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Preethi Reddi April 14, 2020

A Prospective Investigation of the Associations Between Mild Traumatic Brain Injury, Gonadal Steroid Hormones, and Posttraumatic Stress Disorder

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An abstract of
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of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Department of Biology

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Abstract

A Prospective Investigation of the Associations Between Mild Traumatic Brain Injury, Gonadal Steroid Hormones, and Posttraumatic Stress Disorder
By Preethi Reddi

Posttraumatic stress disorder (PTSD) is a debilitating condition that may develop after experiencing a traumatic event. Mild traumatic brain injury (mTBI) has previously shown to be a risk factor for PTSD. In order to develop better prognostic tools and treatment methods for PTSD, it is important to understand the mechanisms behind the relationship between PTSD and mTBI. Sex differences are prevalent in both PTSD and mTBI, with women more likely to experience worse outcomes from PTSD and mTBI while men are more likely to acquire PTSD and mTBI. Gonadal steroid hormones, such as estradiol, testosterone, and progesterone, may play a role in this relationship. This study aims to (i) further understand the relationship between PTSD and mTBI, (ii) examine the relationship between PTSD symptom clusters and mTBI, and (ii) explore the role of estradiol, testosterone, and progesterone in the relationship between PTSD and mTBI. This study includes 504 participants who presented to the Emergency Department (ED) of Grady Memorial Hospital following a traumatic event. PTSD diagnosis and symptoms were assessed at 1, 3, 6, and 12 months following trauma. Chi-square and Mann Whitney U tests were used to examine the relationship between PTSD and mTBI. Linear regression models were used to understand the use of mTBI, hormones, the interaction between mTBI and hormones, and gender in predicting PTSD symptoms. This study found that those with mTBI showed increased PTSD symptom reporting compared to those without mTBI at 3, 6, and 12 months. Those with mTBI reported increased avoidance symptoms at 3 and 6 months compared to those without mTBI. At 3, 6, and 12 months, those with mTBI reported increased intrusive symptoms compared to those without mTBI. At 3 months, those with mTBI reported increased hyperarousal symptoms compared to those without mTBI. Women reported higher PTSD scores compared to men at all time points. At 6 months, there was a negative association between testosterone levels and PSS scores for those with mTBI and those without mTBI. Our findings have potential to create more efficient prognostic and treatment tools for PTSD.

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Acknowledgements

I would like to thank the Grady Trauma Project for providing me the opportunity to conduct this study. Thank you to the Grady Trauma Project biomarkers study for collecting data related to this study. Thank you to the AURORA project and Sam Vincent, the Emory on-site AURORA study coordinator, for providing me with the training necessary to work on this project. I would like to thank Dr. Nicole Gerardo and Dr. Douglas Terry for agreeing to serve on my thesis committee and providing valuable feedback on this project. Lastly, I would like to thank my thesis advisers, Dr. Jessica Maples-Keller and Dr. Jennifer Stevens for all of their support and guidance in this project.

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Introduction

Posttraumatic Stress Disorder (PTSD) is a debilitating condition that may develop after experiencing war, natural disaster, or any other traumatic event. PTSD symptoms include re-experiencing of the traumatic event, avoidance of threat-related stimuli, negative alterations in cognitions and mood, and alterations in arousal and reactivity.[1] This disorder is characterized by a heightened response to threatening stimuli and insufficient control over heightened sensitivity to threatening stimuli.[2] In adults, 60% of men and 50% of women experience at least one trauma in their lives. However, only about 7-8% of the population will acquire PTSD at some point in their lives[3], demonstrating the importance of investigating PTSD risk and resilience following trauma exposure. In order to better understand and treat PTSD, it is important to identify and analyze factors associated with risk for PTSD following trauma exposure.

The WHO Collaborating Centre for Neurotrauma Task Force on Mild Traumatic Brain Injury (mTBI) provides the following operational definition of mTBI:

> mTBI is an acute brain injury resulting from mechanical energy to the head from external physical forces. Operational criteria for clinical identification include: (i) one or more of the following: confusion or disorientation, loss of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24 hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery; (ii) Glasgow Coma Scale score of 13-15 after 30 minutes post-injury or later upon presentation for health care.

It is estimated that 42 million people worldwide suffer from mTBI every year.[4] Studies have shown that traumatic brain injury of any severity (TBI) may be a risk factor for

PTSD.[5,6] One large study of U.S. Army soldiers was conducted 3 to 4 months after their return from a year-long deployment. This retrospective study showed that 44% of soldiers with mTBI screened positive for PTSD while 16% of soldiers with other injuries screened positive for PTSD.[7] PTSD may also have an association with worse TBI outcomes, including increased reporting of postconcussive symptoms.[7–9] Although studies demonstrate there is an important relationship between PTSD and TBI, the mechanisms behind this relationship have yet to be fully understood. PTSD and TBI share several symptoms, including anxiety, irritability, insomnia, personality changes, and memory problems.[10] Therefore, it is important to explore potential biological overlap between PTSD and TBI.

Sex differences are observed in both PTSD and TBI. While literature shows that men are more likely to experience potentially traumatic events than women,[11,12] studies have consistently shown that women are more likely than men to develop PTSD following a traumatic event.[8,11–13] Similar to findings in PTSD, men have shown to have a higher likelihood of acquiring a TBI while women have shown to have worse outcomes following a TBI. A large meta-analysis of 15 studies including 25,134 adults showed that men had more than twice the odds of having had a TBI compared to women.[14] Studies have shown that women report worse outcomes following TBI compared to men, including worse post-concussion symptoms,[15] higher mortality,[16] and higher percentages of manifesting symptoms, such as loss of consciousness, ear or nose bleeding, seizures, headache, and limb weakness.[16] One study shows that women show elevated PTSD symptoms as assessed by the PTSD checklist for civilians (PCL-C[17]) scores compared to men following TBI.[15] However, the findings in this area have been mixed, for example, a large retrospective study shows that female gender is associated with reduced mortality and decreased

complications following TBI.[18] Sex differences are important to investigate when examining the relationship between PTSD and TBI given their prevalence in both conditions.

Gonadal steroid hormones, such as estrogens, progesterone, and testosterone, may play a role in explaining sex differences and the mechanisms behind the relationship between PTSD and TBI. Estradiol is the primary female sex hormone and is responsible for the development of female reproductive organs and secondary sex characteristics.[19] Progesterone is involved in pregnancy, embryogenesis, and the menstrual cycle.[20] Testosterone is the primary male sex hormone and is involved in the development of male reproductive organs and secondary sex characteristics.[21] Estradiol, the primary estrogen hormone released by the ovaries, is present in higher levels in women compared to men.[22] Therefore, studies have examined the role of estradiol in sex differences in PTSD. In humans, a number of studies found that lower estradiol levels were associated with impaired fear inhibition, a hallmark characteristic of PTSD.[23–27] In TBI, estrogens may be neuroprotective. Animal studies have shown decreased mortality and better functional outcomes following induced TBI in mice and rats receiving estradiol treatment compared to placebo.[28] One animal study found improved neurological outcome in male rats, but worsened neurological outcome in female rats following estradiol administration prior to TBI.[29] In a human study, individuals with severe TBI (sTBI) demonstrated lower estradiol levels compared to healthy controls, potentially reflecting hypogonadism following TBI. In addition, those with higher estradiol to testosterone ratios showed lower mortality and better Glasgow Outcome Scale (GOS) scores, which measures recovery after neurotrauma, 6 months post-injury.[30]

