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Katarina Bajic Bartel March 27, 2017

C-Reactive Protein as a Potential Risk Factor for Post-Traumatic Stress Disorder Symptoms and Diagnosis: A Prospective Study

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

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Abstract

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By Katarina Bajic Bartel

Background: Post-traumatic stress disorder (PTSD) is a debilitating psychological disorder with potentially severe psychological, physiological, and community-wide impacts. Physiological co-morbidities with PTSD have led to research showing that heightened inflammation is associated with PTSD symptoms and diagnosis. While it has previously been demonstrated that PTSD can dysregulate the hypothalamic-pituitary-adrenal (HPA) axis and lead to heightened inflammation, more recent findings show that heightened baseline inflammation, specifically heightened levels of the pro-inflammatory marker C-reactive protein (CRP), may also contribute to PTSD symptom development.

Methods: Participants were recruited from the Marcus Trauma Center at Grady Memorial Hospital in Atlanta, GA. During enrollment, blood samples were taken from participants for blood plasma CRP concentration determination. As well, participants underwent various psychological evaluations. All participants were invited to follow-up assessments every three months for the year following enrollment. Psychological assessments were repeated during follow-up appointments to track PTSD symptom progression.

Results: No significant effect of CRP level (high vs. low) was found for any measure of PTSD outcome (total symptoms, symptom clusters, diagnosis, and trajectory).

Conclusion: The present study undertakes one of the first prospective explorations of inflammation and PTSD in a civilian population. Compared to a similar prospective study examining the relationship between CRP levels and PTSD outcomes, the current sample is extremely limited by extensive demographic variation. Moreover, the present study is limited by a small enrollment sample size and low retention rate throughout the year post enrollment. Further investigation within the ongoing project should re-examine the effect of CRP levels on PTSD outcomes. With larger sample sizes, future analysis will be better able to account for population variability and will yield a more generalizable result.

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Acknowledgements

I would like to thank Dr. Jovanovic for allowing me to conduct research in her laboratory at Emory University.

I would like to give special recognition and thanks to Dr. Michopoulous for allowing me to assist with her research. I am so grateful to have worked on a fascinating project with an incredibly dedicated and supportive advisor. Your invaluable mentorship and guidance have been immeasurably appreciated.

I would like to thank all members of the Grady Trauma Project for welcoming me onto their team and for allowing me to participate in their incredible research endeavors.

I would like to thank committee members Dr. McQuaide and Dr. Frenzel for their thoughtful feedback and enthusiastic participation.

I would like to thank my mother, Sylvia Bartel, and my fellow Honors Students at Emory University for their support.

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INTRODUCTION

Post-Traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) is an anxiety disorder caused by exposure to a traumatic event(s) resulting in extreme fear, horror, or helplessness (American Psychiatric Association, 2013). In fact, 7.8% of the population will go on to develop PTSD after a traumatic event, making PTSD the fourth most common psychiatric disorder (Breslau et al. 2004, Keane et al., 2009). In order to meet Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V) qualifications for PTSD, an individual must have been exposed to, witnessed, or have been confronted with actual or threatened death, serious injury, or sexual violence (American Psychiatric Association, 2013). A closely related anxiety disorder, acute stress disorder (ASD) may precede the onset of PTSD. ASD has the same symptomology of PTSD, except it is diagnosed within one month of trauma exposure (American Psychiatric Association, 2013). Often, many ASD and PTSD symptoms presenting soon after a trauma resolve within one month and do not result in a diagnosis. However, individuals who maintain symptomatic one-month post trauma may receive a diagnosis of ASD and are at a greater risk for subsequently developing PTSD (Bryant and Harvey, 1997).

Three main symptom clusters characterize PTSD; re-experiencing trauma (Intrusion symptoms), avoiding trauma-related stimuli (Avoidance symptoms), and psychophysiological alterations post-trauma (Hyperarousal symptoms) [American Psychiatric Association, 2013]. Symptoms from each of these three categories are required for a PTSD diagnosis. Intrusion symptoms include recurrent and distressing memories, dreams, flashbacks, psychological distress, and physiologic reactions to reminders of the traumatic event (American Psychiatric Association, 2013). Avoidance symptoms include both internal and external avoidance;

avoidance of thoughts feelings and memories associated with the traumatic event, as well as avoidance of external reminders of the trauma such as people, places, situations, and activities (American Psychiatric Association, 2013). Psychophysiological alterations include negative emotional states, negative beliefs about one's self, feelings of detachment and isolation, inability to experience positive emotion, irritability, restlessness, self-destructive behavior, hypervigilance, heightened startle response, and sleep disturbance (American Psychiatric Association, 2013). To meet a PTSD diagnosis, these symptoms must cause significant impairment of important areas of functioning such as occupational and interpersonal functioning (American Psychiatric Association, 2013). A diagnosis of PTSD consists of exposure to a significant traumatic event leading to a diverse array of all-encompassing psychophysiological symptoms that ultimately result in significant external functional impairment.

PTSD Risk, Prevalence, and Comorbidity

Not all individuals who experience trauma go on to develop PTSD. In one of the first studies to examine the risk of PTSD by considering a complete account of civilian trauma experience, Breslau et al. (1996) explored why certain people might be more vulnerable to PTSD after experiencing trauma. Since previous research regarding civilian PTSD risk has only focused on the correlation between PTSD and perceived worst trauma, this work was able to consider PTSD vulnerability more objectively than previous studies by employing an approach unbiased to the participants' perceived severity of trauma. Breslau et al. (1996) studied a representative sample of randomly selected people in the Detroit metropolitan area, ranging from 18 to 45 years of age. Participants were interviewed to evaluate their exposure to traumatic events as well as to determine PTSD symptomology. Breslau et al. (1996) found that 89.6% of their sample experienced at least one traumatic event in their lifetime. Moreover, women were

found to have a significantly greater risk of developing PTSD post-trauma than men (13.0% vs. 9.2%) [Breslau et al., 1996]. As well, assault violence traumas were correlated with the greatest risk of PTSD (20.9%) [Breslau et al. 1996].

In a similar investigation, Kessler et al. (1995) corroborate that while a large percentage of the population is likely to experience a traumatic event, a significantly smaller proportion will subsequently develop PTSD. Using the National Comorbidity Survey, Kessler et al. (1995) found lifetime prevalence of PTSD in the United States to be 7.8%, and that more than one third of people that have, or have had, PTSD will fail to recover for several years. In a later study, Kessler (2000) found that PTSD increases the risk of dropping out of high school or college in addition to increasing the risk of suicide completion by 6 fold. Moreover, these authors determined that the repercussions of PTSD symptoms in their population indicate in an annual loss of productivity of about three billion dollars per year in the United States (Kessler, 2000). A more recent nationwide epidemiological study reports similar findings. Goldstein et al. (2016) found that 68.6% of people have experienced at least one potentially traumatic event. Similar to the findings of Breslau et al. (1996), which found assault violence traumas to be correlated with a greater risk of developing PTSD, sexual abuse was determined to be the most commonly endorsed traumatic event among those diagnosed with PTSD (Goldstein et al., 2016).

Goldstein et al. (2016) also found high co-morbidity between PTSD and many other physical and psychological issues. Significant psychological co-morbidities were found between PTSD and substance use disorders, mood disorders, and personality disorders with the strongest significant correlations between PTSD and borderline and schizotypal personality disorders (Goldstein et al., 2016). Similarly, significant physiological co-morbidities have been extensively associated with PTSD. Cardiovascular disease, diabetes, gastrointestinal disease, fibromyalgia,

chronic fatigue syndrome, and musculoskeletal disorders are among those found to be associated with PTSD (Boscarino et al., 2004). Since PTSD is associated with predominantly neuroendocrine and immune alterations, inflammation is thought to play a role in many of these physiological co-morbidities (Boscarino et al., 2004). For example, prospective studies of Vietnam veterans found PTSD to be a significant risk factor for developing heart disease as well as a significant predictor of heart disease mortality (Boscarino et al., 2008, Vaccarino, 2013). It is clear that PTSD is a complicated, nuanced disorder with the potential to be severely debilitating to both effected individuals, as well as larger communities. As previous research has brought to light patterns associated with PTSD risk, prevalence, and outcomes, the present study aims to contribute to the growing body of research by elucidating the biological mechanisms of increased risk for the development of PTSD symptoms in the aftermath of trauma.

