoing debate about the appropriate model for post-traumatic disorders, it would be beneficial to examine current hypotheses using other models of PTSD in addition to the 2-factor model used in this study. Similarly, although there were no significant differences in time of cortisol collection between our subgroups, a single measurement of blood cortisol with limited standardized time frame for sample collection may be affected by stressors unrelated to the trauma and circadian patterns, thereby making it less informative regarding the overall HPA response specific to trauma. Blood cortisol assay appeared appropriate for this study considering our goal of assessing acute cortisol response to trauma. With more research and controlled measurements acute blood cortisol has the potential to be a clinical biomarker for post-trauma psychopathology, yet future studies should consider other means of cortisol assessment (e.g., hair samples assay) to discern long-term cortisol sequelae of trauma exposure and related stressors. While the sample size of this study is large for a study of post-trauma risk factors, the numbers are low for machine learning approaches like cluster analysis and resulted in small sample sizes in some subgroups. Lastly, we tried to capture depression that is unique to trauma exposure, as has been hypothesized that dysphoric symptoms as we have measured them here may reflect consequences of resource loss following trauma (Hunt et al., 2018). However, trauma survivors are also exposed to several everyday life stressors that are not related to the traumatic event. Therefore, the presentation of depression in trauma survivors may not be limited to what is addressed in our study and attempts to unify – or separate – post trauma depression and PTSD based on our available data may be an over-simplified approach. More should be done to understand nuanced and complex post-traumatic psychiatric symptoms.

Despite these limitations we provide compelling evidence for the relationship between acute post-injury cortisol and divergent psychopathological development following traumatic injury. Although there is extensive research on the role of neuroendocrine stress responses in the development of post-traumatic psychopathology, this study is among the few that examined acute post-trauma cortisol while also accounting for the heterogeneity and comorbidity of resulting PTSD and depression symptoms in a single study design. Moreover, we add to the literature that assesses the relationship between physiological factors and PTSD in a relatively rare study sample, similar to other studies and national archives of datasets (e.g., ICCP, PACT) that have also recruited acutely hospitalized individuals following a trauma exposure.” The study highlights two major findings related to the neuroendocrine risk factors for depression and PTSD symptom severity. First, we provide evidence that in trauma survivors, development of symptoms of depression is heightened in individuals who exhibit post-trauma hypercortisolism. Second, the study emphasizes that the hypocortisolism commonly seen in PTSD is not just a characteristic feature condition, but also a potential risk marker for symptom development, particularly in individuals who go on to develop severe PTSD/depression comorbidity. Similarly, the relationship that low cortisol shares with severe symptoms of PTSD and comorbid depression suggest that it may be promising for future clinical research to focus on preventative pharmacological interventions immediately after trauma exposure (e.g., hydrocortisone administration to trauma survivors, see Sijbrandij et al., 2015 for a detailed review). Finally, although cortisol may not single-handedly dictate development of psychopathology in trauma survivors, this study indicates the potential re-entry of acute post-trauma cortisol to the growing literature of

early screening tools for risk predictors. Considering the impairments associated with comorbid PTSD and depression, identification of early intervention plans targeting the HPA pathway is a potential area for continued research.

## Funding

This research was supported by National Institute of Mental Health (NIMH) R21MH102838 (PIs: deRoon-Cassini, Hillard).

## Declaration of interests

CJH is a member of the Scientific Advisory Boards of Phytects, Inc and Formulate Biosciences and has equity in Formulate Biosciences.

## Submission declaration and verification

This work has not been published previously and is not under consideration for publication elsewhere.

## Ethics approval

All study procedures were approved by the Medical College of Wisconsin Institutional Review Board.

## Consent to participate

Informed consent was acquired from all participants prior to initiation of study procedures, and it was emphasized that participants could withdraw at any time without penalty.

## Consent for publication

All authors have provided consent for publication of the manuscript in its current form.

## Data availability

All data generated or analyzed during this study are available upon request by contacting the corresponding author.

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