Studies have shown that progesterone may have neuroprotective effects in individuals with TBI while it may exacerbate symptoms of PTSD. In a randomized human subject clinical trial, progesterone treatment, compared to placebo treatment, improved neurologic outcomes for up to 6 months post-injury in patients with a Glasgow Coma Scale (GCS) score of 8 or less following TBI. In addition, the group that received progesterone treatment showed lower mortality than the group that received placebo treatment. Sub-group analysis for women showed significantly favorable outcomes for women receiving progesterone treatment compared to women in the placebo group.[31] While this study showed improved outcomes following progesterone treatment after TBI, other large randomized studies have shown no difference between placebo and progesterone treatment in individuals following TBI.[32,33] In these studies, participants with a GCS of 3 were excluded, which may have resulted in different findings. A systematic review showed that high levels of estradiol and progesterone, present in the luteal phase of the menstrual cycle, were associated with higher re-experiencing symptoms in those with PTSD.[22] Given this research indicating that hormones may play a significant role in both PTSD and TBI, it is important to further understand how hormones impact the relationship between both conditions.

Studies have demonstrated low levels of testosterone following TBI[34,35] and a correlation between increased testosterone levels and increased avoidance symptom scores in those with PTSD.[36] A case study shows that gonadal hormones may play an important role in the relationship between PTSD and TBI. Hypogonadism has been found in patients following TBI.[35] Based on this finding and the overlap between symptoms of hypogonadism and PTSD, a male veteran with PTSD, a history of two concussions, and

initially low levels of testosterone was treated with testosterone supplements. Following treatment, his PTSD symptoms were lessened. In this case, the patient reported improved sleep, less irritability and explosiveness, and improved concentration.[37] While there are limitations to the generalizability of this case study, including the inability to rule out a placebo effect, this study demonstrates the importance of examining the role of gonadal hormones in PTSD and TBI.

While it is clear that there is a relationship between PTSD and TBI, there is a gap in knowledge of the mechanisms behind this relationship. Further, there is a lack of literature following a prospective study design. In order to provide more accurate prognostic tools and to develop more efficient treatment methods in the care of PTSD and TBI, it is important to further explore their co-occurrence. This study aims to understand the mechanisms behind the relationship between PTSD and mTBI by further examining the direction of this association and the roles of sex differences and hormone levels.

Here, we use a prospective study design in a sample of patients presenting to the emergency department (ED) of a level 1 trauma center. Initial psychological assessments and hormone levels were assessed in the peri-traumatic period within hours of index trauma during hospitalization and follow-up assessments of PTSD symptoms occurred 1, 3, 6, and 12 months following traumatic event. Based on previous literature, we hypothesized that there would be a strong relationship between PTSD and mTBI, with those affected by mTBI more likely to develop greater severity of PTSD symptoms than those unaffected by mTBI. We also investigated the relationship between mTBI and PTSD symptom clusters. Lastly, we hypothesized that high levels of gonadal steroid hormones, including estradiol,

progesterone and testosterone, would have a protective effect in mTBI patients, predicting fewer PTSD symptoms in the time following injury.

Materials and Methods

Procedure

Participants were recruited as part of a prospective study in the emergency department (ED) of Grady Memorial Hospital in Atlanta, GA, the largest Level 1 trauma center in GA, USA. For the purpose of this study, DSM-IV criterion was used in order to define a traumatic event.[38] Based on this definition, patients were asked if they had experienced an event in which they thought they (or a family member or friend) would be killed or seriously injured. Patients were eligible for this study if (i) they had experienced a traumatic event in the past 24 hours, (ii) they thought they (or a family member or friend) would be killed or seriously injured during the accident, and (iii) they were alert, oriented, and able to provide informed consent and complete assessment instruments. Patients were excluded if (i) they were non-English speaking, (ii) they had current or past history of mania, schizophrenia, or other psychoses, (iii) they had current (past month) prominent suicidal ideation or recent (past three months) parasuicidal behavior or other self-injurious behavior, or (iv) they were acutely intoxicated or altered to the degree that they could not accurately complete study assessments or participate in study procedures. All participants underwent assessment of trauma exposure and baseline depression and PTSD symptoms by trained research staff in the ED. PTSD diagnosis and symptom severity were assessed at 1-, 3-, 6-, and 12-month post-trauma follow-up assessments using the PTSD Symptom Scale (PSS).[39] A PSS score of 21 or higher was categorized as a PTSD diagnosis. Blood samples were collected from participants upon enrollment. Progesterone, testosterone, and estradiol levels were all measured following blood sampling. Blood was drawn an average of 3.8 hours following trauma (SD=3.9).

In order to classify participants with mTBI, the operational definition provided by the WHO Collaborating Centre for Neurotrauma Task Force on Mild Traumatic Brain Injury was used.[40] Participants were asked the following: "During the event, did you lose consciousness at all? If so, how long (in minutes)? Do you have a memory of the event?"

All study procedures were reviewed and approved by the Emory Institutional Review Board the Grady Hospital Research Oversight Committee. All data were captured and managed using Health Insurance Portability and Accountability Act-compliant, REDCap electronic data capture tools hosted by Emory University.

Participants

Participants (n=504) were 18-65 years of age upon enrollment in this study. The mean age of the sample was 35.3 years (SD=13.0). 46.8% of the participants were women. Self-reported racial/ethnic background of participants was: Black (72.1%), White (19.1%), Asian (1.4%), American Indian or Alaska Native (0.2%), Mixed (4.0%), and other (2.6%). The mean time between trauma and enrollment in this study was 5.1 hours (SD=3.6). The mechanisms for injury were as follows: motor vehicle collision (49.6%), pedestrian vs. automobile collision (8.1%), non-sexual assault (7.5%), sexual assault (7.5%), gunshot wound (6.0%), fall (4.8%), industrial/home accident (4.6%), motorcycle collision (4.4%), bike accident (3.0%), stabbing (2.6%), animal bite/attack (1.0%), fire/burn (0.6%), sports injury (0.2%), and other (0.2%).

All participants in this study had a GCS of 15. For the present study, participants were classified with mTBI based on the presence of loss of consciousness, loss of memory, or both loss of consciousness and loss of memory following the traumatic event. Table 1 describes the participants of this study based on classification with or without mTBI.

