The hormonal correlates of stress and posttraumatic stress disorder symptoms in female veterans

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THE HORMONAL CORRELATES OF STRESS AND POSTTRAUMATIC STRESS DISORDER SYMPTOMS IN FEMALE VETERANS

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ABSTRACT

The Hormonal Correlates of Stress and Posttraumatic Stress Disorder in Female Veterans

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With the increase of female veterans serving in the military, a better understanding of posttraumatic stress disorder is needed to provide comprehensive treatment. This study examines salivary cortisol in female veterans with PTSD, female veterans without PTSD, civilian females with PTSD and healthy controls. The Posttraumatic Stress and Beck Depression Inventory-II were used to assess posttraumatic stress and depressive symptoms. Saliva samples were collected at bedtime and awakening, as well as in response to the Trier Social Stress Test. Significant results were not found for diurnal cortisol levels \( F(3, 11)= .979, p < .05 \) or stress cortisol levels \( F(3,12) = 1.140, p>.05 \). Insignificant results may be due to low sample size, TSST schedule, and confounding anxiety.
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CHAPTER 1
INTRODUCTION

The participation of women in the military is steadily increasing. Although the presence of women in the military is not a new concept, more and more women are exposed to direct combat every year (Newhouse, 2008). Since 1994, female military personnel have been cleared to serve in non-combat related specialties on the front lines (Klein, 2005). The Department of Veterans Affairs states the population of female veterans residing in the United States totaled 1,824,198 in 2009. Currently, about ten percent of all troops deployed to Afghanistan and Iraq are female (Murdoch et al., 2006). Along with combat exposure, female military personnel have reported being sexually assaulted or raped while serving. Annual reporting of sexual trauma within the military for women is around 25% and 5% for men (Kimerling et al., 2003). This exposure to combat and sexual assault increases the prevalence of Posttraumatic stress disorder (PTSD) in active duty female military personnel (Zinzow, Monnier, Suffoletta-Maierle, & Frueh, 2007). Few studies conducted to date have assessed women diagnosed with PTSD. No study published to date addresses the biological underpinnings of PTSD in women in the military.

Purpose of the Study

In the past, researchers have focused on the biological effects of combat trauma on male combat veterans (Baker et al., 2005; Boscarino, 1996; Lauc, Vuksic-Mihaljevic & Flogel, 2003; Mason et al., 2002). This study will examine how the effects of combat and military sexual trauma (MST) on female veterans impact the hypothalamic-pituitary-adrenal axis (HPA-axis) in relation to PTSD. With the growing number of women
serving in areas of conflict, understanding the female experience of PTSD is imperative. Studies have shown differences in symptom expressions in women and men (Luxton, Skopp, & Maguen, 2010; Irish et al., 2011). A better understanding of the biological correlates of PTSD in female veterans will lead to more comprehensive treatment. The collection of empirical data on this subject will fill a much-neglected gender gap in PTSD research.

This study will address the HPA-axis stress response in women with military experience. We will utilize the Trier Social Stress Test (TSST) to assess the effects of stressors similar to those encountered in daily life. Salivary cortisol samples will be collected upon waking and before bed to assess circadian secretion. Saliva samples will also be collected before and after the TSST to assess the HPA-axis stress response. Assessing both functions will improve the understanding of the biological underpinnings of PTSD as well as contribute to a more gender specific treatment model.

**Research Questions**

Much of the existing literature on the biological correlates of PTSD has found discrepancies in cortisol levels among veterans with PTSD (Baker et al., 2005; Boscarino, 1996; Lauc, Vuksic-Mihaljevic & Flogel, 2003; Mason et al., 2002). These studies were conducted on a strictly male population. No study to date examines the relationship between cortisol reactivity and PTSD in the female veteran population. Hormonal studies conducted with the female population have examined the relationship between cortisol and PTSD from childhood and domestic abuse. This study will examine how neuroendocrine function differs in female veterans with PTSD versus female veterans without PTSD and healthy controls.
Considering the current findings in both male veteran studies and female abuse studies, it is expected that our PTSD positive female veterans will exhibit higher basal cortisol levels compared to the healthy controls and PTSD negative female veterans. We also posit that the TSST will elicit a blunted cortisol response in PTSD positive female veterans compared to controls.
CHAPTER 2