The present study investigates PTSD symptoms and diagnoses in an inner-city, predominantly African American, impoverished community in Atlanta, GA. This population has been studied extensively in one of the first large scale investigations of civilian specific trauma and PTSD (Jovanovic et al., 2005, Gillespie et al., 2009, Gapen et al., 2011, Donley et al., 2012, Powers et al., 2015). Previously, Gillespie et al. (2009) found that individuals of low socioeconomic status (SES) were at high risk of exposure to traumatic events in this population. Additionally, a 46.2% lifetime prevalence of PTSD was determined, a frequency significantly higher than the predetermined national lifetime prevalence of 7.8% (Gillespie et al., 2009, Kessler et al 1995). As was found in the National Comorbidity Survey, Gillespie et al. (2009) found that females are significantly more likely than males to develop PTSD in their population (Kessler et al., 1995). Moreover, adult and childhood trauma exposure were both found to be a significant predictor of adult PTSD (Gillespie et al., 2009). Since the population of interest is

clearly at high risk for both trauma exposure and PTSD diagnosis, a goal of the present study is to achieve a deeper understanding of the risk factors for PTSD in order to reveal the underlying biological mechanisms at play. Specifically, the high risk nature of this population provides a unique opportunity to investigate the physiological components of PTSD and how environmental experiences and biological vulnerability factors impact psychological well-being following trauma exposure.

Stress Sensitization and The Psychophysiology of PTSD

Stress sensitization

Considering the symptoms of PTSD and ASD, heightened stress sensitization is a significant component of the psychophysiological manifestation of trauma-related psychological disorders (American Psychiatric Association, 2013). Defined as an increased reactivity to stressful stimuli caused by exposure to trauma, stress sensitization is a key component of PTSD symptoms (Smid et al., 2014). Since the majority of people who experience a traumatic event do not develop PTSD, heightened stress sensitization is likely the result of impaired stress coping mechanisms (Yehuda, 2009). In a study of combat exposed soldiers, Smid et al. (2014) found that immune reactivity mediated the impact of stressful life events post-deployment on those reporting high exposure to combat trauma. In other words, stressful life events occurring post-trauma impacted the progression of PTSD symptomology when accompanied by a heightened immune response to the stressors (Smid et al. 2014). This suggests that stress sensitization is mediated by the immune system and that dysregulation of the immune response to stress is likely the biological mechanism underlying the impaired ability to cope with trauma that causes certain individuals to develop PTSD. Since only a fraction of the people who experience trauma will go on to develop PTSD, these results support that PTSD is the result of impaired recovery due to a

dysfunctional stress coping mechanism, rather than a natural response to trauma exposure (Breslau et al. 2004, Goldstein et al., 2016, Yehuda, 2009). The present study takes a prospective approach at examining the relationship between immune system dysregulation and PTSD symptom development in the aftermath of trauma exposure.

Fear potentiated startle (FPS) and transfer of fear inhibition

An exaggerated startle response has been widely researched as a physiological measure of PTSD symptom severity and is thought to be associated with the hyperarousal symptoms of PTSD via stress sensitization. To investigate exaggerated startle, Jovanovic et al. (2005) utilized a fear conditioning paradigm. Derived from a conditional discrimination procedure previously used in animals, they adapted a paradigm capable of assessing fear potentiation and fear inhibition in humans. They used an unpleasant blast of air to the larynx to condition participants to associate various shapes with either danger or safety. Subsequent test trials were then used to measure the intensity of participants' startle response to sound bursts in the presence of the safety and danger signals comparatively (Jovanovic et al., 2005). In their initial study of a healthy population (lacking PTSD diagnoses), two key findings elucidated the nature of a healthy fear response. First, they observed significantly stronger startle responses to a sound burst when accompanied by the danger signal as compared to the safety signal; in other words, the danger signal potentiated participants' fear response (Jovanovic et al., 2005). In addition to a significant fear potentiated startle (FPS), they also observed significant transfer of fear inhibition (Jovanovic et al., 2005). More specifically, the conditioned danger signal prompted an increased fear response to the sounds burst, the danger and safety signal prevented this increase when presented together (Jovanovic et al., 2005). These results show that psychologically healthy individuals demonstrate a transfer of fear inhibition; a learned safety signal can prevent a conditioned fear

response in the presence of a learned danger signal. Understanding FPS and fear inhibition is crucial to learning more about the psychopathology of PTSD as these mechanisms reveal how experiences with various types of stressful stimuli impact how individuals respond to subsequent stressful stimuli.

Ultimately, the fear conditioning paradigm adapted by Jovanovic et al. (2005) was intended to compare FPS in individuals who either had or had not experienced trauma and either had or had not developed PTSD. Thus, subsequent studies have applied this paradigm to learn more about the underlying biological mechanisms of PTSD symptoms. In order to understand the overactive fear response characteristic of PTSD symptoms, work has been done to examine fear inhibition in PTSD versus healthy controls. Jovanovic et al. (2009) studied participants from the Veterans' Affairs (VA) medical center in Atlanta, GA being treated for PTSD and found that those with a history of PTSD, but with low current symptoms, demonstrated significant transfer of fear inhibition, as did healthy participants in their previous study (Jovanovic et al., 2009, Jovanovic et al., 2005). However, participants with severe PTSD symptoms demonstrated significantly greater fear potentiation and significantly lesser transfer of fear inhibition (Jovanovic et al., 2009). More specifically, an impaired transfer of fear inhibition was most strongly correlated with the Intrusion and Avoidance PTSD symptom clusters (Jovanovic et al. 2009). Since these symptom clusters have to do with exaggerated fear related arousal, the same biological mechanism that underlies an impaired transfer of fear inhibition may also be responsible for exaggerating arousal in response to trauma reminders.

This fear potentiation paradigm was then used in one of the first studies to examine the effect of time since trauma on psychophysiological measures such as startle and fear inhibition. Jovanovic et al. (2013) studied a Croatian population comparing fear inhibition in groups of

people with either ASD or PTSD. The ASD (30 days or less since trauma) and PTSD (10 or more years since trauma) groups both demonstrated significant FPS responses as compared to healthy controls, however, neither group demonstrated a transfer of fear inhibition. Interestingly, these two symptomatic groups were able to correctly assess for safety, even while an exaggerated startle response indicated that safety was not learned. Jovanovic et al. (2013) hypothesized that the PTSD group would be more likely to demonstrate impaired fear inhibition, assuming that the biological mechanisms of PTSD would require time to develop such impairments. However, rather than impaired fear inhibition being developed over time, they found that startle magnitude was highest in the ASD group (Jovanovic et al., 2013).

Hypothalamic-pituitary-adrenal (HPA) axis and PTSD

The HPA axis is one of the primary hormone systems responsible for the physiological response to stress. Since stress has been shown to impact memory formation and consolidation, these systems are thought to play a role in the biological mechanism of PTSD symptomology, specifically via glucocorticoids and catecholamines (Pace and Heim, 2010). There is a lot of variability in findings relating cortisol levels and PTSD diagnosis (Pace and Heim, 2010). Increased cerebrospinal levels of corticotrophin releasing hormone (CRH) have been found to be associated with PTSD, whereas decreased peripheral levels of cortisol have been found to be associated with PTSD (Bremner et al., 1997, Wessa and Rohleder, 2007). While the specific nature of HPA axis dysregulation in PTSD remains to be unclear, the mechanism is thought to involve glucocorticoid receptors (GRs) [Yehuda, 2009]. The HPA axis may be dysregulated in PTSD via GR hypersensitivity, possibly due to an increased number of GRs or altered receptor binding properties in PTSD (Jovanovic et al., 2011). Dexamethasone suppression has been used to examine the effects of reduced cortisol release by blocking GRs (Jovanovic et al., 2011). In

the first study to examine the effect of a dexamethasone suppression test on FPS, Jovanovic et al. (2011) found that dexamethasone administration reduced the exaggerated FPS previously observed in patients with PTSD, supporting the role of HPA dysregulation in PTSD pathophysiology via GR sensitivity (Jovanovic et al., 2009, Jovanovic et al., 2011).