Table 1. Demographics for participants classified with or without mTBI.

	mTBI	**No mTBI**
	N (%) or Mean (SD)	N (%) or Mean (SD)
Total	129 (25.6%)	375 (74.4%)
Gender	-	-
Men	73 (56.6%)	194 (51.7%)
Women	56 (43.4%)	179 (47.7%)
Missing	0 (0%)	2 (0.5%)
Ethnicity	-	-
Hispanic/Latino	6 (4.7%)	17 (4.6%)
Not Hispanic/Latino	123 (95.3%)	356 (95.4%)
Missing	0 (0%)	2 (0.5%)
Race	-	-
Black	93 (72.1%)	269 (72.1%)
White	27 (20.9%)	69 (18.4%)
Asian	1 (0.8%)	6 (1.6%)
American Indian or Alaska Native	1 (0.8%)	0 (0%)
Native Hawaiian or Other Pacific Islander	0 (0%)	0 (0%)
Mixed	2 (1.6%)	18 (4.8%)
Other	4 (3.1%)	9 (2.4%)
Missing	1 (0.8%)	2 (0.5%)
Trauma Type	-	-
Non-sexual assault	10 (7.8%)	28 (7.5%)
Motor Vehicle Collision	78 (60.5%)	172 (45.9%)
Motorcycle Collision	5 (3.9%)	17 (4.5%)
Pedestrian vs. Automobile Collision	11 (8.5%)	30 (8.0%)
Gunshot Wound	2 (1.6%)	28 (7.5%)
Stabbing	0 (0%)	13 (3.5%)
Fire/Burn	0 (0%)	3 (0.8%)
Industrial/Home accident	5 (3.9%)	18 (4.8%)
Fall	6 (4.7%)	18 (4.8%)
Animal Bite/Attack	0 (0%)	5 (1.3%)
Sports Injury	0 (0%)	1 (0.3%)
Bike accident/Bike vs. auto	4 (3.1%)	11 (2.9%)
Other	0 (0%)	1 (0.3%)
Sexual assault	8 (6.2%)	30 (8.0%)
Missing	0 (0%)	0 (0%)
Age (years)	36.4 (12.6)	35.0 (12.6)
Time since trauma (minutes)	315.7 (235.3)	298.7 (213.0)
Time between trauma and blood draw (minutes)	221.9 (224.6)	238.7 (256.7)
Patient-rated severity	3.98 (1.0)*	3.66 (1.27)*
Clinician-rated severity	2.70 (1.0)	2.71 (1.02)

*$p<0.05$

Statistical Analysis

Using SPSS software, Chi-square analysis was used to determine whether there is an association between mTBI and gender, ethnicity, race, and trauma type. A Mann-Whitney U test was used to determine whether there was an association between mTBI and age, time since trauma, time between trauma and blood draw, patient-rated trauma severity, and clinician-rated trauma severity. Chi-square analysis was used to determine whether there was an association between gender and PTSD diagnosis at 1, 3, 6, and 12 months following trauma. Chi-square analysis was also conducted to determine whether there was an association between mTBI and PTSD diagnosis at 1, 3, 6, and 12-month assessment time points compared to those without mTBI as baseline. A Mann-Whitney U test was used to determine whether testosterone, progesterone, and estradiol levels were associated with mTBI groups. A Mann-Whitney U test was also used to determine whether PSS scores differ at 1, 3, 6, and 12 months between those who presented with mTBI at initial assessment and those who did not have a mTBI at initial assessment. Total PSS scores were analyzed as well as PSS scores for intrusive, avoidance, and hyperarousal symptom clusters.

At each assessment time point, three linear regression models were created. Each of these models was created based on the hormones estradiol, testosterone, or progesterone. The dependent variable for each model was PSS score at the given time point. The independent variables for each model were mTBI, hormone level, the interaction between mTBI and hormone level, and gender. At 6 months, Spearman correlation was used to determine the relationship between testosterone levels and PSS scores in both mTBI and no mTBI groups.

Results

Of the 504 participants, 129 (25.6%) were classified with mTBI and 375 (74.4%) were classified with no mTBI. Chi-square analysis showed mTBI was independent of gender (X^2=0.807, p=0.413), race (X^2=6.513, p=0.368), ethnicity (X^2=0.002, p=0.565), and trauma type (X^2=18.42, p=0.142). A Mann-Whitney U test showed that there was no significant association between mTBI and age (U=22791.5, p=0.40), time since trauma (U=21460.5, p =0.32), time between trauma and blood draw (U=20964.0, p=0.59), and clinician-rated trauma severity (U=23789.5, p=0.96). However, one-way ANOVA analysis showed a significant positive association between mTBI and patient-rated trauma severity (U=20954.0, p=0.032), with patients with mTBI rating symptoms with a higher severity than those without mTBI (Figure 1). A one-way ANOVA analysis showed no significant difference at the time of the ED visit in testosterone (p=0.841, F=0.040), progesterone (p=0.162, F=1.96), and estradiol (p=0.194, F=1.69) between the group with mTBI and the group without mTBI.

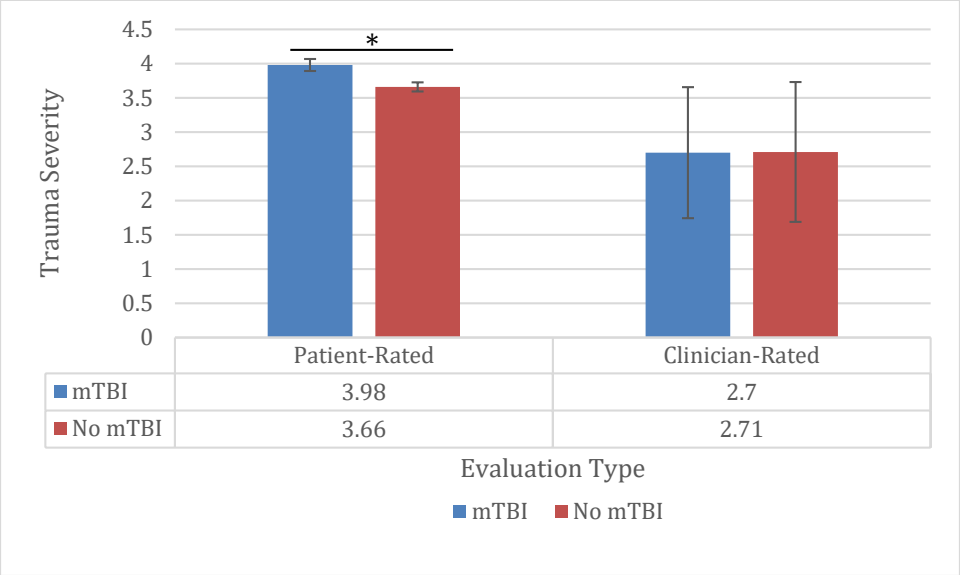

Figure 1. Mean patient-rated and clinician-rated trauma severity scores for participants with and without mTBI at baseline. Error bars represent standard error of mean. (mTBI: N=129, No mTBI: N=370). *p<0.05

Chi-Square analysis showed a positive association between mTBI and PTSD diagnosis at 1 month ($p=0.025$, $X^2=5.48$), 3 months ($p=0.038$, $X^2=4.77$), and 12 months ($p=0.005$, $X^2=8.92$) following trauma. No association between mTBI and a PTSD diagnosis was found at 6 months post-trauma ($p=0.118$, $X^2=2.50$). A Mann-Whitney U test showed a significant elevation in PSS total score for individuals with mTBI compared to individuals without mTBI at 3 months ($U=6135.0$, $p=0.003$), 6 months ($U=6027$, $p=0.045$), and 12 months ($U=5367.0$, $p=0.049$), but not 1 month ($U=9803.5$, $p=0.097$) (Figure 2).