REVIEW OF RELATED LITERATURE

Posttraumatic Stress Disorder

PTSD is a disorder that occurs when individuals are exposed to a life-threatening situation. Any situation that involves threatened death or serious injury, which causes an individual to feel horrified, terrified, or helpless, can result in the development of PTSD (APA, 2000). Events that most commonly result in PTSD are combat, physical and sexual assault, and natural and man-made disasters. PTSD symptoms intensify with physical or emotional proximity to the event. Symptoms will be more intense if an individual experiences the triggering event first hand. Individuals usually develop symptoms within three months of exposure to the traumatic event. The symptoms are divided into three categories: re-experiencing and emotional numbing, avoidance and hyperarousal. Re-experiencing symptoms can be manifested in nightmares, unwanted intrusive thoughts, or a dissociative state in which the individuals relives the traumatic experience. The dissociative state is what is commonly known as a “flash-back” and can last from seconds to hours. Triggers, physical or symbolic reminders of the traumatic event, usually cause re-experiencing symptoms.

Emotional numbing and avoidance occur when an individual feels detached from others and their own emotions. Individuals may avoid situations that are reminiscent of the trauma, such as talking to people who experienced the trauma with them, talking about the trauma, and specific or symbolic stimuli relating to the trauma. Peritraumatic dissociation is a form of avoidance that consists of symptoms like depersonalization or derealization (Fullerton et al., 2001). Individuals that experience more peritraumatic
dissociation symptoms are at a higher risk for developing chronic PTSD (Ursano et al., 1999). Emotional numbing may present itself as a lack of planning for the future.

The last criterion, hyperarousal, is marked by physiological experiences of anxiety. Individuals will experience symptoms such as persistent anxiety and depression. These symptoms are typically expressed as difficulty sleeping, hypervigilance, and difficulty concentrating. Hyperarousal may be expressed as an exaggerated response to noise or sudden movement. Individuals experiencing symptoms may have difficulty falling or staying asleep. Hyperarousal may result in symptoms of gastrointestinal problems, suppressed immune function and chronic pain.

The duration of PTSD is specified as being acute, chronic, or delayed onset (APA, 2000). The specifier “acute” is used if the symptoms last less than three months and “chronic” is used when symptoms last for three months or longer. “Delayed onset” is a term used when symptoms do not appear for six months or longer.

PTSD has a lifetime prevalence of around 8% of the population in the United States (Department of Veteran Affairs, 2009). This disorder affects men and women at different rates. Women have a lifetime prevalence almost double that of men. Community studies indicate that women have a lifetime prevalence of PTSD at the rate of 9.7% and men at 3.6%. Differences in prevalence may stem from type of trauma experienced and perception of the trauma (Pereira, 2002; Ennis, Kelly & Lambert, 2001; Irish et al., 2011).

Men are more likely to experience assault; however, women are more likely to develop PTSD following assault (Breslau, Chilcoat, Kessler, Peterson & Lucia, 1999). This difference may stem from perception of the female’s ability to defend herself against
the perpetrator Pereria, 2002). Additionally, females are more likely to experience sexual trauma than men (Kimerling et al., 2003). Sexual trauma is the greatest predictor of PTSD in both men and women (Seedat et al., 2005). Female veterans are at a high risk for experiencing military sexual trauma (MST). Twenty-seven percent of men reported experiencing MST whereas 60% of women reported similar trauma (Department of Defense, 2006).