Glucocorticoids and memory formation

Glucocorticoids and catecholamines are two major stress hormones thought to play a role in the biological mechanism of PTSD symptoms (Pace and Heim, 2010). Specifically, previous research has extensively investigated the role of glucocorticoids in memory formation and consolidation (Buchanan and Lovallo, 2001, Buchanan et al., 2006, Andreano and Cahill, 2006). In a study of the impact of exogenous cortisol on emotional memory formation, Buchanan and Lovallo (2001) administered either cortisol or a placebo to participants prior to showing them images of varying emotionality. They found that cortisol administration specifically enhanced long-term recall of the more emotionally arousing images. In a later study, Buchanan et al. (2006) extended these findings, examining the relationship between stress, endogenous cortisol, and memory retrieval. Subjects were exposed to a stressor after learning various words (Buchanan et al. 2006). Comparing word recall to measured cortisol release post stress exposure, they found greater cortisol release to be associated with significantly worse memory test performance (Buchanan et al., 2006). Andreano and Cahill (2006) used a similar paradigm to further specify the relationship between cortisol and memory formation, revealing a quadratic relationship between endogenous, stress-induced cortisol release and performance on a memory test (post stress exposure). The highest and lowest measured cortisol spikes in response to a stressor, were associated with the poorest recall, whereas the relatively moderate cortisol spikes were associated with the best recall (Andreano and Cahill, 2006). Since the Intrusion and

Avoidance symptoms of PTSD are marked by dysfunctional reactions to trauma reminders, the relationship between cortisol and memory formation are likely involved in the biological mechanism of PTSD symptoms. Moreover, since cortisol release is also associated with biological stress response, it is likely that cortisol plays a role the relationship between memory and stress sensitization that is characteristic of PTSD

The immune system, C-reactive protein (CRP), and PTSD

Considering the research showing that immune cell GR sensitivity is altered in PTSD, an altered GR sensitivity in immune cells may also influence the pathophysiology of PTSD (Yehuda et al., 2012, Rohleder et al., 2007). Recent research suggests that inflammation may be involved in the biological basis of stress sensitization, potentially predisposing individuals to PTSD after a traumatic event (Neigh and Ali, 2016). Cytokines and interferons are pro-inflammatory markers that act as key signaling molecules in the periphery as well as the central nervous system (Passos et al., 2015). In the first meta-analyses elucidating the role of pro-inflammatory markers in PTSD, Passos et al. (2015) found levels of interleukin 1β, interleukin 6, and interferon γ to be elevated in individuals with PTSD. It has also been shown that heightened levels of CRP, a systemic biomarker of the pro-inflammatory response to stress and injury, are also associated with PTSD symptomology (Spitzer et al., 2010, Miller et al., 2001). Additionally, Michopoulos et al. (2015) found high CRP levels to be positively associated with greater PTSD symptoms as well as a higher likelihood of PTSD diagnosis in traumatized individuals. It is thought that the high comorbidity observed between inflammatory disease and cardiovascular disease with PTSD is linked with the hypothesized inflammation-stress sensitization mechanism (Boscarino, 1997).

FPS and fear inhibition have been used as biomarkers for the hyper-vigilance and exaggerated startle that characterize PTSD. In regards to inflammation as a predictor of PTSD, CRP has been shown to be associated with impaired inhibition of FPS in the presence of a safety signal (Michopoulos et al., 2015). More specifically, evidence reveals a genetic contributor to heightened CRP that has been linked to increased PTSD risk (Michopoulos et al., 2015). Michopoulos et al. (2015) examined the *CRP* gene in an inner-city, primarily African American, and low-socioeconomic population (the population of interest in the present study), finding genetic variation in the *CRP* gene to correlate with increased hyper-vigilance and elevated CRP blood plasma levels. They found one single nucleotide polymorphism (SNP) in the *CRP* gene that was significantly associated with elevated blood plasma CRP as well as PTSD symptomology. This genetic locus was especially associated with individuals being overly alert, implicating the role of this SNP in hyper-vigilance and stress sensitization via influencing blood plasma CRP concentrations. Moreover, this *CRP* SNP is specifically thought to mediate PTSD severity via increased inflammation (Michopoulos et al., 2015). These findings suggest a genetically based vulnerability, or potential risk factor, for developing PTSD in the event that an individual experiences trauma. Considering that not everyone who experiences trauma develops PTSD, the *CRP* SNP may contribute to a genetically based risk that makes some individuals more vulnerable or more resilient to PTSD. Considering the established relationship between CRP and physiological PTSD co-morbidities (such as heart disease), as well as the association of CRP and PTSD symptoms, the present study chose CRP as an indicator of inflammation and potential prospective risk factor for PTSD (Harvard Health Publications, 2009).

To date, most of the work assessing the association between inflammation and PTSD has been cross-sectional in nature (Spitzer et al., 2010, Miller et al., 2001). However, a recent

prospective study reported that elevated basal CRP is associated with increased prospective risk for PTSD development in the aftermath of trauma exposure (Eraly et al., 2014). Eraly et al. (2014) measured the severity of PTSD symptoms experienced by marines before deployment, as well as three- and six-months after deployment. Their results showed that elevated CRP levels before deployment were associated with increased likelihood of developing PTSD symptoms post deployment. Notably, CRP levels proved to be a predictor of the presence versus the absence of symptoms, rather than a predictor of the extent of symptoms (Eraly et al., 2014). While high concentrations of peripheral CRP seem to indicate an individual's vulnerability to developing PTSD symptoms, other factors, such as trauma type, probably determine symptom severity (Eraly et al., 2014).

Considering Eraly et al. (2014) studied a healthy, military cohort comprised of young males, a replication of this finding would strengthen the generalizability of their result. Thus, the goal of the current project was to conduct a prospective examination of whether blood plasma CRP levels immediately post trauma predict the development of PTSD symptoms one-, three-, six-, and twelve-months post trauma. We hypothesized that elevated blood plasma CRP concentrations immediately post trauma would significantly predict PTSD symptom severity in the aftermath of trauma. Additionally, we predicted to observe a positive association between heightened blood plasma CRP concentration at the time of enrollment and greater odds for a PTSD diagnosis one-, three-, six-, and twelve-months post trauma. Upon examining PTSD trajectories of our participants for one-year post trauma, we also expected to find a significant association between high blood plasma CRP levels and chronic PTSD outcomes.

METHODS

Participants

Participants (N=287) were recruited from the Marcus Trauma Center of Grady Memorial Hospital in Atlanta, GA. To qualify for this study, participants must have experienced a criterion A trauma within the last 6 hours, as defined by DSM-VI, during which the patient experienced, witnessed, or was confronted with an event that involved actual or threatened death or serious injury, or a threat to the physical integrity of the patient or others (American Psychiatric Association, 2000). Moreover, the patient's response to the event must have involved intense fear, helplessness, or horror (American Psychiatric Association, 2000). All qualifying participants were between 18 and 65 years old, understood and spoke English proficiently, were able to provide informed consent, and were contactable by phone to schedule follow-up appointments. Patients were excluded from this study if they reported prominent suicidal ideation within the past month or a current or past history of psychosis including mania or schizophrenia. As per the Glasgow Coma Scale (GCS), patients who were not alert, oriented, and coherent, or who were presently intoxicated, were excluded from this study. Moreover, patients experiencing severe pain, active labor, respiratory distress, medical instability, hemodynamic complications, or who were anticipating immediate admission or surgery were excluded from this study. All procedures were reviewed and approved by the Emory Institutional Review Board and the Grady Hospital Research Oversight Committee.