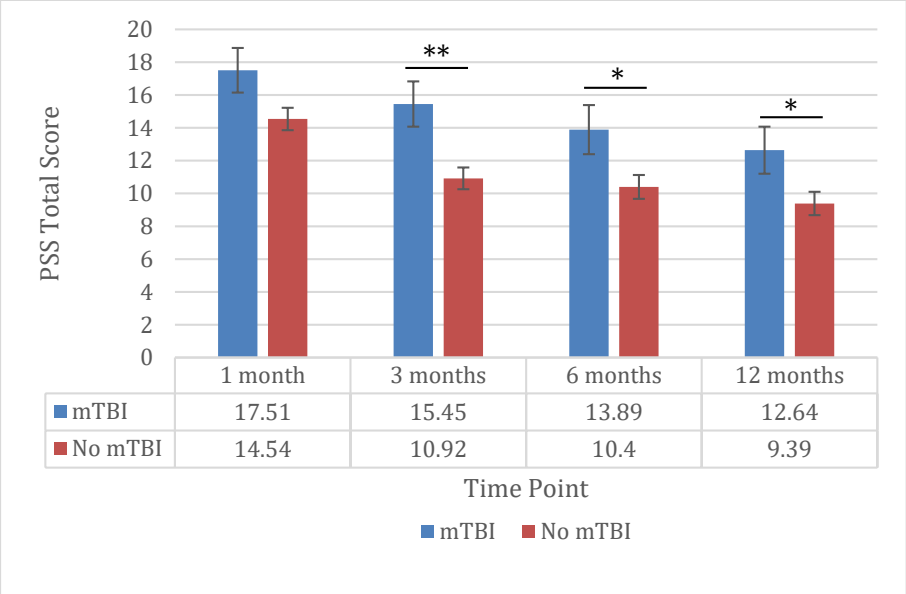

Figure 2. Mean PSS total scores for participants with and without mTBI at 1, 3, 6, and 12 months post-trauma. Errors bars represent standard error of mean. (1 month: n=90 mTBI, n=247 no mTBI; 3 months: n=71 mTBI, n=225 no mTBI; 6 months: n=70 mTBI, n=205 no mTBI; 12 months: n=67 mTBI, n=191 no mTBI). *p<0.05 **p<0.01

A Mann-Whitney U test showed differences between mTBI and no mTBI groups in PSS symptom subtypes. Those with mTBI had significantly higher avoidance scores than those without mTBI at 3 months (U=6736.0, p=0.045) and 6 months (U=6037.0, p=0.044), but not at 1 month (U=9785.0, p=0.091) and 12 months (U=5568.5, p=0.11) (Figure 3).

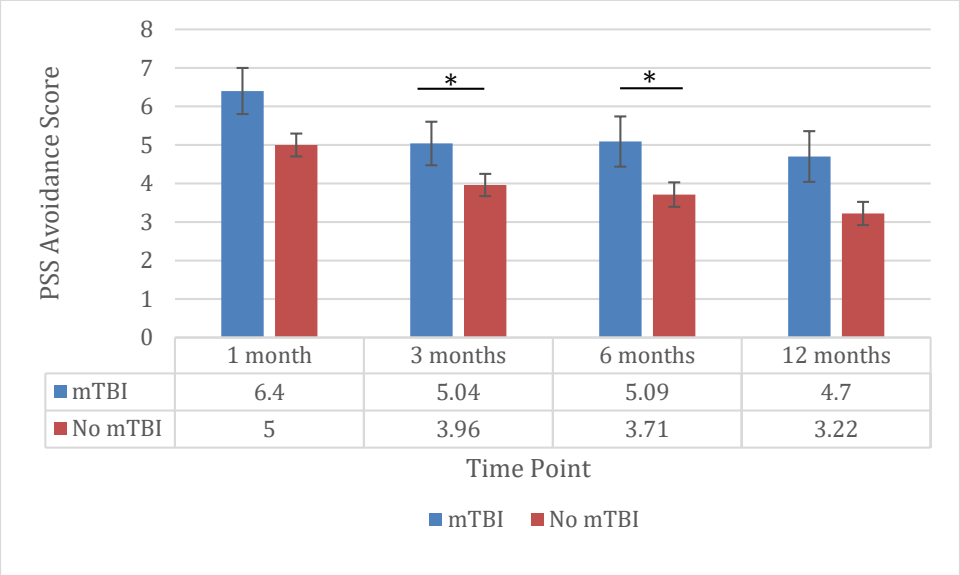

Figure 3. Mean PSS avoidance scores for participants with and without mTBI at 1, 3, 6, and 12 months post-trauma. Error bars represent standard error of mean. (1 month: n=90 mTBI, n=247 no mTBI; 3 months: n=71 mTBI, n=225 no mTBI; 6 months: n=70 mTBI, n=205 no mTBI; 12 months: n=67 mTBI, n=191 no mTBI). *p<0.05

Those with mTBI showed greater intrusive symptom severity compared to those without mTBI at 3 months (U=6320.5, p=0.007), 6 months (U=5809.0, p=0.014), and 12 months (U=5385.0, p=0.042), but not at 1 month (U=10050.5, p=0.175) (Figure 4).

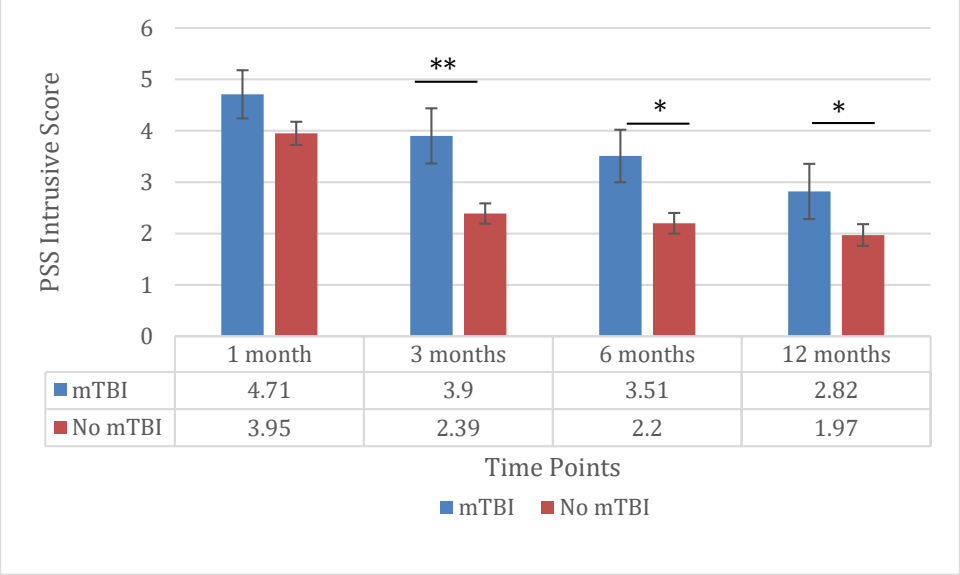

Figure 4. Mean PSS intrusive scores for participants with and without mTBI at 1, 3, 6, and 12 months post-trauma. Error bars represent standard error of mean. (1 month: n=90 mTBI, n=247 no mTBI; 3 months: n=71 mTBI, n=225 no mTBI; 6 months: n=70 mTBI, n=205 no mTBI; 12 months: n=67 mTBI, n=191 no mTBI). *p<0.05 **p<0.01

Those with mTBI showed greater hyperarousal symptom severity compared to those without mTBI at 3 months (U=6031.5, p=0.002), but not at 1 month (U=10067.0, p=0.184), 6 months (U=6411.0, p=0.180), or 12 months (U=5636.5, p=0.143) (Figure 5).