Although women have a greater lifetime prevalence of PTSD, they are often underdiagnosed, especially within military settings (Pereira, 2002). Female veterans are diagnosed at low rates throughout the VA healthcare system; however, the incidence of female veterans with PTSD does not differ from trends found in the general U.S. population. Pereira (2002) believes this discrepancy may be a result of the perception of female military duties and gender stereotypes. Lilly et al. (2009), found female police officers are trained to suppress emotions when in traumatic situations. Female veterans undergo similar training and may also suppress emotions when witnessing or experiencing traumatic situations. This suppression may lead to more somatic complaints and fewer PTSD symptoms. Women also experience comorbid depression more often than men (Haskell, et al., 2010). Symptoms of depression and PTSD overlap, which may result in more diagnoses of Depression than PTSD. The type of stress varies by gender. Women face a variety of stressors unique to their gender. For example, women are often the sole caretakers in their families and work fulltime. Women also face significantly more sexual harassment in the workplace (Street, Gradus, Stafford, & Kelly, 2007). Pregnancy, domestic violence, and stress from single parenting are also more likely to effect women than men. Biological reaction to stress varies in men and women. This
may lead to a difference in the physical and psychological experience of stress. The
differences in stress response may be due to variations in hormonal phase. Studies have
shown women respond differently to stress during the different phases of the menstrual
cycle (Childs, Dlugos, De Wit, 2010; Ennis, Kelly & Lambert, 2010; Kudielka &
Kirschbaum, 2003). In conclusion, gender differences in PTSD may be related to the
type of trauma experienced, comorbid psychopathologies, severity and perception of
trauma, biological alterations and/or additional life stressors.

The Neuroendocrinology of PTSD

The neuroendocrine system involved with PTSD is the hypothalamic-pituitary-
adrenal axis (HPA-axis) (Yehuda, 1998). The HPA-axis is a main component in
regulating the body’s response to stress (Lightman, 2008). When a person encounters a
stressor, the sympathetic nervous system’s fight or flight response activates by releasing
adrenaline. The HPA-axis acts in response to the stressor following the release of
adrenaline. When an individual experiences stress, the hypothalamus releases
corticotrophin-releasing hormone (CRH), which then activates the anterior pituitary to
release adrenocorticotrophin (ACTH). ACTH acts on the adrenal glands to prompt the
release of cortisol into the bloodstream. Cortisol, a glucocorticoid, binds to
glucocorticoid receptors in the body, blood, and brain. Glucocorticoid receptors are
heavily concentrated in the hippocampus and hypothalamus. Once cortisol binds to
glucocorticoid receptors in the hypothalamus, CRH secretion is stopped, and thus
completing a negative feedback loop. The body’s response to stress works to maintain
homeostasis during a stressful event (Johnson, Kamaris, Chrousos, & Gold, 1992).
The stress reaction causes increased alertness and sensory threshold, suppressed hunger, digestion, reproduction and immune function. While these physiological alterations are adaptive in the context of short-term stress, prolonged exposure to stress can have detrimental effects on physical and psychological health. Constant exposure to a stressor, like combat, can cause a dysregulation in the HPA-axis, leading to negative side effects like hypertension, diabetes, and immune suppression. Many studies have found distinct gender differences in stress reactivity. Studies that measure cortisol release in response to laboratory stressors found that men have an increased response to stress when compared to women in the follicular phase of the menstrual cycle but not with women in the luteal phase (Zimmer et al., 2003; Childs, Dlugos, & De Wit, 2010). The follicular phase of the menstrual cycle is marked by the release of estrogen and ends with ovulation. The luteal phase is marked by the release of progesterone and ends with menses. These studies also found that men recover from stress quickly as evident by rapidly decreasing cortisol levels, but women in both phases of the menstrual cycle cortisol release decreases slowly over time. This difference in stress recovery may explain the role the stress hormones play in the gender differences in stress related diseases. Men are more prone to heart disease, which is associated with hypercortisolism, while women are more prone to autoimmune and inflammatory diseases, which are associated with hypocortisolism (Kudielka & Kirschbaum, 2005).

HPA-axis function can be investigated by measuring cortisol in blood plasma cerebral spinal fluid (CSF), urine, and saliva (Lundberg, 2005). When cortisol is measured through saliva or urine, it is considered a free hormone fraction because it is not bound to proteins (Mendel, 1989). When cortisol is free from bound proteins, it can bind to
glucocorticoid receptors. This allows a more accurate measure of HPA-axis function. Cortisol measured through plasma can give an inaccurate picture of cortisol release because of protein binding. If cortisol is bound to proteins, it cannot bind to glucocorticoid receptors. Therefore, cortisol collected from plasma or CSF will provide lower levels of active cortisol. Cortisol release follows a circadian rhythm as well as being released during a stress response (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). Cortisol measurements are highest 30-minutes after waking and steadily decrease throughout the day (Kudielka & Kirschbaum, 2003). When an individual is exposed to a stressor, cortisol can be measured to assess the HPA-axis function in response to stress (Kirschbaum & Hellhammer, 1993).

