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Evaluation of Cortisol and DHEA as Biomarkers for Stress

By: Jennifer A Smith

Master's Thesis

Mentor: Dr. Roberta Moldow

Seton Hall University

August 2008

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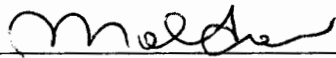
Abstract:

Stress significantly alters the way our bodies respond to different situations. Biomarkers can help determine how the body reacts to different types of stress. Cortisol and DHEA are two steroid hormone biomarkers under investigation, cortisol being the standard biomarker and DHEA as a possibility. In this study, salivary samples were collected anonymously from subjects undergoing psychological or physical stressors. Psychological stressors included undergraduate college exams and a disaster drill organization. Physical stressors consisted of TASER[®] hits accompanying police training and a military training obstacle course. The samples were analyzed via ELISAs. Subjects were also asked to fill out surveys, such as BORG Ratings of Perceived Exertion survey, the Spielberger State/Trait Anxiety Inventory (STAI), and the Thayer Activation-Deactivation Checklist (ADCL). Statistical analysis revealed a significant increase in both cortisol and DHEA in the majority of subjects undergoing physical stressors. Overall, subjects undergoing psychological stressors showed no significant changes in cortisol or DHEA levels, although there were some individual responders. Findings suggest that both cortisol and DHEA respond to physical stressors, with TASER[®] hits as a suitable positive control for the stress response. Findings also suggest the psychological tasks set to the subjects in this study were not stressful enough to illicit a stress response. Further studies are needed to determine the effect of confounding variables on the hormone biomarkers and the stress response.

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Jennifer A. Smith

July 31, 2008



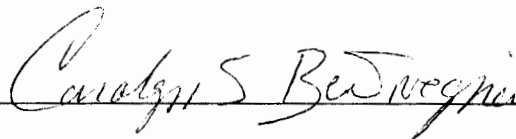
Roberta L. Moldow, Ph.D. – Mentor



Heping Zhou, Ph.D. – Committee Member



Kevin Beck, Ph.D. – Committee Member



Carolyn Bentivegna, Ph.D. – Department Chairperson

Acknowledgements

This thesis is dedicated to my mother, who has been my pillar of strength and shoulder to cry on throughout this demanding chapter of my life. I could not have completed this without her by my side.

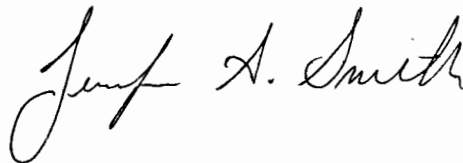
To my loving friends and family, I cannot express my gratitude for the patience you have had, nor the silent support you have all given me through my educational career.

I want to thank Dr. Roberta Moldow, my mentor, for steadfast guidance in the course of my research.

Dr. Allen Blake, thank you for advising me through both my undergraduate and graduate studies. Your sense of humor and forward personality always kept me pushing on.

Dr. Heping Zhou and Dr. Kevin Beck, thank you for sitting on my Thesis Committee and making this experience less daunting.

Again, thank you everyone. My heart goes with you all.

A handwritten signature in black ink that reads "Jennifer A. Smith". The signature is written in a cursive style with a large, looping initial "J".

Jennifer A. Smith

Introduction

One of the most studied responses in the physiology or endocrinology is the stress response. Stress refers to any external force than changes the body's state of equilibrium (Ganong, 1989/2005). Stress can be separated into physical stress or psychological stress. Physical stress can be equated with physical exertion, such as exercise (running, lifting weights, etc.) or physical training (body drags, obstacle course, etc.). With psychological stress, physical exertion is not a factor, but is more about mental or emotional stress, such as anticipation or anxiety such as the feelings experienced before an exam or oral presentation.

A person's ability to reason and react is drastically altered when under stress. By studying the stress response in training activities, we can determine if the exercises are successful in preparing the responders for real-life emergencies, such as the tragedies of September 11th or Hurricane Katrina. The goal of this study is to develop biomarkers to determine the intensity of the response as well as try to differentiate between physical and psychological stress.

The hormones in question to serve as biomarkers are cortisol and dehydroepiandrosterone (DHEA). Cortisol is the major stress hormone of the body, and is a glucocorticoid (steroid hormone) released from the adrenal cortex in response to the presence of adrenocorticotrophic hormone (ACTH) (Ganong, 1989/2005; Petrides, et al., 1994). When the body undergoes a stressful event, CRH (corticotropin releasing hormone) is released from the hypothalamus, triggering the production and secretion of ACTH from the anterior pituitary into the bloodstream (Herman, et al., 1997). ACTH triggers the secretion of cortisol from the adrenal cortex, which leads to numerous changes in the physiology of the body so that it can respond to stress such as a

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permissive effect of catecholamines, which results in the rearrangement of blood flow to brain, heart and skeletal muscles, alertness, and more (Ganong, 1989/2005; Herman, et al., 1997; Petrides, et al., 2007). DHEA is also a steroid hormone along the same synthetic pathway as cortisol. DHEA is considered a sex hormone since it is the precursor to all the sex hormones (testosterones, estrogens, progesterone, etc.) (Wellman, et al., 1999). DHEA is under consideration because studies have shown it to have anxiolytic effects, and the ratio of DHEA/cortisol has been suggested as a marker for the level of military performance (Morgan, et al., 2004; Van Niekerk, et al., 2001).