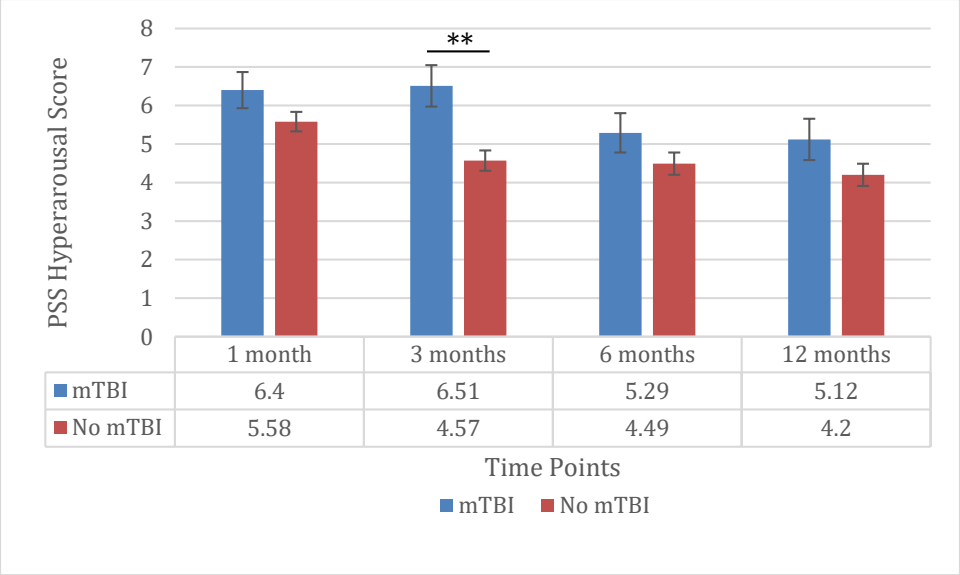

Figure 5. Mean PSS hyperarousal scores for participants with and without mTBI at 1, 3, 6, and 12 months post-trauma. Error bars represent standard error of mean. (1 month: n=90 mTBI, n=247 no mTBI; 3 months: n=71 mTBI, n=225 no mTBI; 6 months: n=70 mTBI, n=205 no mTBI; 12 months: n=67 mTBI, n=191 no mTBI). **p<0.01

At 1 month, women had higher PSS total scores than men in all hormone models (p<0.001). However, mTBI, hormone levels, and an interaction of mTBI and hormone levels were not significant predictors of PSS total scores at 1 month (Table 2).

Table 2. Linear regression models for PSS scores at 1 month assessment.

		B	**St. Error**	**Beta**
Model 1 Estradiol $R^2 = 0.092$ F = 6.43 p<0.001	(Constant)	4.97	2.25	-
	mTBI	3.55	1.96	0.14
	Estradiol levels (pgml)	-0.001	0.002	-0.33
	mTBI x Estradiol levels (pgml)	-0.013	0.018	-0.054
	Gender	6.52	1.40	0.282***
Model 2 Testosterone $R^2 = 0.098$ F=6.56 p<0.001	(Constant)	4.19	3.30	-
	mTBI	2.47	1.94	0.095
	Testosterone levels (ngml)	0.050	0.307	0.015
	mTBI x Testosterone levels (ngml)	-0.299	0.473	-0.053
	Gender	6.72	1.80	0.298***
Model 3 Progesterone $R^2 = 0.103$ F=6.90 p<0.001	(Constant)	4.19	3.30	-
	mTBI	2.47	1.94	0.095
	Progesterone levels (ngml)	0.050	0.307	0.015
	mTBI x Progesterone levels (ngml)	-0.299	0.473	-0.053
	Gender	6.72	1.80	0.298***

*p<0.05 ***p<0.001

At 3 months, those with mTBI had higher PSS scores in the estradiol (p=0.031) and testosterone (p=0.027) models, but not the progesterone model (p=0.091). Women had higher PSS scores than men in all models (p<0.001). However, hormone levels and the interaction between mTBI and hormone levels were not significant predictors of PSS score (Table 3).

Table 3. Linear regression models for PSS scores at 3 month assessment.

		B	St. Error	Beta
Model 1 Estradiol $R^2 = 0.080$ F = 4.94 p = 0.001	(Constant)	3.32	2.16	-
	mTBI	4.13	1.91	0.167*
	Estradiol levels (pgml)	-0.002	0.002	-0.044
	mTBI x Estradiol levels (pgml)	-0.010	0.017	-0.046
	Gender	5.11	1.36	0.242***
Model 2 Testosterone $R^2 = 0.065$ F = 4.81 p = 0.001	(Constant)	2.02	3.31	-
	mTBI	4.51	2.02	0.177*
	Testosterone levels (ngml)	0.228	0.315	0.067
	mTBI x Testosterone levels (ngml)	-0.732	0.539	-0.117
	Gender	5.58	1.82	0.262***
Model 3 Progesterone $R^2 = 0.081$ F = 4.76 p = 0.001	(Constant)	2.58	2.26	-
	mTBI	3.00	1.77	0.118
	Progesterone levels (ngml)	-0.048	0.039	-0.082
	mTBI x Progesterone levels (ngml)	-0.244	0.639	-0.027
	Gender***	5.70	1.42	0.267***

*p<0.05 ***p<0.001

At 6 months, women had higher PSS scores than men in the estradiol (p<0.001), testosterone (p=0.006), and progesterone (p<0.001) models. Those with mTBI had significantly higher PSS scores than those without mTBI in the testosterone (p=0.005) and progesterone (p=0.042) models, but not the estradiol model (p=0.146). Hormone levels were not significant predictors of PSS scores in any models. The interactions between mTBI and estradiol levels and mTBI and progesterone levels were not significant predictors of PSS scores (Table 4). The interaction between mTBI and testosterone levels was a significant negative predictor of PSS scores (p=0.044). Testosterone levels had a significant negative linear correlation with PSS scores in those with mTBI (ρ=-0.291, p=0.043) and in those without mTBI (ρ=-0.162, p=0.041) (Figure 6).

Table 4. Linear regression models for PSS scores at 6 month assessment.

		B	St. Error	Beta
Model 1 Estradiol $R^2 = 0.072$ F = 4.19 p = 0.003	(Constant)	2.13	2.34	-
	mTBI	2.94	2.02	0.117
	Estradiol levels (pgml)	-0.002	0.003	-0.042
	mTBI x Estradiol levels (pgml)	0.003	0.018	0.014
	Gender	5.56	1.46	0.254***
Model 2 Testosterone $R^2 = 0.089$ F = 4.96 p = 0.001	(Constant)	1.47	3.51	-
	mTBI	6.33	2.22	0.243**
	Testosterone levels (ngml)	0.257	0.339	0.072
	mTBI x Testosterone levels (ngml)	-1.14	0.563	-0.189*
	Gender	5.29	1.93	0.243**
Model 3 Progesterone $R^2 = 0.076$ F = 4.17 p = 0.003	(Constant)	1.73	2.45	-
	mTBI	3.74*	1.83	0.143
	Progesterone levels (ngml)	-0.045	0.050	-0.062
	mTBI x Progesterone levels (ngml)	-0.358	0.637	-0.039
	Gender	5.69	1.53	0.257***