Distinct neuroendocrine changes have been found in individuals with PTSD (Lauc, Zvonar, Vuksic-Mihaljevic & Flogel, 2004; Baker, Ekhator, Kaschkow, Dashevsky, Horn, Bednarik, & Geracioti, 2005). It is not known whether the changes occur as a result of trauma or are present prior to symptom onset and predispose the individual for developing PTSD. Previous research has represented conflicting results in cortisol levels related to posttraumatic stress disorder. There are currently two theories regarding cortisol secretion in PTSD: hypercortisolism (increased levels of cortisol) and hypocortisolism (blunted levels of cortisol) (Mason et. al., 2001). Simeon and colleagues (2007) found individuals with PTSD exhibited blunted negative-feedback response to the dexamethasone (DEX) suppression test compared to healthy controls, but exhibited no difference in basal cortisol levels. Boscarino (1996) found similar results with male Vietnam veterans through the analysis of serum cortisol. This study found basal cortisol levels were blunted in male veterans with current PTSD but not among male veterans.
with a lifetime diagnosis. A study conducted with male and female victims of motor vehicle accidents found individuals who had lower urinary cortisol levels directly after an accident developed PTSD symptoms later. (Delahanty, Raimonde, & Spoonster, 2000). Lauc, Zvonar, Vuksic-Mihaljevic, and Flogel (2004) found combat veterans who suffered from PTSD have 29% more incidences of flat diurnal cortisol cycles than veterans without. Kanter and colleagues (2001) found lower basal cortisol levels in veterans with PTSD when compared to healthy controls. This effect was found by using Metyrapone to block cortisol and reintroducing cortisol through injection with male Vietnam veterans.

Similarly conducted studies found an increase in basal plasma cortisol levels. Lindley, Carlson and Benoit (2004), found elevated basal cortisol levels in males and females diagnosed with PTSD resulting from childhood abuse. Additionally, Baker and colleagues (2005) found higher levels of basal cortisol compared to healthy controls. Most of the studies conducted do not address cortisol fluctuation in PTSD in response to a stressor. The studies that examined cortisol levels in military personnel have been conducted with a strictly male population. Our study purposed to elucidate this reaction in a purely female population.

Very few studies have examined stress responsivity and HPA-axis function with regards to PTSD in a female population. The studies conducted focus on intimate partner violence and childhood abuse. According to a review of the literature, no study examines cortisol levels in female veterans. Young, Tolman, Witkowski, and Kaplan (2004), examined salivary cortisol in low-income women with recent PTSD, past PTSD and healthy controls. This study collected cortisol at awakening and bedtime as well as during participants’ visit to the research site. The researchers found that individuals with
recent trauma had higher levels of cortisol, however, they did not find a significant effect between individuals with a lifetime diagnosis of PTSD and controls. Inslicht et al. (2006), examined salivary cortisol in women with intimate partner violence (IPV) related PTSD and abused women with no history of PTSD. They found women with PTSD had higher levels of diurnal cortisol than women without. Lemieux and Coe (1995) examined urinary cortisol in women diagnosed with PTSD from childhood sexual abuse, those who experienced sexual abuse but did not develop PTSD and healthy controls. After analyzing diurnal cortisol levels, it was found that women with PTSD exhibited higher levels of cortisol compared to women without PTSD and healthy controls. Conversely, MacMillian et al. (2009) found lower cortisol reactivity in adolescents exposed to abuse compared to healthy controls in response to the TSST.

Previous studies that examined male veterans found significant, albeit discrepant, results when compared to healthy controls. Studies conducted with male veterans found either blunted or heightened levels of cortisol (Baker et al., 2005; Boscarino, 1996; Lauc, Vuksic-Mihaljevic & Flogel, 2003; Mason et al., 2002). Similarly, studies conducted with a female population found significant results that differed from the results found within a male population (Inslicht et al., 2006; Lemieux & Coe, 1995; MacMillan et al., 2009). Female veterans face a unique set of stressors when entering the military. They are exposed to combat and have an increased risk of sexual trauma (Zinzow et al., 2007). Therefore, it is important to examine how PTSD alters the HPA-axis within this population. The purpose of this study is to examine this phenomenon by measuring basal cortisol levels as well as in response to stress in female veterans with PTSD symptoms, female veterans without PTSD symptoms and healthy controls.
CHAPTER 3
METHODOLOGY