Saliva samples were used to study these potential biomarkers. Collection of saliva samples is a fast, noninvasive process that allows for easy repetitive sampling. The hormone concentration in saliva is reflective of the free/unbound active form of the hormone found in blood plasma, so it can be a more accurate reading of the active form (Hofman, 2001; Takai, et al., 2004). In this study, we examined these biomarkers in before and after tasing (physical stress and possible psychological stress or combination) (training drills (psychological stress or combination), before and after students taking exams (psychological stress).

Methods

Subjects

Taser

The taser study was performed in collaboration with UMDNJ and various police forces cross-country that were undergoing training, part of which was tasing. These studies occurred in multiple sites across the nation; each group is designated with a label associated with the location

(FC, CH, SF, NB, FFX and WH). The study involved a supervised five (5) second hit – except for WH group (1-5 seconds) – with a taser (Model X26E) with a peak loaded voltage of 1,200V and an average current of 2.1mA (TASER® X26E, 2007) followed by a controlled fall to avoid injury. Samples were collected at three time points: 1) a baseline sample taken in the early morning the day of the tasing – between 6:30am and 9:30am – or immediately prior to tasing (FC group only), 2) a sample taken 20 minutes post-tasing (all groups), and 3) a sample taken early the following morning between 6:00 and 8:00am (all groups). Table 1 below displays the demographics for the Taser groups presented.

Group	Number of Subjects (N)	Females	Males	Approx. Exposure Time (Military Time)	Age Range	Age Mean
FC	14	2	12	0700h – 0900h	21-35	24.7
CH	14	5	9	0700h or 1100h	20-48	25.9
SF	17	1	16	0800h or 1200h	22-50	31.7
NB	12	5	7	1000h or 1500h	23-44	28.4
FFX	12	4	8	0800h or 1200h	22-28	25.0
WH	9	1	8	0900h – 1000h	22-40	30.0

Table 1. Demographics of Taser Groups

Disaster Drill (PAD group)

The disaster drill was a training exercise to prepare and train regional volunteer Community Emergency Response Teams (CERT) how to respond efficiently to an emergency. However, the subjects were not from the CERT teams, but the personnel organizing and running the drill. Samples were collected in the early morning the day of the drill (approximately 7:00am) and again in the afternoon around 1:00pm. This particular group contained seven subjects (n=7) with 7 males and 0 females. The age range was 26-65yrs with a mean of 46.3 yrs.

Military Training Facility (FIG group)

This study took place on a military training base where a group of trainees were running through an obstacle course. The course run was approximately 10-15 minutes long and involved strenuous activity, such as wall jumps, rope climbs, and inclined-wall scaling, and running between each obstacle. Two samples were collected, the first around noon and the second taken immediately at the end of the obstacle course, about 10 minutes after the start of the course (about 4:30pm). There were seventeen subjects ($n=17$) with 5 females and 12 males. The age range was 18-24, with a mean of 19.8 years old.

Exam Groups

This particular study recruited volunteers who were taking an exam for a college course within their major. All groups had samples collected immediately prior to and immediately following the exam. Three groups (E2, E3, and E4) had additional samples collected on a normal class day, immediately prior to and following the class to stand as controls. The E1pm group had the first sample taken at 3:00pm and the second taken at 4:30pm [the E1pm group is designated as such to differentiate it from another set of subjects who took exams for the same course material, but in the morning (designated E1am)]. Groups E2 and E3 had the first sample taken at 1:00pm and the second at 2:30pm. Group E4 had the first sample taken at 5:30pm and the second taken around 7:00pm. Table 2 (below) contains the demographics for these groups.

Group	# of Subjects (N)	# of Females	# of Males	Age Range	Mean Average
E1pm	6	5	1	18-20	19
E2	10	8	2	19-21	19.5
E3	5	5	0	19-20	19.2
E4	4	2	2	20-22	20.8

Table 2. Demographics of Exam groups.

Samples were collected from volunteer subjects anonymously for all studies, except for the tasing studies, after the consent form had been read and any questions pertaining to the study were answered. The consent form remained unsigned, as this would be the only way to identify the subjects who were to remain anonymous. Signed consent forms were obtained for the tasing study. Subjects were also reminded that participation was voluntary and could stop at any time for any reason without penalty. All of this was performed in accordance with the proposal approved by the Institutional Review Boards (IRB) from Seton Hall University (SHU), New Jersey Medical School (NJMS) and/or U.S. Army Medical Research and Materiel Command (USAMRMC) when appropriate. Demographic information such as gender and age were obtained through surveys. These surveys collected information pertaining to any food, beverages and/or medications taken that may have an effect on cortisol or DHEA levels.

Psychological Assessment Tools

All subjects except for those participating in the taser study filled out psychological assessment tools. Subjects were asked to fill out a series of surveys to attain perceived exertion levels [BORG Ratings of Perceived Exertion (RPE)]; information about anxiety was obtained from Spielberger State-Trait Anxiety Inventory (STAI) and Thayer Activation-Deactivation check list (ADCL) from all subjects.