Procedures & Measures

After initial medical evaluation and clearance, eligible patients were approached and offered the opportunity to participate in the present study. After receiving informed consent from the prospective participant, trained enrollment assessors collected information regarding

demographics, history of prior traumas, substance abuse, smoking, medical and psychiatric history, current and past depression and PTSD symptoms, and details regarding their most recent trauma. Measures administered included the Standardized Trauma Interview (STI) (Foa and Rothbaum, 1998), PTSD Diagnostic Scale (PDS) (Foa et al., 1997), and Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 1994). At the time of enrollment, a blood sample was obtained for assessment of CRP concentrations. One, three, six and twelve months after enrollment, participants were invited to return for follow-up assessment sessions. Lasting 60-90 minutes, these follow-up assessments measured PTSD and depression symptoms and diagnoses using the modified PTSD Symptom Scale (mPSS) and the Beck Depression Inventory (BDI) (Foa et al., 1993, Beck et al., 1996). An mPSS score of 21 or greater was considered indicative of a PTSD diagnosis (Foa et al., 1993). Participants were compensated $50 for each follow-up assessment.

CRP Assays and Concentrations

CRP concentrations were derived from participant blood samples taken in the Marcus Trauma Center within three hours after trauma exposure. Until the time of CRP assay, blood plasma samples were stored at negative eighty degrees Celsius. An immunoturbidometric assay from Sekisui Diagnostics (Lexington, MA) was used to measure blood plasma CRP concentration on the Beckman AU480 chemistry analyzer, with an interassay coefficient of variation (CV) of 5.2% and an intra-assay CV of 3.1%. Individuals with blood plasma CRP concentrations of 20 mg/L or greater were excluded from analysis as levels of this magnitude indicate an acute immune response not indicative of baseline peripheral inflammation (Michopoulos et al., 2015). Blood plasma CRP concentrations averaged 2.975 mg/L (SD=0.2376, range: 0.0109 – 18.9400 mg/L). A CRP concentration cutoff point of 3mg/L was

used to differentiate individuals with high (≥3 mg/L) versus low (<3 mg/L) blood plasma CRP concentrations in subsequent data analysis. According to the American Heart Association, a blood plasma CRP concentration of 3 mg/L or higher is indicative of high peripheral inflammation and is a significant risk factor for cardiovascular disease (Harvard Health Publications, 2009). Additionally, previous work showing an association between low grade peripheral inflammation, as indicated by blood plasma CRP concentration, defined an inflammatory experimental group as all participants having blood plasma CRP concentrations of 3mg/L or greater (Spitzer et al., 2010, Michopoulos et al., 2015).

Statistical Analysis

For analysis of the relationship between CRP level and PTSD outcomes, a categorical CRP variable (high vs. low) was used throughout, as described above. To examine differences in continuous outcome variables, including demographic information and PTSD symptom severity, at one-, three-, six-, and twelve-months post-trauma between participants who enrolled with elevated versus non-elevated baseline blood plasma CRP, analysis of variance (ANOVA) was used. Similarly, the effect of CRP (high vs. low) on individual PTSD symptom cluster severity was analyzed with ANOVA tests. Chi-square analysis, logistic regression, and the calculation of odds ratios and 95% confidence intervals [OR(95%CI)] were used to further assess the differences between participants who presented with elevated basal blood plasma CRP concentrations at the time of enrollment versus those who did not, on categorical demographic variables as well as a categorical variable of PTSD diagnosis. As well, logistic regression was used to determine the effect of CRP levels on PTSD trajectory. PTSD trajectory (Chronic, Recovery, or Resilient) was determined by a colleague for all participants who attended at least two follow-up assessments (at one-, three-, six-, or twelve-months post trauma). Using latent

growth mixture modeling (LGMM), participants were assigned to either a Recovery, Resilience, or Chronic PTSD outcome group (Kloet et al., 2009). Qualifications for each trajectory group were determined based on criteria specified in previous work. Individuals in the Recovery group initially exhibited relatively high PTSD symptom severity, with symptom severity decreasing throughout the study (Galatzer-Levy et al., 2017). Individuals in the Resilience group maintained relatively low symptom severity throughout the study and individuals in the Chronic PTSD group demonstrated relatively high PTSD symptom severity throughout the study (Galatzer-Levy et al., 2017). This data analysis was completed using RStudio (v.1.0.136) and alpha level was set at $p \leq 0.05$ for statistical significance.

RESULTS

Demographic Characteristics

A total of 489 patients were recruited from the Marcus Trauma Center and enrolled in the present study. Of this initial sample, 202 were excluded from analysis for either not attending any follow-up assessments, lacking blood plasma CRP concentration data, or having a baseline blood plasma CRP concentration of 20 mg/L or higher. The remaining 287 participants averaged 36 years old and consisted of slightly more females than males (51.23% vs. 48.78%) [Table 1]. Additionally, this sample was predominantly African American (71.13%) with the most common educational level being completion of some college (36.93%) [Table 1]. About half of our sample reported a monthly household income of $2,000 or more and just under half of participants reported prescription medication use (Table 1, Table 2). As well, 69.02% of participants reported current tobacco use (Table 2). The average BMI in our sample was 28.69 and the average baseline blood plasma CRP concentration was 2.975 mg/L (Table 2). Additionally, the average baseline AUDIT-C, BDI, and PSS scores did not meet criteria for the associated diagnoses (Table 2). The average CTQ score was 42.31, indicating high childhood trauma exposure, and an assessment of baseline trauma (experienced pre-enrollment) revealed an average STI score of 2.826 (Table 2). For most participants (57.14%), a motor vehicle crash (MVC) was their most recent trauma prior to enrollment (Table 3). Only 47 participants (16.38%) experienced interpersonal violence prior to enrollment, and of these individuals, 8 had experienced intimate partner violence (Table 3).

CRP Level and PTSD Outcomes

When analyzing the effect of CRP levels on each continuous demographic variable, the only significant effect found was between BMI and CRP level (high vs. low), where BMI levels

were significantly higher among individuals with high CRP levels (p=8.85e-12, r=50.73). Similarly, examination of the effect of CRP level on each categorical demographic variable revealed only one significant effect; high CRP levels were associated with female sex (x-squared=10.302, df=1, p=0.001). Due to these significant effects, sex and BMI were controlled for as covariates in all subsequent analyses. Baseline trauma was also controlled for as a covariate. Even though no significant effect of CRP level on baseline trauma was determined, all trauma experienced prior to enrollment would likely influence subsequent PTSD outcomes (ANOVA, p=0.747, correlation = 0.12). Since the present study is concerned with PTSD outcomes due to the most recent trauma at the time of enrollment, controlling for baseline trauma was essential (Eraly et al., 2014).

Analysis revealed no significant effect of CRP on total PTSD symptom severity at any time point post trauma; all p-values are >0.05 (one-month: 0.850, three-months: 0.407, six-months: 0.322, twelve-months: 0.317) [Figure 1]. Since no significant effect of CRP on total PTSD symptom severity was determined, the effect of CRP level (high vs. low) on the severity of individual symptom clusters (Intrusive, Avoidance, and Hyperarousal) was examined. No significant effects of CRP level (high vs. low) were determined for any of the symptom clusters; all p-values are >0.05 (Figure 2). Similarly, no significant effect was found of CRP (high vs. low) on PTSD diagnosis for any time point post trauma (one-month: 0.999, three-months: 0.494, six-months: 0.197, twelve-months: 0.165) [Figure 3]. As well, no significant effect of CRP level on PTSD trajectory throughout the study was found (p=0.842) [Figure 4].