Participants

The sample in this study consists of three groups: control (women without military experience whose Posttraumatic Diagnostic Scale (PDS) scores were less than or equal to 10), Veterans without PTSD (women with military experience whose PDS scores were less than or equal to 10), or Veterans with PTSD (women with military experience whose PDS scores were greater than 10). Participants in the control group were students in introductory psychology classes at the University of Nevada, Las Vegas participating for credit. All participants were between the ages of 18-35. Individuals were excluded from participation if they had a diagnosis of major depressive disorder (MDD) within the past year or if their score on the Beck Depression Inventory II (BDI-II) was greater than 17 (minimal depression). Individuals were excluded from participation in the control group if they had ever been diagnosed with PTSD or if their score on the PDS was greater than 10 (mild PTSD symptoms). The participants were recruited through flyers posted in the community and on websites.

Psychological Assessments

Questionnaires were given to participants to complete on the first day of the study. The participants were asked to complete a demographics form. The participants were then administered the Beck Depression Inventory II (BDI-II) and the PDS. Participants were given the BDI-II to account for level of depression. Depression may alter cortisol levels differently than PTSD. The BDI-II allowed the researchers to account for this
change and co-vary out co-morbid depression. The PDS was given to participants to assess PTSD symptoms.

**Procedures**

Informed consent was obtained and participants were asked to fill out a demographics survey and psychological assessments. Based on demographic information and scores on the PDS and BDI-II, participants were assigned to one of three groups: veterans without PTSD, veterans with PTSD or the age matched control group. The BDI-II score was used as an exclusion criterion. If selected for further participation, the participant was given a packet containing instructions on evening and morning saliva collection and Salivette collection devices. The participants were also verbally instructed on saliva collection procedures. Participants were asked to report to the study location the following day with the saliva samples they had collected. Participants who were not able to return the following day were provided with a pre-addressed, postage-paid envelope in which to return their saliva samples. Instructions in the saliva collection packet addressed sample storage and directions for the option to return by mail.

On day two, the participants arrived to the study location. After turning in their saliva samples, participants were given a brief verbal description of the tasks were to complete. They were informed that audio and video recordings would be made for later analysis of their performance on these tasks. The researchers administered the Trier Social Stress Test (TSST) (Kirschbaum, Pirke & Hellhammer, 1993). This test was divided into three sections. The first portion of the TSST was a ten-minute habituation period, after which the baseline saliva sample was collected. The participant was then given a paper and pencil and instructed to spend the next ten minutes preparing the speech on why they
would be the most qualified candidate for a management position. After ten minutes, the participant was escorted to a room containing a committee. The committee consisted of three trained research assistants from the principle investigator’s lab. The participant was greeted by the committee and asked to begin the prepared speech. The committee followed a specific protocol and script if the participant stopped speaking or finished the speech before five minutes had elapsed. After completion of the public speaking task, the participant began the mental arithmetic section. A committee member verbally instructed the participant to start at 1,793 and count backward (serially subtract) in increments of 13. The participant was advised to give answers aloud as quickly and accurately as possible. The committee followed a script and protocol for handling participants' errors, after an error was made, the participant was instructed to begin again. After the TSST was completed, the researcher led the participant to a private room to collect a post-stress saliva sample. Before leaving the study location, the researcher debriefed the participant by informing the participant that no video or audio recordings were made during the Trier Social Stress Test. The researcher explained that the purpose of the supposed recordings was to ensure that the task would elicit a measurable hormonal stress response. The participants were thanked for their participation, asked if they had any questions and were given written and verbal instructions for setting up optional, complimentary counseling sessions through the Department of Counselor Education. All counseling services were conducted by student interns under the supervision of a Counselor Education faculty member.
Saliva Collection

All participants were given two salivettes and instructions for at-home collection. Participants collected samples of saliva before bedtime and within thirty minutes of awakening. The participants were advised to refrain from eating, drinking, smoking and using chewing tobacco for thirty minutes prior to collection. Abstention from brushing or flossing teeth 30 minutes before sample collection was requested as to limit blood contamination. Also, participants were instructed to refrain from using medications containing steroids immediately before the collection. Participants were asked to provide a list of any medications they were taking on the demographics form. Collection of saliva occurred three times during the Trier Social Stress Test. Participants provided samples upon arrival, after the speaking and arithmetic tasks, and after a short waiting period upon completion of debriefing.