Biochemical Analysis

Samples were analyzed using specific kits for each salivary substance, cortisol and DHEA. These kits were purchased from Salimetrics® and stored in a 4°C freezer until use. Samples were labeled and stored in a -70°C freezer until they could be assayed. Both the cortisol and

DHEA kits were colorimetric ELISAs (Enzyme-Linked ImmunoSorbent Assay) specifically designed for saliva. These kits were the Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, LLCa, State College, PA) and Salivary DHEA Enzyme Immunoassay Kit (Salimetrics, LLCb, State College, PA).

Statistical Analysis

Data gathered through the ELISA assays were analyzed using the NCSS: Number Crunchers software. Error bar charts were generated for each marker in comparison to the times the samples were collected. Linear regression between cortisol and DHEA were also generated, as well as repeated measures ANOVA (rmANOVA) followed by a Tukey-Kramer Multiple-Comparison Test to determine if a specific time point was statistically significant from the others. If the study consisted of only two groups, a paired t-test was used in lieu of the rmANOVA. The Wilcoxon Signed-Rank test was used instead of the paired t-test when the psychological data was analyzed (BORG, ADCL, etc.)

Results

Taser

According to the rmANOVA, the FC group showed a significant increase in cortisol [$F(2,41)=19.980$, $P=0.001^*$] and DHEA [$F(2,41)=15.280$, $P=0.001^*$] in time point 20 minutes after the tasing, compared to samples taken before tasing and the morning after (Figure 1), with cortisol levels peaking around 750pg/100 μ l and DHEA peaking around 400pg/ml. The linear regression of DHEA versus cortisol showed a significant positive correlation between the two, [$R^2=0.263$, $F(2,41)=14.301$, $P=0.001^*$] indicating a rise in cortisol levels coincided with a rise in DHEA

levels. Repeated-measures ANOVA was also performed on the DHEA/Cortisol ratios (D/C). The analysis showed no significant change in D/C ratios between time points [$F(2,41)=2.380$, $P=0.112$].

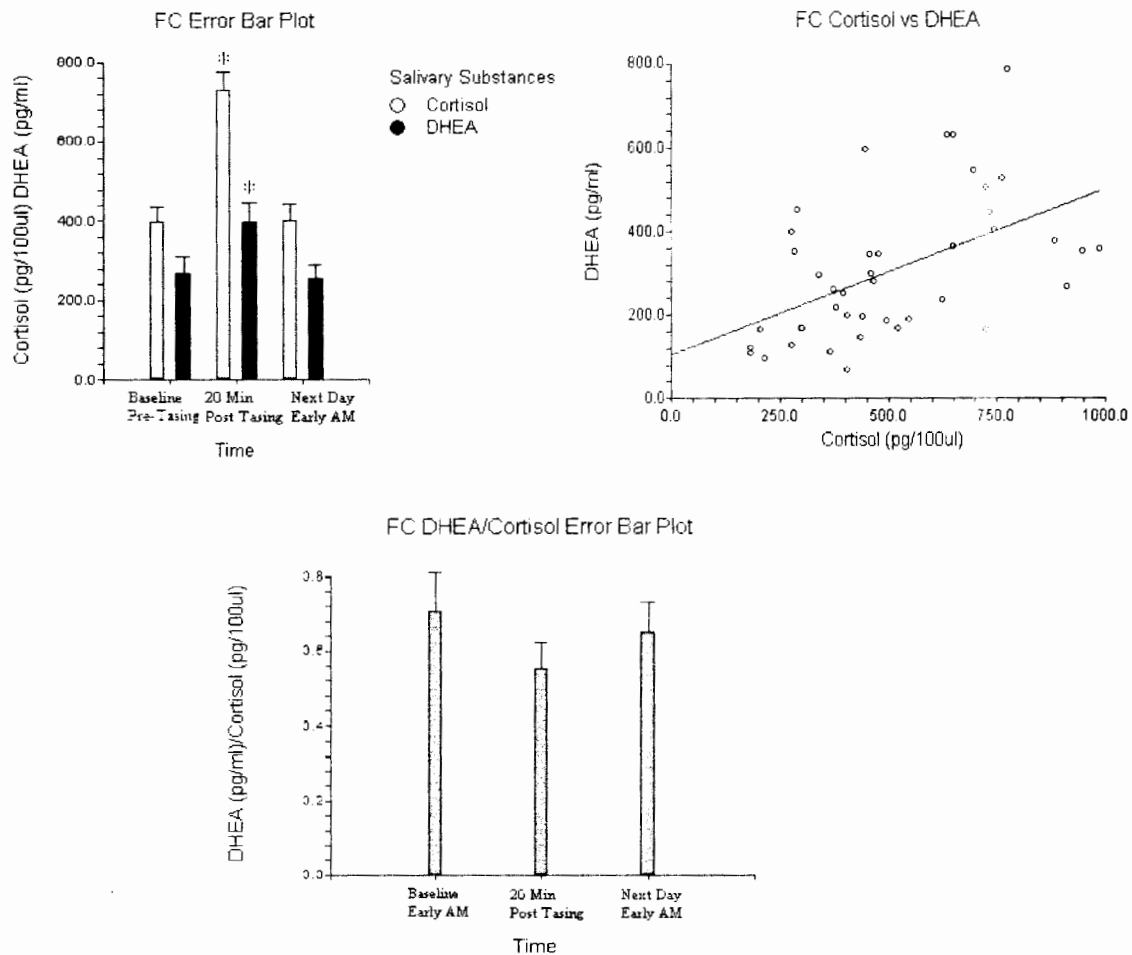


Figure 1. FC Taser Group. Asterisks (*) indicate statistical significance.