DISCUSSION

PTSD is a heterogeneous disorder with debilitating physical, psychological, and social consequences (American Psychiatric Association, 2013, Kessler, 2000, Goldstein et al., 2016). Considering the crucial role stress hormones play in learning and memory, there is a wide body of evidence linking biological dysregulation to PTSD symptoms (Buchanan and Lovallo, 2001, Buchanan et al., 2006, Andreano and Cahill, 2006). While not fully established, current research demonstrates a substantial role of the HPA axis and the immune system in PTSD (Pace and Heim, 2010). Pro-inflammatory markers, specifically CRP, have been shown to be associated with psychophysiological components of PTSD symptoms such as FPS and inhibition of safety transfer (Michopoulos et al., 2015). Moreover, cross-sectional studies have linked PTSD with elevated peripheral inflammation (Spitzer et al., 2010, Miller et al., 2001). The present study examined an urban population with a heightened vulnerability to trauma exposure and PTSD Gillespie et al., 2009). A prospective study design was used to explore increased CRP as a potential risk factor for developing PTSD, with study enrollment taking place just hours post-trauma. High blood plasma CRP concentrations were hypothesized to be positively associated with PTSD symptoms and diagnosis at one-, three-, six-, and twelve-months post trauma. Similarly, high blood plasma CRP concentrations were hypothesized to be associated with a yearlong trajectory of chronic PTSD post trauma. No significant associations were found between high blood plasma CRP concentrations and PTSD outcomes at any time point post trauma.

Limitations

While these results do not support CRP as a prospective risk factor for PTSD, several limitations of the present study hinder the conclusiveness of this result. Firstly, CRP levels were

measured from blood samples taken within 3 hours of trauma exposure. Since CRP is a systemic marker of inflammation, rather than a primary reactor to stress exposure, blood plasma CRP concentration should not increase immediately post trauma (Lenz et al., 2007). However, physical injury and trauma exposure are both known to increase cytokines such as IL-6, which may increase blood plasma CRP concentration over time (Lenz et al., 2007, Lemieux et al., 2008, Vidovic et al., 2007). While blood is taken shortly post trauma in the present study, it is uncertain whether or not trauma exposure would increase CRP concentration within hours. As such, the blood plasma CRP concentrations obtained and analyzed in the present study may not reliably indicate baseline peripheral inflammation. Since most research linking PTSD and inflammation has been cross-sectional, this is a potentially significant limitation when it comes to addressing causality (Spitzer et al., 2010, Miller et al., 200). Moreover, the association of trauma exposure and increased inflammation is much more established than the effect of increased inflammation on the development of PTSD in the aftermath of trauma (Lenz et al., 2007, Gill et al., 2008). When an individual experiences a trauma, the HPA axis is activated, enabling the immune system to cope with the stressor and leading to an increase in inflammation (Gill et al., 2008). Soon after, a negative feedback loop is activated, inhibiting the HPA axis so that the body is not exposed to prolonged inflammation (Gill et al., 2008). As such, alterations to the HPA axis in PTSD lead to disinhibition of pro-inflammatory processes, a potential pathophysiological link between PTSD and the associated physical comorbidities (Spitzer et al., 2010). However, more recent research suggests that individuals with higher levels of peripheral inflammation may be at greater risk for PTSD (Early et al., 2015). Investigating this requires a prospective approach that truly captures baseline CRP in order to distinguish between the effect of trauma on inflammation increase, and the effect of inflammation on PTSD development. Even

though CRP should not spike as readily as other indicators of inflammation, it is still unclear whether or not experiencing a trauma would impact CRP levels within hours post trauma (Lenz et al., 2007). As such it is uncertain whether any subsequent PTSD symptoms would be due to heightened baseline trauma or the increase in inflammation that follows a trauma.

Compared to previous work studying CRP as a potential prospective risk factor for PTSD, several aspects of the population of interest pose limitations to the present investigation. Eraly et al. (2015) found CRP to be a prospective risk factor for PTSD in a military population. Comparatively, the civilian sample in the present study entails much greater variability in regards to life style (military vs. civilian), physical fitness, and trauma type. For example, both males and females are included in the present study, as opposed to the all-male military sample (Eraly et al., 2015). Additionally, trauma exposure in the present study encompasses a wide range of events including accidental injury, interpersonal violence, and intimate partner violence, whereas the military population was exposed to only combat related trauma (Eraly et al., 2015). Additionally limiting is the sample size in the present study. Eraly et al. (2015) showed a significant effect of CRP as a prospective risk factor for PTSD with a sample size of 2,548 participants, whereas the present study considered only 287 individuals. A small sample size may impair our statistical power to uncover effects of CRP on PTSD outcomes post trauma. Small sample size also limits the extent to which our dataset can be broken down into subsets in order to account for some of the variability. For example, about half of our sample had experienced an MVC prior to enrollment (Table 3). While limiting our analysis to only those who had experienced an MVC would have reduced the variability of trauma exposure in our sample, this would have drastically reduced the statistical power of our analysis as well as the generalizability of our result. Additionally, since our sample included a lot of variability in sociodemographic characteristics,

there may have not been enough statistical power to see the effect of certain confounding variables. BMI and sex were controlled for due to the significant effect of CRP on these variables, however, with a larger sample size, additional or different covariates may be elucidated. A larger sample size could both reveal additional confounding variables as well as an effect of CRP on PTSD outcomes.

Since the present study employs a prospective design, follow-up assessments during the year post trauma, are essential. While the 287 enrolled participants included in analysis were present for an initial evaluation of PTSD diagnosis and symptoms, not all participants returned for all of the follow-up appointments. In general, attendance decreased for each follow-up appointment throughout the post-trauma year; 232 participants returned one month after enrollment, 201 returned after three months, 174 returned after six months, and only 144 returned after one year (Figure 2). Since only about half of participants returned for all of the follow-up appointments, it is even harder to obtain generalizable results. While it is unlikely that future investigation will obtain 100% attendance at each follow-up assessment, the difference between participants that are and are not attending follow-up appointments needs to be considered. The relationship between PTSD risk and self-selection to participate in follow-up appointments is under investigation in the ongoing project. Generally, individuals in our sample who are exposed to high risk trauma exposure (such as interpersonal violence) and significant past trauma and mental health histories are less likely to return for follow-up appointments (unpublished result). Since high-risk individuals are the most likely to self-select out of post-enrollment participation, the current study is likely observing reduced PTSD symptoms in the follow-up appointments. Thus, the rates of PTSD observed post enrollment may not be representative of the actual PTSD outcomes for our total sample.

Other limitations to the present study include potential confounding variables that were not considered. While we assessed prescription medication use, we did not account for specific medications in our analysis that may impact baseline, systemic inflammation. We also did not account for cardiovascular disease, which could be a confounder because it is known to be associated with heightened inflammation (Harvard Health Publications, 2009). Despite these limitations, the design of the present study enables a more generalizable approach to exploring inflammation as a prospective risk factor for PTSD than has ever been employed. The present study is a prospective design with multiple follow-up assessments allowing for PTSD trajectory to be considered. Moreover, multiple potential confounders were controlled for. Most importantly, this study established a reliable framework for prospectively associating inflammation with PTSD in a high-risk, civilian population that will undoubtedly yield extensive future investigation.

Future Directions

While the nuances of our study sample hinder the generalizability of our result, the present study brings attention to a high need population. As previously described, the ongoing project has extensively studied population samples recruited from Grady Memorial Hospital. Unlike the present study, previous investigations have recruited participants specifically from Grady outpatient clinics, rather than the emergency department. As Grady Memorial Hospital is one of two level-one trauma centers in the greater Atlanta area, the Marcus Trauma Center treats many individuals who are not primary care patients at Grady (Georgia Trauma Commission, 2015). Therefore, it is likely that our sample consists of individuals from a wider range of communities and of broader demographic backgrounds than previous investigation. For instance, Gillespie et al. (2009) found the majority of their participants recruited from outpatient clinics to

be of very low socioeconomic status, reporting 88.1% of their sample to have an average monthly income of fewer than $2,000. In contrast, the present study reports about half of our sample to have an average monthly income of fewer than $2,000 (Table 1).