Treatment of Data

Saliva was stored in salivette tubes at 4 C until spun down in an Eppendorf centrifuge (5810R, Brinkmann Instruments Inc.) at 3000 rpm for 5 minutes. Afterwards, the saliva samples were transferred to eppendorf tubes and stored at -20 C. Cortisol assays were performed using the Assay Designs cortisol enzyme immunoassay (Enzo Life Sciences, Plymouth Meeting, PA) according to the manufacturers instructions, and results were read on a multi-mode microplate reader (SpectraMax M2, Molecular Devices).
Descriptive and multivariate statistics were performed using SPSS. All statistical analyses were conducted using a mixed analysis of variance (ANOVA). For measurement of diurnal cortisol levels, the between group factors were military/PTSD status (female veterans with PTSD, female veterans without PTSD, and controls). The within subject variable was time of saliva sample collection (nighttime and morning). A separate mixed ANOVA was run for the samples collected from the TSST. The between group factors are the same as outlined above for diurnal cortisol. The within subject variables were the three cortisol samples collected during the TSST.

At the time of data analysis, 17 individuals participated in the study. Out of these individuals, 12 were control participants, 2 were female veterans with PTSD and 1 female veteran without PTSD. One veteran and one control participant dropped out of the study after day one. Participant’s ages ranged from 18-39, the mean age being 22.06. The original research question sought to investigate the differences in stress response in female veterans with PTSD, female veterans without PTSD and with age matched controls. However, four control participants scored above the PDS cutoff. This new finding allowed us to break up the control group into control participants with PTSD ($n=4$) and control participants without PTSD ($n=8$).

The control participants ranged 18-24 in age (M=20.25). Control participants came from introductory Psychology courses and were enrolled in classes at the University of Nevada, Las Vegas. Veteran participants were recruited from the community through
At the time of data collection, one female veteran without PTSD and two female veterans with PTSD participated in the study. All veteran participants were enrolled in college level courses in the Las Vegas, NV area. Veteran participants ranged 23-39 years in age (M=27.75).

### Demographics

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### Statistical Analysis of Research Questions

A repeated measures ANOVA was conducted to measure the difference of basal (awakening and bedtime) levels of cortisol between groups. A significant effect was found with regards to the time the sample was collected ($F(1,3)= 0.00$, $p < 0.05$). No significant difference was found between groups for basal levels [$F(3,11)= .979$, $p >$ 0.05].
A separate repeated measure ANOVA was conducted to assess the difference in stress reactivity between groups. No significant effect was found between time of measurement \((F(2, 12) = .176, p > 0.05)\). No significant effect was found between groups for stress reactivity \((F(3,12) = 1.140, p>0.05)\). A Pearson Correlation was run on BDI-II and PDS scores. BDI-II and PDS scores were positively correlated \(r(.612)= .020 p < 0.05\).

![Basal Cortisol](image)

Figure 1. Comparison of basal cortisol levels in control participants without PTSD \((n=8)\), participants with PTSD \((n=4)\), female veterans without PTSD \((n=1)\) and female veterans with PTSD \((n=2)\). Cortisol levels differed significantly in relation to time \((F(1,13)= 1.01, p < 0.05)\). No significance was found in levels between participants \([F(3, 11)= .979, p < .05]\).
Figure 2. Cortisol concentrations in response to the Trier Social Stress Test in controls without PTSD (dashed line square, $n=8$), controls with PTSD (line square, $n=4$), female veterans without PTSD (dashed line diamond, $n=1$), and female veterans with PTSD (line diamond, $n=2$). Cortisol reactivity to stress was not significant ($F(3,12) = 1.140, p > .05$). No significance was found between subjects ($F(2,12) = .176, p > .05$).
CHAPTER 5
SUMMARY CONCLUSIONS AND RECOMMENDATIONS

Discussion of Results

At the time of data analysis, we were unable to disprove the null hypothesis. This could be a result of a few factors. First, our sample size was small for each group. Originally, we only intended to include three groups: controls, female veterans with PTSD and female veterans without PTSD. However, many control participants scored above the cut off for the PDS. This allowed us to break up the drastically uneven groups into smaller, more even groups. Even with the addition of a new group, our sample sizes were still too small to elicit a significant result.