The CH group also showed a significant increase, with rmANOVA, in both cortisol [$F(2,41)=17.050$, $P=0.001^*$] and DHEA [$F(2,41)=15.050$, $P=0.001^*$] 20 minutes after tasing (Figure 2) with cortisol and DHEA peaking at 650pg/100 μ l and 225pg/ml, respectively. Again, the linear regression of DHEA versus cortisol showed a positive correlation [$R^2=0.272$, $F(2,41)=14.916$, $P=0.001^*$], indicating that cortisol and DHEA rose together. RmANOVA

analysis of the D/C ratios showed no significant change across time points [$F(2,41)= 3.130$, $P=0.060$].

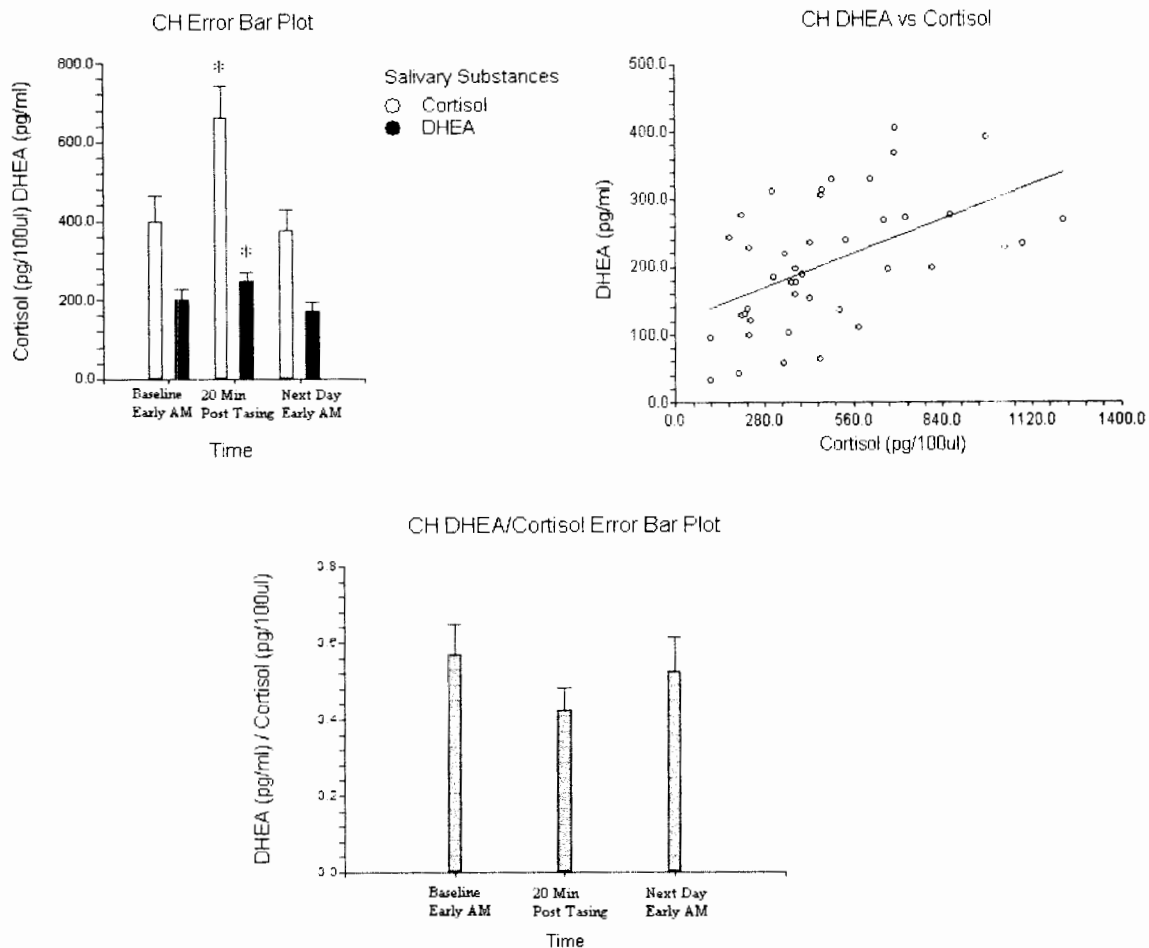


Figure 2. CH Taser Group. Asterisks (*) indicate statistical significance.

The rmANOVA showed that the SF group (Figure 3) displayed a significant increase in cortisol 20 minutes after tasing [$F(2,50)=7.560$, $P=0.002^*$] with a peak around $550\text{pg}/100\mu\text{l}$. However, DHEA did not show a significant increase 20 minutes after tasing [$F(2,50)=1.350$, $P=0.272$], with levels staying relatively constant throughout this particular study. Linear regression of between cortisol and DHEA yielded no significant positive correlation [$R^2=0.054$, $F(2,50)=2.814$, $P=0.100$]. The D/C ratios were analyzed using rmANOVA; the test showed a

significant change from baseline sample to the post-20 minute sample [$F(2,50)= 3.790$, $P=0.033^*$].