While the high risk population previously studied is better captured via recruitment from outpatient clinics, emergency department recruitment enables a prospective approach. This population captures highly vulnerable, low-income individuals that are at increased risk for both childhood and adult trauma exposure, putting much of this population at greater risk of developing PTSD (Gillespie et al., 2009). Moreover, this population has a high concentration of disadvantaged neighborhoods with many individuals belonging to communities with high rates of neighborhood disorder, which has been shown to be associated with PTSD (Gapen et al., 2011). Civilian trauma, as well as mental health in low-income communities mostly consisting of ethnic minorities, is severely understudied. In order to focus analysis on specifically the high PTSD risk individuals in this population, the ongoing project could examine the effect of CRP on PTSD outcomes separately for different levels of socioeconomic status. Since much of this population lacks adequate resources and has limited access to intervention, future investigation of the specific subsets of this population will hopefully inform how to better assist communities with high PTSD risk and prevalence, ultimately uncovering how to better mitigate risk, intervene early, and reduce the burden of PTSD on individuals and communities.

To our knowledge, the present study is the first to examine CRP as a potential prospective risk factor for PTSD in a civilian population. As we considered both males and females in our analysis, the present study is also the first to undergo such investigation in a population that includes females. This is important because previous research has demonstrated several differences in the health risk for PTSD in males versus females. In general, females are

about twice as likely as males to develop PTSD (Gillespie et al., 2008, Alim et al., 2006, Kessler et al., 1995). Additionally, females in the population of interest are at higher risks for certain types of trauma exposure. Specifically, females in this population are more likely to report sexual assault and intimate partner violence (Gillespie et al., 2008). This is significant because sexual abuse was determined to be the most commonly endorsed traumatic event among those diagnosed with PTSD (Goldstein et al., 2016, Breslau et al., 1996). Moreover, females are at greater risk for increased inflammation, as they tend to show higher levels of pro-inflammatory markers, on average, as compared to men (Yang and Kozloski, 2010). The present study corroborates these findings as we report a significant association between high CRP levels and female sex ($p=0.001$). Additionally, high CRP levels have been associated with negative psychological outcomes, such as depression, in females (Mathews et al., 2010). The framework of the ongoing project provides useful opportunity to assess sex specific risk factors for PTSD, contributing to a more comprehensive understanding of PTSD risk and resilience. Once a larger body of data has been collected from the ongoing project, it is imperative that the association between CRP and PTSD outcomes be analyzed specifically for differences between males and females.

The present study is part of an ongoing project that continues to enroll new participants. Once a greater number of participants have enrolled and attended follow-up appointments, future investigation should prioritize a reassessment of the relationship between CRP level and PTSD outcome. Additionally, some components of the ongoing project that were not available for analysis in this preliminary study should be investigated in the future. Variation in the *CRP* gene as described by Michopoulos et al. (2015), as well as FPS and inhibition of safety transfer have been associated with blood plasma CRP levels in traumatized individuals. As such, a framework

has been established for expanded exploration of PTSD in this prospective cohort. As it is of the capacity of the ongoing project to assess *CRP* gene variability, future investigation should examine the relationship between PTSD outcome and *CRP* gene variability. Variation in the *CRP* gene (that is associated with elevated blood plasma CRP concentration) could contribute a more reliable indication of baseline CRP level. Since injury and trauma immediately pre-enrollment may alter blood plasma CRP concentrations, the present study makes a limited attempt to measure baseline CRP levels. Therefore, analysis of the *CRP* gene, which is less likely to be impacted within hours post trauma, could enhance the relevance of our result. Also, in the ongoing project, FPS and inhibition of transfer of safety are examined during the three-month follow-up assessment. Future investigation should examine the effect of CRP level on FPS and inhibition of transfer of safety in recently traumatized individuals. While the association of high CRP with FPS and impaired transfer of safety has previously been shown, such an investigation in the ongoing project would uniquely contribute a prospective approach to this association by assessing the effect of baseline CRP levels on FPS and inhibition of transfer of safety throughout the year post trauma (Michopoulos et al., 2015).

The current study undertakes one of the first prospective explorations of inflammation and PTSD in a civilian population. We examined the correlation between the pro-inflammatory marker, CRP and PTSD in an inner-city, low-income population with significant vulnerability to trauma exposure. In a prospective approach, high blood plasma CRP levels were assessed for an effect on PTSD symptoms, diagnosis, and trajectory for one year following a trauma. Additionally, the relationship between CRP level and PTSD trajectory was also assessed for the post-trauma year. No significant associations were found between high blood plasma CRP concentrations and PTSD symptom severity and diagnosis. As the population of interest

experiences high rates of PTSD, future investigation is required in order to gain a deeper understanding of biological risk and resilience in PTSD. While the present study focuses on the immunology of PTSD pathophysiology, the complex, intricate, and severely disabling psychophysiological impairments of PTSD clearly demand further interdisciplinary investigation.

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TABLES AND FIGURES

Table 1. Baseline selected demographic characteristics of the sample for which CRP data was obtained (N=287). Sex, race, education level, marital status, and monthly income breakdown (by percentage), as well as average age, are reported.

Characteristics	No.	Mean (SD or %)
Demographics		
Sex	287	
Male	140	48.78
Female	147	51.23
Age (years)	287	36.09 (0.776)
Ethnicity	287	
Non-Hispanic	271	94.43
Hispanic	16	5.575
Race	284	
European	57	20.07
African American	202	71.13
Asian American	5	1.761
Mixed	11	3.618
Other	9	3.169
Highest educational level	287	
Grammar school	2	0.687
Some high school	45	15.68
High school	75	26.13
Some college	106	36.93
BA, BS, or equivalent	41	14.29
Some graduate school	5	1.742
MA, MS, or equivalent	11	3.833
PhD, MD, or equivalent	2	0.687
Marital status	287	
Single	134	46.69
Married, domestic partner	81	28.22
Co-habitating	28	9.756
Divorced or separated	40	13.94
Widowed	4	1.394
Monthly Income	277	
$0-$249	20	7.220
$250-$449	16	5.776
$500-$999	37	13.36
$1,000-$1,999	65	23.47
$2,000 or more	139	50.18

Table 2. Baseline selected medical and psychological characteristics of the sample for which CRP data was obtained (N=297). Prescription medication and tobacco use breakdown (by percent) and average blood plasma CRP concentration (mg/L), BMI, AUDIT-C score (substance abuse inventory), BDI (depression inventory) score, PTSD symptoms (measured using the PSS), CTQ score (childhood trauma assessment), and baseline trauma (STI score) are reported.

Characteristics	No.	Mean (SD) or %
Demographics		
Prescription medications	251	
Yes	103	41.04
No	148	58.96
Tobacco use	255	
Yes	176	69.02
No	79	30.98
CRP, mg/L	296	2.975 (0.238)
BMI	284	28.69 (0.416)
AUDIT-C score	216	3.833 (0.349)
DAST score	219	1.119 (0.108)
BDI score		
Baseline	279	13.05 (0.666)
One month	232	12.9 (0.698)
Three months	201	10.94 (0.715)
Six months	174	11.09 (0.815)
Twelve months	144	9.812 (0.836)
PTSD		
Baseline	247	10.86 (0.665)
One month	232	15.16 (0.767)
Three months	200	11.73 (0.765)
Six months	174	11.32 (0.861)
Twelve months	144	9.938 (0.870)
CTQ total	250	42.31 (1.253)
Baseline trauma	304	2.826 (0.118)

Table 3. Types of trauma experienced by our sample for which CRP data was obtained (N=296). Trauma types experienced by participants immediately prior to arrival at the Marcus Trauma Center are reported (broken down by percentage).