Control participants were divided into two groups, controls with PTSD and controls without. The change resulted from a large number of control participants scoring above the cut off on the PDS. The current lifetime prevalence for females with PTSD in the general population is 9.7% (APA, 2000). Thirty-three percent of our control group scored above the cutoff for PTSD. Our sample may indicate an underreporting of PTSD. Posttraumatic stress disorder is underreported within the military and may be underreported in the general population (Feczer, 2008).

Our population may have reported higher levels of PTSD for the following reasons. First, our participants were provided the highest standard of confidentiality when completing their diagnostic forms. The privacy and anonymity provided for each participant may have resulted in increased reporting of traumatic events. Similar results were found when examining reporting rates in recently deployed Navy veterans (McLay et al., 2008). Veterans were given asked to fill out the PTSD Checklist voluntarily and
anonymously. More responses indicated symptoms of PTSD when the PTSD Checklist was given anonymously than when a name was required.

Additionally, feelings of guilt and shame are associated with traumatic experiences (Leskela, Dieperink, & Thuras, 2002; Vidal & Pitrak, 2007). Individuals may not seek help with symptoms of PTSD in the aftermath of a trauma due to feelings of shame, guilt, or depression. Furthermore, individuals may not have correct knowledge or understanding of PTSD. Individuals may not understand how symptoms of PTSD affect their daily life. This may contribute to disassociation or a disconnection between the traumatic event and symptom expression (Hepp et al., 2006). Individuals with delayed onset of PTSD may not experience symptoms for 6-months after the trauma (APA, 2000). With this delay, individuals may not connect the symptoms with the event.

Basal cortisol changes were significant over time. These results support previous findings (Spath-Schwalbe et al., 1991). Cortisol levels in the morning were significantly higher than measurements taken at night. However, cortisol levels did not differ significantly between participants. Control participants with PTSD had slightly lower cortisol levels than control participants without PTSD. Had this difference been significant it would have supported studies that found a blunted cortisol effect in females with PTSD (MacMillian et al., 2009). The basal response was not significant between the veterans with PTSD and without. The between subjects analysis was not significant when comparing all groups. Cortisol levels in response to the TSST were not significant, nor were levels different between participants. However, the cortisol levels trended towards significance. Cortisol levels rose in response to stress but did not decrease consistently.
Every participant who scored above the cut off on the PDS scored above the cut off for the BDI-II. A significant correlation score supported this finding. This may be due to high comorbid depression (Brady et al., 2000). Additionally, PTSD and depression share symptoms. Due to the low number of participants, we were unable to exclude data from individuals who scored above PDS the cut off. It is unknown whether comorbid depression altered our cortisol findings.

Conclusions and Recommendations for Further Studies

Several factors played in to the success of the current statistical results. Results for both baseline and stress response appear to be trending towards statistical significance. To adequately analyze the current hypotheses, more participants are needed in each group. The original sample size for the planned groups was 20 (controls, veterans, veterans with PTSD). However, there were fewer than 10 participants in each group. Additionally, proper exclusion protocol should be followed. Depression may alter cortisol levels differently. Therefore, participants should be excluded from the PTSD group if they score above the cut off from mild depression. Although adding an extra control group allowed us to bring our sample sizes down to similar levels, the original hypothesis did not call for civilians with PTSD. For future studies, if comparison between civilian women with PTSD and veterans is desired, the civilian group should be larger. However, if this is not the desired direction, researchers should follow the exclusion protocol.

The TSST results did not coincide with other studies. Typically, salivary cortisol begins to decrease a half hour after the stressor (Kirschbaum & Hellhammer, 1993). About half of our participants’ cortisol levels rose 30-minutes after the TSST. It is not
known whether this is a result of time or a confounding stressor. An example of a confounding stressor is time constraints. Control participants were scheduled for a one-hour session. Often times, a participant may be tardy or exceed the allotted question and answer time. Participants may not become anxious if time runs over. For future studies, more time should be given for participants to destress after the TSST. Additionally, extra time should be added to the schedule to cushion any extra time spent on questions or tardiness.