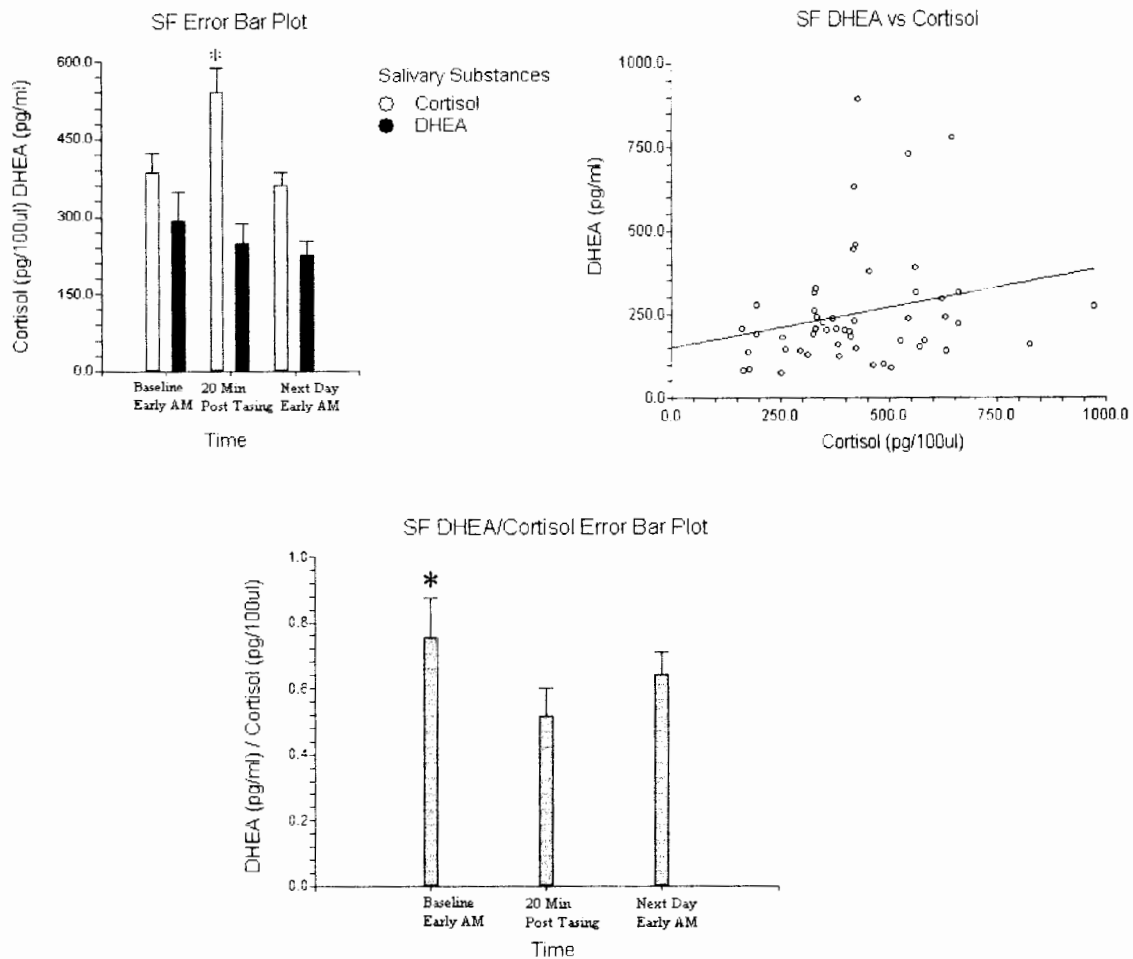


Figure 3. SF Taser Group. Asterisks (*) indicate statistical significance.

Using rmANOVA to analyze the NB group (Figure 4), the test showed a significant change in the cortisol [$F(2,35)= 15.400$, $P=0.001^*$] and DHEA [$F(2,35)= 13.42$, $P=0.001^*$] levels in the 20 minute post-tasting sample than the baseline and morning-after samples. Cortisol peaked around 700pg/100 μ l and DHEA peaked around 350pg/ml. A linear regression showed a significant positive correlation between cortisol and DHEA [$R^2=0.330$, $F(2,25)= 16.767$, $P=0.001^*$]. This

indicates a simultaneous rise between cortisol and DHEA. Analysis of the D/C ratios using rmANOVA yielded no significant change across time points [$F(2,35)= 1.390$, $P=0.269$].