*p<0.05 **p<0.05 ***p<0.001

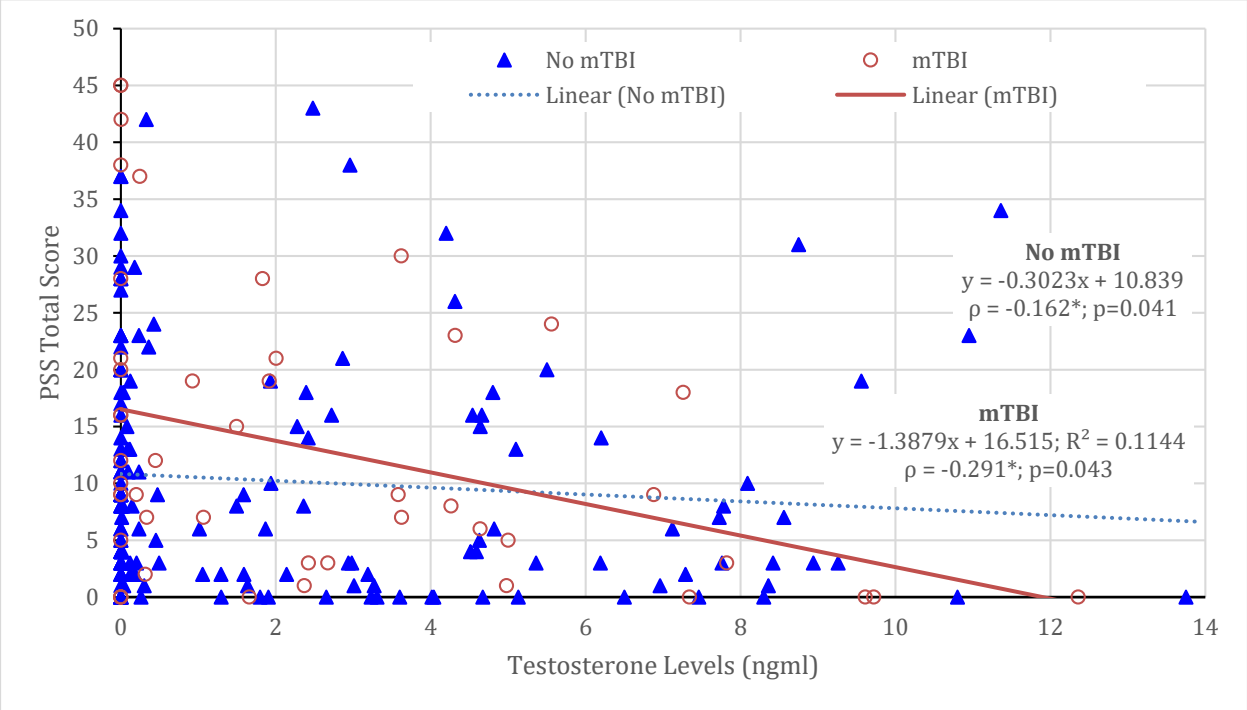

Figure 6. The relationship between testosterone levels (ngml) and PSS total scores at 6 months for mTBI and no mTBI groups. (mTBI: n=72, No mTBI: n=159). *p<0.05

At 12 months, women had higher PSS scores than men in estradiol (p=0.001), testosterone (p=0.020), and progesterone (p=0.001) models. Those with mTBI had higher

PSS scores than those without mTBI in the testosterone model (p=0.041), but not the estradiol (p=0.081) or progesterone (p=0.102) models. Hormone levels and the interaction between mTBI and hormone levels were not significant predictors of PSS scores in any models.

Table 5. Linear regression models for PSS scores at 12 month assessment.

		B	**St. Error**	**Beta**
Model 1 Estradiol $R^2 = 0.063$ F = 3.43 p = 0.010	(Constant)	2.16	2.27	-
	mTBI	3.32	1.89	0.144
	Estradiol levels (pgml)	0	0.002	-0.193
	mTBI x Estradiol levels (pgml)	-0.008	0.017	-0.040
	Gender	4.55	1.40	0.223**
Model 2 Testosterone $R^2 = 0.077$ F = 4.03 p = 0.004	(Constant)	2.53	3.32	-
	mTBI	4.16	2.02	0.177*
	Testosterone levels (ngml)	-0.024	0.306	-0.008
	mTBI x Testosterone levels (ngml)	-0.501	0.459	-0.107
	Gender	4.25	1.82	0.208*
Model 3 Progesterone $R^2 = 0.071$ F = 3.68 p = 0.007	(Constant)	1.30	2.34	-
	mTBI	2.83	1.72	0.120
	Progesterone levels (ngml)	-0.023	0.041	-0.040
	mTBI x Progesterone levels (ngml)	-0.098	0.617	-0.012
	Gender	5.09	1.46	0.249**

*p<0.05 **p<0.01

Discussion

Similar to prior studies,[7–10] our results showed a significant relationship between PTSD and mTBI. Our results also show that testosterone may have protective effects in PTSD for patients who have suffered from mTBI, consistent with a prior case study.[37] Our study adds to very few currently existing prospective studies that examine the relationship between PTSD and mTBI. This particular study includes recent trauma survivors who were assessed during emergency medical care and followed for 12 months. While one large prospective study also shows increased risk for PTSD following mTBI in a civilian population,[41] our prospective study is the first to analyze the role of gonadal steroid hormones in the relationship between PTSD and mTBI.

Our study showed increased reporting of PTSD symptoms in those with mTBI compared to those without mTBI at 3, 6, and 12 months following trauma. Increased PTSD symptom reporting in TBI patients is consistent with a prior study.[15] A significant relationship between mTBI and PTSD symptom reporting was not found at 1 month, but it is important to note that PTSD symptoms must persist longer than 1 month in order to be diagnosed as PTSD.[38] A significant association between mTBI and likely PTSD diagnosis was found at 1, 3, and 12 months. Although a significant association was not found at 6 months, this may be due to lower statistical power using a categorical outcome. Overall, the results suggest that mTBI is associated with increased PTSD symptom severity in recent trauma survivors presenting to an emergency department. It is notable that the association between mTBI and PTSD symptom severity remained significant at each timepoint across a 12 month follow-up given that symptoms of mTBI are highly unlikely to persist beyond 6 months.[42]

Study results found that PTSD symptom cluster severity scores differed in mTBI and no mTBI groups throughout the 12-month follow-up time period. PSS avoidance scores were significantly elevated in those with initial mTBI compared to those without initial mTBI at 3 and 6 months post-trauma. PSS hyperarousal symptoms were elevated for those with mTBI compared to those without mTBI at 3 months, but no significant difference in PSS hyperarousal scores between these groups was observed at other time points. PSS intrusive scores were elevated at 3, 6, and 12 months for those with initial mTBI compared to those without mTBI, but no significant difference in PSS intrusive scores was observed at 1 month and 6 months. Studies have shown that approximately 95% of people experience PTSD symptoms in the time period closer to a traumatic event, and differentiation between PTSD and natural recovery occurs at the 3 month time point.[43] In addition, symptoms of mTBI are highly unlikely to persist past 6 months.[42] Therefore, it is likely that avoidance, hyperarousal, and intrusive symptoms of PTSD have a relationship to the co-occurrence between PTSD and mTBI.

This study shows no significant association between gender and mTBI. These results are inconsistent with the results of a study conducted by Frost et. al.[14] However, gender was a strong predictor of higher PSS total scores at all points, with women reporting higher PSS scores than men. These findings are consistent with several previous studies that show that women are more likely than men to develop PTSD.[8,13] Our results do not show significant differences in levels of hormones between individuals with and without mTBI. In addition, hormones themselves were not significant predictors of PSS total scores at any time point.