Characteristics	No.	%
Demographics		
Trauma type	287	
Motor vehicle crash	164	57.14
Other	123	42.86
Interpersonal violence	287	
Yes	47	16.38
No	240	83.62
Intimate partner violence	287	
Yes	8	2.787
No	279	97.21

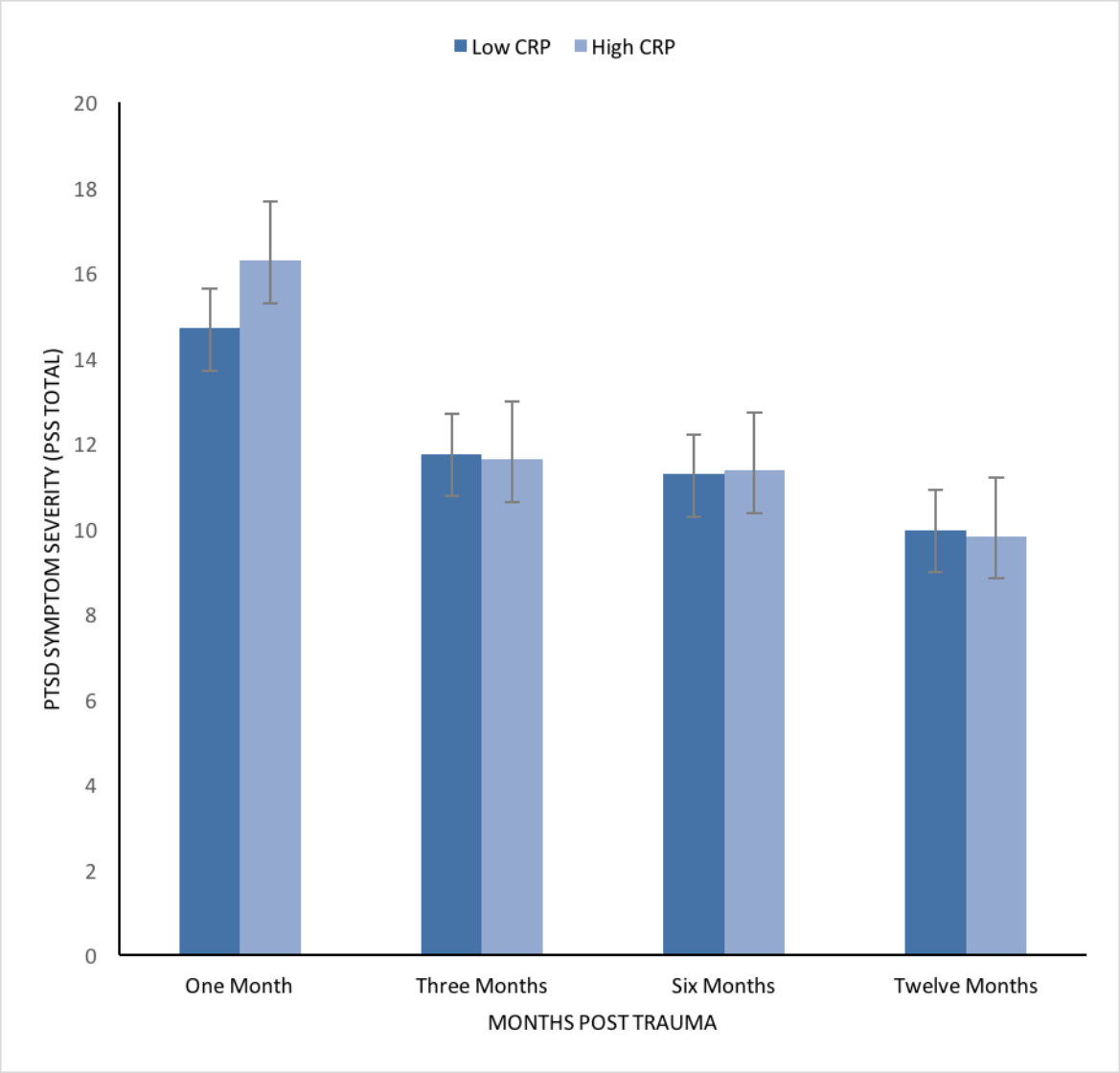
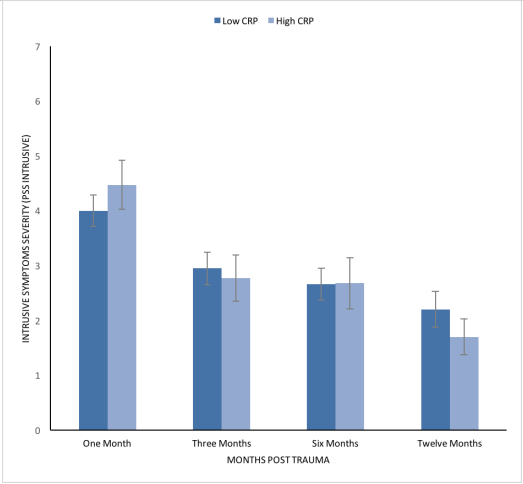
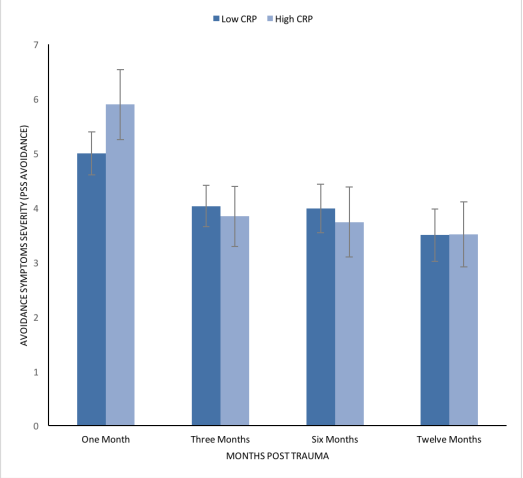


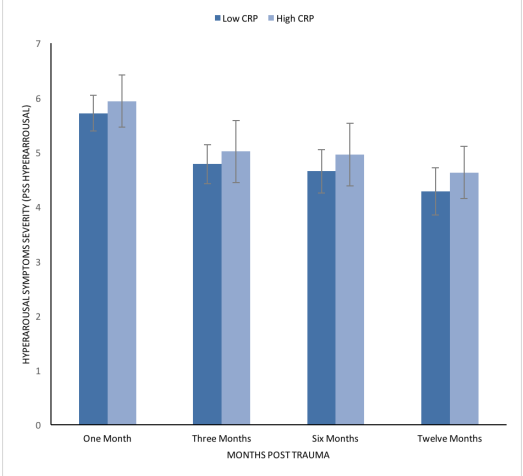
Figure 1. PTSD symptom severity (as indicated by PSS score) at one-, three-, six-, and twelve-months post trauma, broken down by CRP level (high; ≥3mg/L vs. low; <3mg/L, N=296). Error bars indicate standard deviation. No significant correlations were found between PTSD symptom severity (as indicated by PSS score) and high vs. low CRP levels at any of the follow up assessment time points. All p-values are >0.05 (one-month: 0.850, three-months: 0.407, six-months: 0.322, twelve-months: 0.317).



a.) Intrusive symptoms



b.) Avoidance symptoms



c.) Hyperarousal symptoms

Figure 2. PTSD symptom severity for each symptom cluster (as indicated by PSS score) at one-, three-, six-, and twelve-months post trauma, broken down by CRP level (high; ≥3mg/L vs. how; <3mg/L, N=296). Error bars indicate standard deviation. No significant effects of CRP on Intrusive symptoms (a), Avoidance symptoms (b), or Hyperarousal symptoms (c) were found at any of the follow up assessment time points. All p-values are >0.05 (Intrusive: one-month: 0.612, three-months: 0.384, six-months: 0.432, twelve-months: 138. Avoidance: one-month: 0.603, three-months: 0.447, six-months: 0.338, twelve-months: 0.417. Hyperarousal: one-month: 0.454, three-months: 0.572, six-months: 0.349, twelve-months: 0.580).