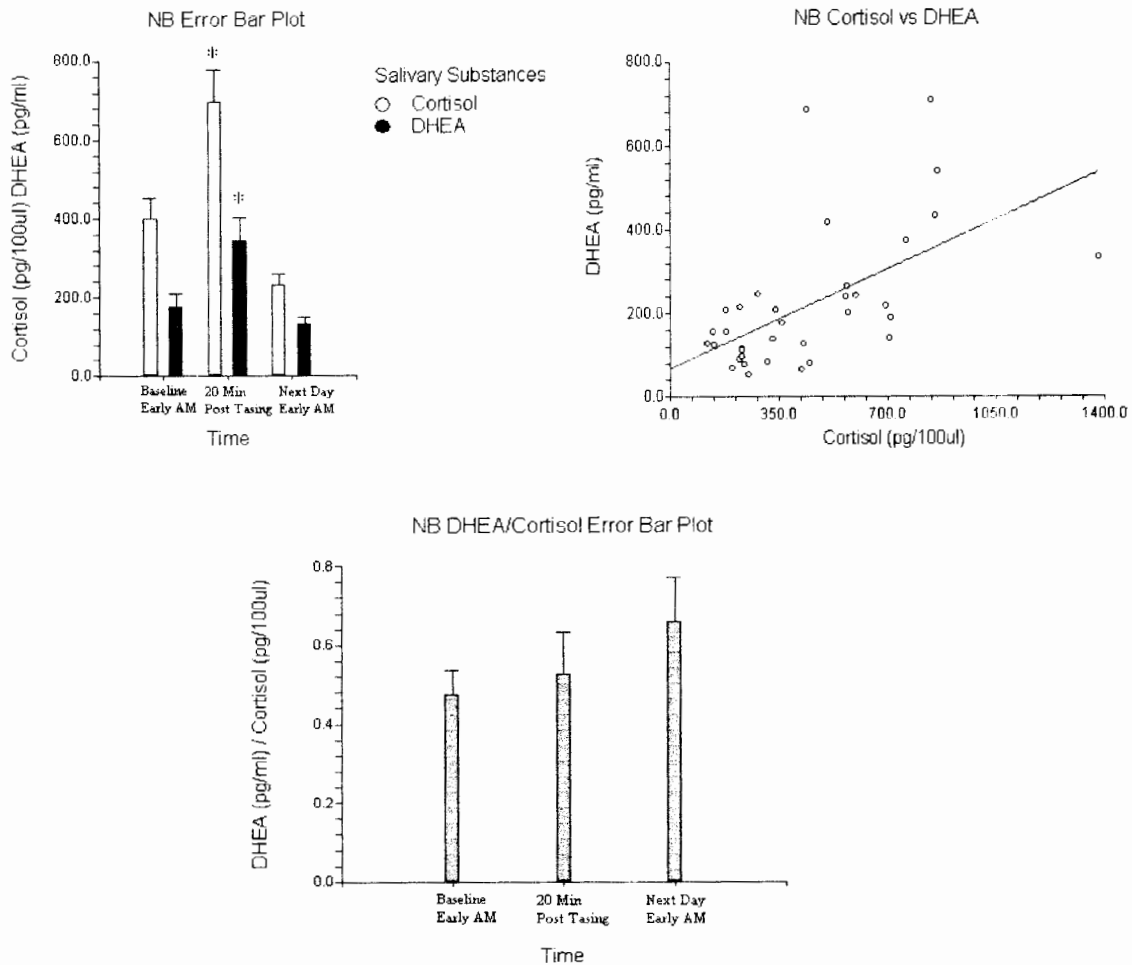


Figure 4. NB Taser Group. Asterisks (*) indicate statistical significance.

The rmANOVA showed a significant increase in cortisol in the 20 minute post-tasing sample [$F(2,35)= 6.830$, $P=0.005^*$] in the FFX group (Figure 5), compared to the baseline and following morning samples. Cortisol peaked around 700pg/100 μ l. DHEA showed a significant increase in the 20 minute post-tasing sample compared to the following morning sample [$F(2,35)= 8.050$, $P=0.002^*$] and peaked around 350pg/ml. Linear regression showed a statistically significant positive correlation between cortisol and DHEA [$R^2=0.315$, $F(2,35)= 15.654$, $P=0.001^*$]

indicating that as cortisol rose so did DHEA. Using rmANOVA, analysis of the D/C ratios showed no significant changes between time points [$F(2,35)= 2.190, P=0.135$].