While the interactions between mTBI and estradiol and mTBI and progesterone did not show to be significant predictors of PSS total score, the interaction between mTBI and testosterone were a significant predictor of PSS total score at 6 months. In addition, there was a significant negative linear correlation found between testosterone levels and PSS scores in both the mTBI group and the group without mTBI. Therefore, higher testosterone levels may have protective effects in PTSD following a mTBI. This finding is consistent with the results of a prior case study in a veteran with PTSD and a history of two concussions, which showed that testosterone supplements improved the veteran's PTSD symptoms.[37] In addition, our findings offer insight into the role of hypogonadism found following mTBI.[30,35,37]

The findings of this study not only add to growing literature on the relationship between PTSD and TBI, but also have potential to influence possible treatments for TBI. This study is one of few[41,44] to use a prospective design to examine the relationship between PTSD and mTBI. In addition, our findings show an important relationship between PTSD symptom clusters and mTBI, which could be used as a prognostic tool for clinicians. Our study also shows important findings in the role of gonadal steroid hormones in the relationship between PTSD and mTBI, which could lead to treatment methods that lessen the symptoms of PTSD following mTBI.

This study has several limitations that should be addressed in future studies. First, only individuals with a GCS of 15 were included. Therefore, our study results do not reflect cases of more severe TBI. Several prior studies in sTBI have shown hypogonadism following sTBI.[30,35] Therefore, we may have found a difference in hormone levels between TBI and no TBI groups if individuals with lower GCS scores were included. In addition, the

inclusion of those with GCS scores lower than 15 in future studies could allow for comparisons between hormone levels and GCS scores. In the future, we hope to incorporate data from the AURORA Study[45] in order to address this limitation. The AURORA Study is a national research initiative that aims to understand more about the process of recovery following a traumatic event. The goal of the AURORA Study is to enroll 5,000 trauma survivors who present to one of several EDs across the nation and follow-up with these participants over the course of 12 months. The AURORA Study has collected data on post-concussive symptoms, which provides a discrete variable to study mTBI symptoms following the traumatic event. Second, it would have been preferable to have larger sample sizes of individuals with both PTSD and initial mTBI. The proportion of individuals classified with mTBI is much lower than the proportion of individuals classified without mTBI. Therefore, it is important to include a larger sample size of individuals with mTBI in a future study. Third, patient-rated trauma severity for the mTBI was significantly higher compared to the no mTBI group. Therefore, the trauma could have been more severe for the group with mTBI, which may have led to higher PTSD symptom reporting. However, clinician-rated trauma severity was not significantly different between mTBI and no mTBI groups. Fourth, our study criteria for classifying participants with initial mTBI were based on participant self-report of the symptoms of loss of consciousness, loss of memory, or both following a trauma. While these symptoms align with diagnostic criteria for mTBI,[46] they could also be caused by mechanisms other than head injury. In future studies, it is important to include clinician diagnosis of mTBI to ensure the sample accurately captures those with mTBI.

Our study adds to the growing literature on the relationship between PTSD and mTBI. It not only shows higher PTSD symptom reporting in those with a mTBI, but also analyzes variations in PTSD symptom clusters at different time points. In addition, this study is the first prospective study to examine the role of gonadal steroid hormones in the relationship between PTSD and mTBI. This study shows that testosterone may have protective effects following mTBI. The finding of the potential role of testosterone in this relationship could lead to potential changes in the treatment of PTSD following a mTBI.

References:

1. American Psychiatric Association. *The Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, VA: American Psychiatric Publishing; 2013.
2. Liberzon I, Sripada CS. The functional neuroanatomy of PTSD: a critical review. *Prog Brain Res*. 2008;167:151-169. doi:10.1016/S0079-6123(07)67011-3
3. How Common is PTSD in Adults? - PTSD: National Center for PTSD. https://www.ptsd.va.gov/understand/common/common_adults.asp. Accessed October 30, 2019.
4. Gardner RC, Yaffe K. Epidemiology of mild traumatic brain injury and neurodegenerative disease. *Mol Cell Neurosci*. 2015;66(PB):75-80. doi:10.1016/j.mcn.2015.03.001
5. Bryant RA, Harvey AG. Relationship between acute stress disorder and posttraumatic stress disorder following mild traumatic brain injury. *Am J Psychiatry*. 1998;155(5):625-629. doi:10.1176/ajp.155.5.625
6. Howlett JR, Stein MB. *Post-Traumatic Stress Disorder: Relationship to Traumatic Brain Injury and Approach to Treatment*. CRC Press/Taylor and Francis Group; 2016. http://www.ncbi.nlm.nih.gov/pubmed/26583182. Accessed August 14, 2019.
7. Hoge CW, McGurk D, Thomas JL, Cox AL, Engel CC, Castro CA. Mild traumatic brain injury in U.S. Soldiers returning from Iraq. *N Engl J Med*. 2008;358(5):453-463. doi:10.1056/NEJMoa072972
8. Schneiderman AI, Braver ER, Kang HK. Understanding sequelae of injury mechanisms and mild traumatic brain injury incurred during the conflicts in Iraq and Afghanistan: persistent postconcussive symptoms and posttraumatic stress disorder.

Am J Epidemiol. 2008;167(12):1446-1452. doi:10.1093/aje/kwn068

9. Porter KE, Stein MB, Martis B, et al. Postconcussive symptoms (PCS) following combat-related traumatic brain injury (TBI) in Veterans with posttraumatic stress disorder (PTSD): Influence of TBI, PTSD, and depression on symptoms measured by the Neurobehavioral Symptom Inventory (NSI). *J Psychiatr Res*. 2018;102:8-13. doi:10.1016/j.jpsychires.2018.03.004
10. Kaplan GB, Leite-Morris KA, Wang L, et al. Pathophysiological Bases of Comorbidity: Traumatic Brain Injury and Post-Traumatic Stress Disorder. *J Neurotrauma*. 2018;35(2):210-225. doi:10.1089/neu.2016.4953
11. Breslau N. Gender differences in trauma and posttraumatic stress disorder. *J Gend Specif Med*. 2002;5(1):34-40. https://www.ncbi.nlm.nih.gov/pubmed/?term=11859685. Accessed March 28, 2020.
12. Tolin DF, Foa EB. Sex Differences in Trauma and Posttraumatic Stress Disorder: A Quantitative Review of 25 Years of Research. 2006. doi:10.1037/0033-2909.132.6.959
13. Breslau N, Anthony JC. Gender differences in the sensitivity to posttraumatic stress disorder: An epidemiological study of urban young adults. *J Abnorm Psychol*. 2007;116(3):607-611. doi:10.1037/0021-843X.116.3.607
14. Frost RB, Farrer TJ, Primosch M, Hedges DW. Prevalence of traumatic brain injury in the general adult population: A meta-analysis. *Neuroepidemiology*. 2013;40(3):154-159. doi:10.1159/000343275
15. Yue JK, Levin HS, Suen CG, et al. Age and sex-mediated differences in six-month

outcomes after mild traumatic brain injury in young adults: a TRACK-TBI study. *Neurol Res*. 2019;41(7):609-623. doi:10.1080/01616412.2019.1602312