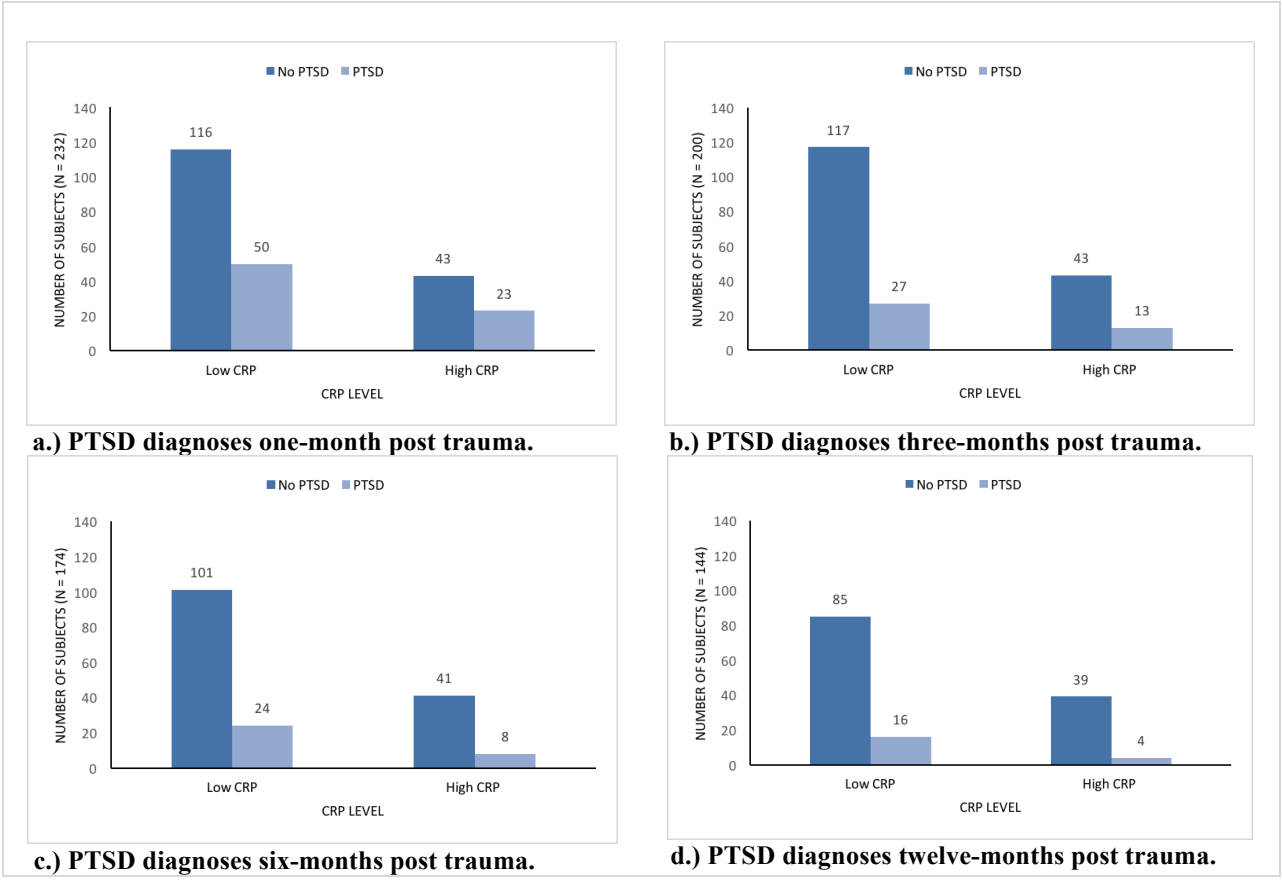


Figure 3. Breakdown of PTSD diagnoses (as determined by PSS score) at one-(a), three-(b), six-(c), and twelve-(d) months post trauma by CRP level (high; ≥3mg/L vs. low; <3mg/L). No significant associations were found between PTSD diagnosis and high vs. low CRP levels at any of the follow up assessment time points. All p-values are >0.05 (one-month: 0.999, three-months: 0.494, six-months: 0.197, twelve-months: 0.165).

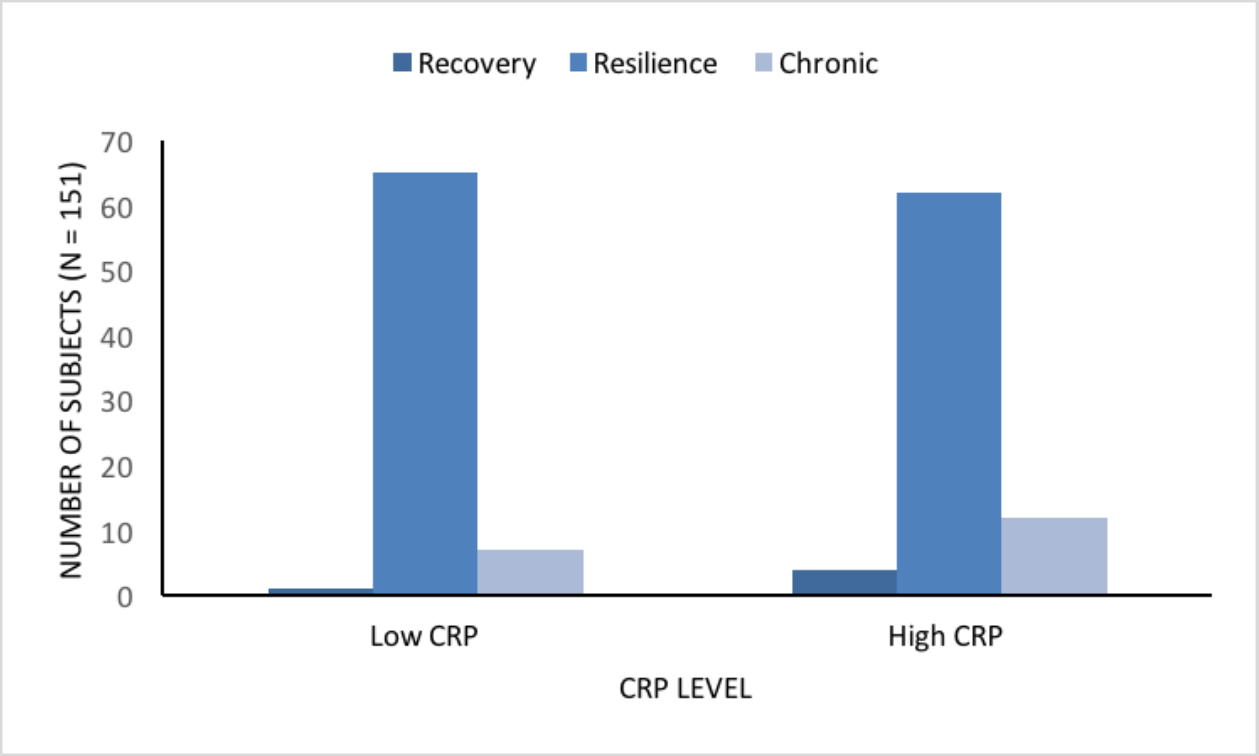


Figure 4. Breakdown of PTSD trajectories (Recovery, Resilience, and Chronic) by CRP level (high; ≥3mg/L vs. how; <3mg/L) for participants who completed at least two follow-up assessments within the year following their enrollment (N=151). PTSD trajectory was determined by a collaborator using latent growth mixture modeling (LGMM). No significant correlations were found between PTSD trajectory and high vs. low CRP levels. The p-value is >0.05 (0.842).