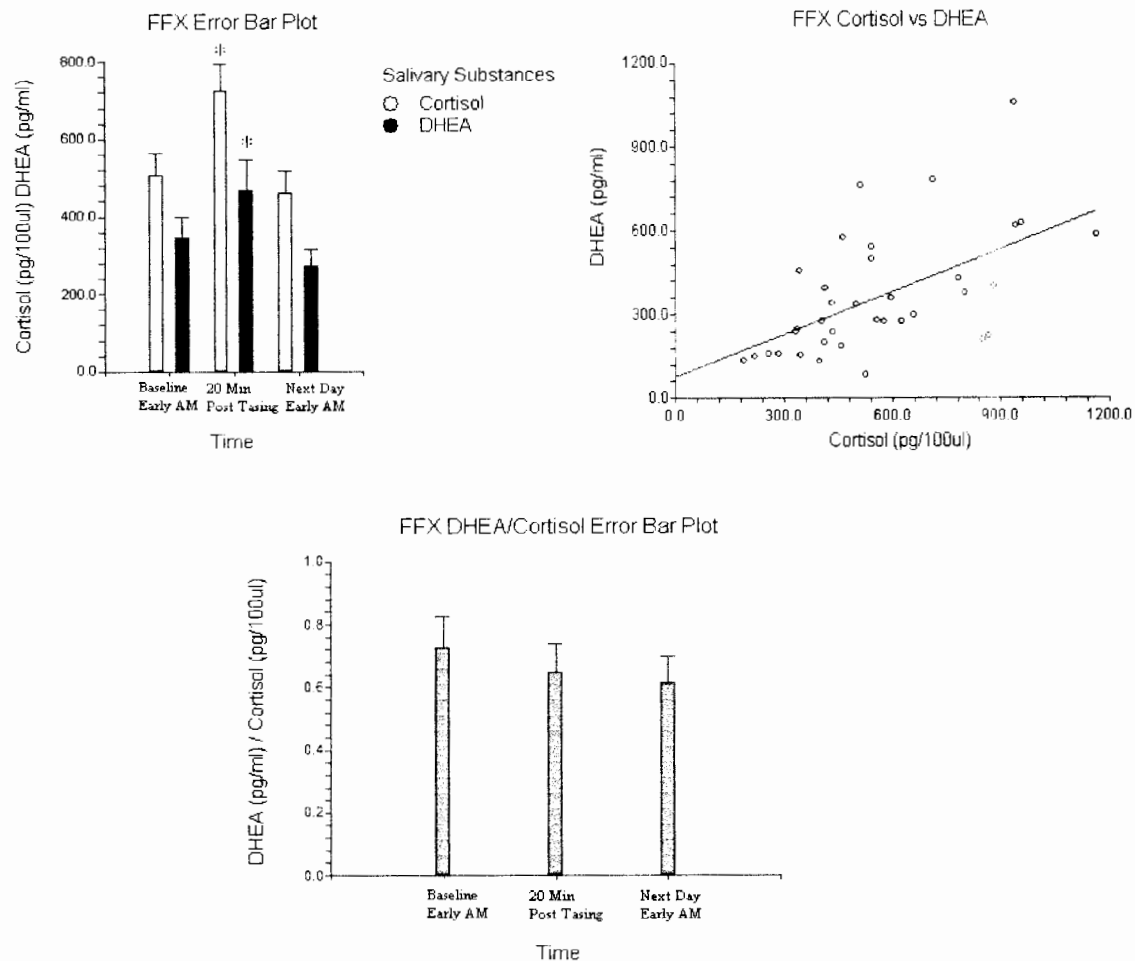


Figure 5. FFX Taser Group. Asterisks (*) indicate statistical significance.

The WH group (Figure 6) displayed a significant increase in cortisol in the 20 minute post-tasing sample compared to the baseline and morning after samples [$F(2,17)= 24.400, P=0.001^*$], and peaked around 525pg/100 μ l. DHEA showed a significant increase in the 20 minute post-tasing sample compared only to the morning after sample [$F(2,17)= 4.850, P=0.023^*$], peaking around 275pg/ml. A linear regression showed a significant positive correlation between cortisol and DHEA [$R^2=0.408, F(2,17)= 17.233, P=0.001^*$] indicating a simultaneous change in cortisol and

DHEA. DHEA/Cortisol ratios were analyzed using rmANOVA. The test showed a significant change in the D/C ratio in the sample the morning after the tasing in comparison to the other two time points [$F(2,17)= 40.540$, $P=0.001^*$]. Additionally, a linear regression was performed between cortisol levels 20 minutes after tasing and the actual exposure duration to the taser. The results show no correlation between cortisol levels and exposure duration [$R^2=0.011$, $F(2,17)= 0.080$, $P=0.785$].