**Limitations**

The limitations to the current study at the time of data analysis were small sample size, time constraints, and recruitment. The low sample size is a direct result of problems with recruitment. The recruitment flyer emphasizes the study of PTSD. This may deter female veterans from participating in the study. Female veterans may be hesitant to participate due to stigma that may be associated with PTSD or have never been formally diagnosed with PTSD. For future recruitment, the researchers will change the verbage of the recruitment flyer to convey PTSD status is not necessary for participation. Due to the low sample size, data analysis was inconclusive. No significant results were found for diurnal levels of cortisol or stress reactivity. The data collection will be continued in order to reach adequate sample sizes. Final data analysis will consist of healthy controls \( n=20 \), female veterans with PTSD \( n=20 \), and female veterans without PTSD \( n=20 \).

Time constraints and limitations may have resulted in skewed cortisol activity during the TSST. Previous studies showed that cortisol levels rise and fall during the progression of the TSST (Kirschbaum & Hellhammer, 1993). Several participants did not follow the typical progression. This may be due to time of TSST, physical activity
prior to TSST, or anxiety. The TSST sessions were scheduled during late morning, early afternoon hours. Cortisol fluctuations may have been skewed due to the lunchtime schedule. Participants may have come from eating or may have been anticipating a meal (Slag et al., 1981; Anderson et al., 1987; Ennis, Kelly & Lambert, 2001). In the future, researchers will schedule TSST appointments during midmorning and later afternoon hours. Participants will be asked to refrain from eating 30 minutes prior to TSST appointment.

Cortisol levels have also been found to increase after exercise (Kirschbaum, Platte, Pirke, & Hellhammer, 1996). A few participants came from the gym or ran to the location to ensure an on time arrival. For future participants, researchers will screen level of physical activity before beginning the TSST. If a participant engaged in exercise before the appointment time, researchers will add 5 minutes to habituation phase before collecting baseline reading. Time constraints and anticipation anxiety may play a role in discrepant progression of cortisol levels during the TSST (Ennis, Kelly, & Lambert, 2001). Future appointment times will be scheduled for 1.5 hours to allow for extraneous circumstances that may arise, such as tardiness and longer question sessions and a longer habituation or destress phase when needed.
APPENDIX 1

IRB APPROVAL

Biomedical IRB – Full Board Review
Approval Notice

NOTICE TO ALL RESEARCHERS:
Please be aware that a protocol violation (e.g., failure to submit or modify protocol for any change(s) to an IRB-approved protocol may result in mandatory remedial education, additional audits, re-consultation, and re-submission of any research protocol or even suspension of additional existing research protocols. Violations of all research conducted under the research protocol as reviewed, and further repercussions may be determined by the IRB and the institutional officials.

DATE: May 13, 2010
TO: Dr. Laurel Pritchard, Psychology
FROM: Office of Research Integrity – Human Subjects
RE: Notification of IRB Action
Protocol Title: Hormonal Correlates of Stress and PTSD Symptoms in Female Veterans
Protocol #: 1002-3377

This memorandum is notification that the project referenced above has been reviewed by the UNLV Biomedical Institutional Review Board (IRB) as indicated in Federal regulatory standards 45CFR46. The protocol has been reviewed and approved.

The protocol is approved for a period of one year from the date of IRB approval. The expiration date of this protocol is April 22, 2011. Work on the project may begin as soon as you receive written notification from the Office of Research Integrity – Human Subjects.

PLEASE NOTE:
Attached to this approval notice is the official Informed Consent/Assent (IC/A) Form for this study. The IC/A contains an official approval stamp. Only copies of this official IC/A form may be used when obtaining consent. Please keep the original for your records.

Should there be any change to the protocol, it will be necessary to submit a Modification Form through ORI – Human Subjects. No changes may be made to the existing protocol until modifications have been approved by the IRB.

Should the use of human subjects described in this protocol continue beyond April 22, 2011, it would be necessary to submit a Continuing Review Request Form 60 days before the expiration date.

If you have questions or require any assistance, please contact the Office of Research Integrity – Human Subjects at IRB@unlv.edu or call 895-2794.

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