16. Munivenkatappa A, Agrawal A, Shukla DP, Kumaraswamy D, Devi BI. Traumatic brain injury: Does gender influence outcomes? *Int J Crit Illn Inj Sci*. 2016;6(2):70-73. doi:10.4103/2229-5151.183024
17. *PTSD CheckList-Civilian Version (PCL-C)*. www.PDHealth.mil. Accessed October 30, 2019.
18. Berry C, Ley EJ, Tillou A, Cryer G, Margulies DR, Salim A. The effect of gender on patients with moderate to severe head injuries. *J Trauma*. 2009;67(5):950-953. doi:10.1097/TA.0b013e3181ba3354
19. Ryan KJ. Biochemistry of aromatase: Significance to female reproductive physiology. *Cancer Res*. 1982;42(8 Suppl.).
20. Jameson JL, DeGroot LJ, De Kretser DM (David M., et al. *Endocrinology : Adult & Pediatric*.
21. Mooradian AD, Morley JE, Korenman SG. Biological actions of androgens. *Endocr Rev*. 1987;8(1):1-28. doi:10.1210/edrv-8-1-1
22. Garcia NM, Walker RS, Zoellner LA. Estrogen, progesterone, and the menstrual cycle: A systematic review of fear learning, intrusive memories, and PTSD. *Clin Psychol Rev*. 2018;66:80-96. doi:10.1016/j.cpr.2018.06.005
23. Glover EM, Mercer KB, Norrholm SD, et al. Inhibition of fear is differentially associated with cycling estrogen levels in women. *J Psychiatry Neurosci*. 2013;38(5):341-348. doi:10.1503/jpn.120129
24. Pineles SL, Nillni YI, King MW, et al. Extinction retention and the menstrual cycle:

Different associations for women with posttraumatic stress disorder. *J Abnorm Psychol*. 2016;125(3):349-355. doi:10.1037/abn0000138

25. White EC, Graham BM. Estradiol levels in women predict skin conductance response but not valence and expectancy ratings in conditioned fear extinction. *Neurobiol Learn Mem*. 2016;134:339-348. doi:10.1016/j.nlm.2016.08.011
26. Wegerer M, Kerschbaum H, Blechert J, Wilhelm FH. Low levels of estradiol are associated with elevated conditioned responding during fear extinction and with intrusive memories in daily life. *Neurobiol Learn Mem*. 2014;116:145-154. doi:10.1016/j.nlm.2014.10.001
27. Glover EM, Jovanovic T, Mercer KB, et al. Estrogen levels are associated with extinction deficits in women with posttraumatic stress disorder. *Biol Psychiatry*. 2012;72(1):19-24. doi:10.1016/j.biopsych.2012.02.031
28. Brotfain E, Gruenbaum SE, Boyko M, Kutz R, Zlotnik A, Klein M. Neuroprotection by Estrogen and Progesterone in Traumatic Brain Injury and Spinal Cord Injury. *Curr Neuropharmacol*. 2016;14(6):641-653. http://www.ncbi.nlm.nih.gov/pubmed/26955967. Accessed October 25, 2019.
29. Emerson CS, Headrick JP, Vink R. Estrogen improves biochemical and neurologic outcome following traumatic brain injury in male rats, but not in females. *Brain Res*. 1993;608(1):95-100. doi:10.1016/0006-8993(93)90778-L
30. Garringer JA, Niyonkuru C, McCullough EH, et al. Impact of aromatase genetic variation on hormone levels and global outcome after severe TBI. *J Neurotrauma*. 2013;30(16):1415-1425. doi:10.1089/neu.2012.2565
31. Xiao G, Wei J, Yan W, Wang W, Lu Z. Improved outcomes from the administration of

progesterone for patients with acute severe traumatic brain injury: a randomized controlled trial. *Crit Care*. 2008;12(2):R61. doi:10.1186/cc6887

32. Skolnick BE, Maas AI, Narayan RK, et al. A clinical trial of progesterone for severe traumatic brain injury. *N Engl J Med*. 2014;371(26):2467-2476. doi:10.1056/NEJMoa1411090

33. Wright DW, Kellermann AL, Hertzberg VS, et al. ProTECT: a randomized clinical trial of progesterone for acute traumatic brain injury. *Ann Emerg Med*. 2007;49(4):391-402, 402.e1-2. doi:10.1016/j.annemergmed.2006.07.932

34. Hohl A, Zanela FA, Ghisi G, et al. Luteinizing hormone and testosterone levels during acute phase of severe traumatic brain injury: Prognostic implications for adult male patients. *Front Endocrinol (Lausanne)*. 2018;9(FEB). doi:10.3389/fendo.2018.00029

35. Hohl A, Mazzuco TL, Coral MHC, Schwarzbold M, Walz R. Hypogonadism after traumatic brain injury. *Arq Bras Endocrinol Metabol*. 2009;53(8):908-914. doi:10.1590/S0004-27302009000800003

36. Spivak B, Maayan R, Mester R, Weizman A. Plasma testosterone levels in patients with combat-related posttraumatic stress disorder. *Neuropsychobiology*. 2003;47(2):57-60. doi:10.1159/000070009

37. Isaacs KH, Geracioti TD. Post-TBI central hypogonadism and PTSD. *Am J Psychiatry*. 2015;172(11):1160. doi:10.1176/appi.ajp.2015.15060750

38. Treatment C for SA. Appendix E: DSM-IV-TR Criteria for Posttraumatic Stress Disorder. 2009.

39. *PTSD Scale-Self Report for DSM-5 (PSS-SR5)*.

40. Holm L, David Cassidy J, Carroll L, Borg J, Neurotrauma Task Force on Mild Traumatic

Brain Injury of the WHO Collaborating Centre. Summary of the WHO collaborating centre for neurotrauma task force on mild traumatic brain injury. *J Rehabil Med*. 2005;37(3):137-141. doi:10.1080/16501970510027321

41. Stein MB, Jain S, Giacino JT, et al. Risk of Posttraumatic Stress Disorder and Major Depression in Civilian Patients after Mild Traumatic Brain Injury: A TRACK-TBI Study. *JAMA Psychiatry*. 2019;76(3):249-258. doi:10.1001/jamapsychiatry.2018.4288
42. Brain Injury Rehabilitation Service. *Recovering from a Mild Traumatic Brain Injury*. https://bianj.org/wp-content/uploads/2014/10/recoveringfrommildtbi.pdf. Accessed February 19, 2020.
43. Rothbaum BO, Foa EB, Riggs DS, Murdock T, Walsh W. A prospective examination of post-traumatic stress disorder in rape victims. *J Trauma Stress*. 1992;5(3):455-475. doi:10.1002/jts.2490050309
44. Meehan A, Lewandowski A, Weaver LK, Hebert D, Deru K. Prospective study of anxiety, post-traumatic stress and depression on postural control, gait, otolith and visuospatial function in military service members with persistent post-concussive symptoms. *Undersea Hyperb Med*. 2019;46(3):271-287.
45. McLean SA, Ressler K, Koenen KC, et al. The AURORA Study: a longitudinal, multimodal library of brain biology and function after traumatic stress exposure. *Mol Psychiatry*. 2020;25(2):283-296. doi:10.1038/s41380-019-0581-3
46. Mild Traumatic Brain Injury Committee of the Head Injury Interdisciplinary Special Interest Group of the American Congress of Rehabilitation Medicine. *Definition of Mild Traumatic Brain Injury*.; 1993. www.ACRM.org.