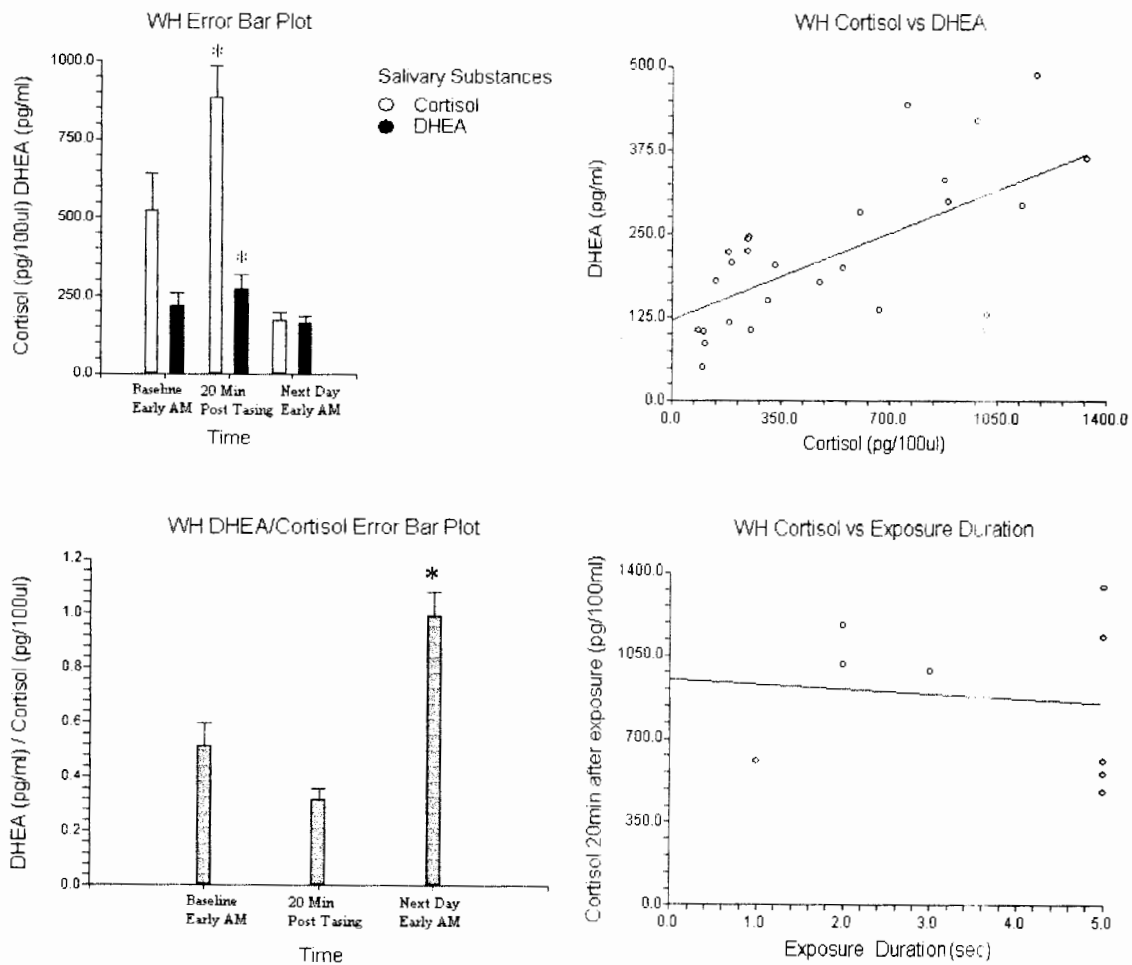


Figure 6. WH Taser Group. Asterisks (*) indicate statistical significance.

Disaster Drill

Using a paired t-test for analysis, the PAD group (Figure 7) showed a significant higher concentration in cortisol [$T=6.738$, $P=0.001^*$] and DHEA [$T=2.693$, $P=0.036^*$] before the drill, compared to after the drill. Cortisol levels were highest around 400pg/100 μ l and DHEA around 150pg/ml. For the BORG Rating of Perceived Exertion (RPE), the Wilcoxon Signed-Rank Test was used, and showed no statistical difference between 'pre-drill' levels and 'post-drill' levels [$T=0.410$, $P=0.682$], staying relatively constant at an RPE of 8.5. A linear regression between cortisol and DHEA showed a significant positive correlation [$R^2=0.623$, $F(1,13)=19.792$, $P=0.001^*$], indicating a simultaneous rise in cortisol and DHEA. A linear regression between cortisol levels and BORG levels was performed, yielding no correlation between the two [$R^2=0.211$, $F(1,13)=0.802$, $P=0.437$]. Using a paired t-test, D/C ratios were analyzed, and showed no significant change across the time points [$T=-1.220$, $P=0.268$]. A linear regression between cortisol and tension (derived from Thayer anxiety survey) yielded no correlation between the two, meaning any changes in cortisol levels did not coincide with changes in tension levels [$R^2=0.036$, $F(1,11)=0.371$, $P=0.556$].

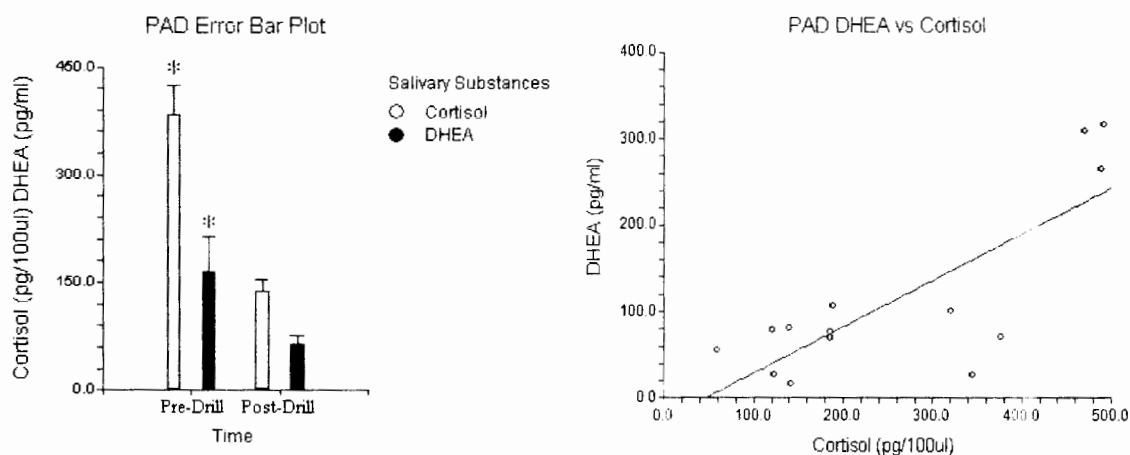


Figure 7. PAD Disaster Drill Group. Asterisks (*) indicate statistical significance.

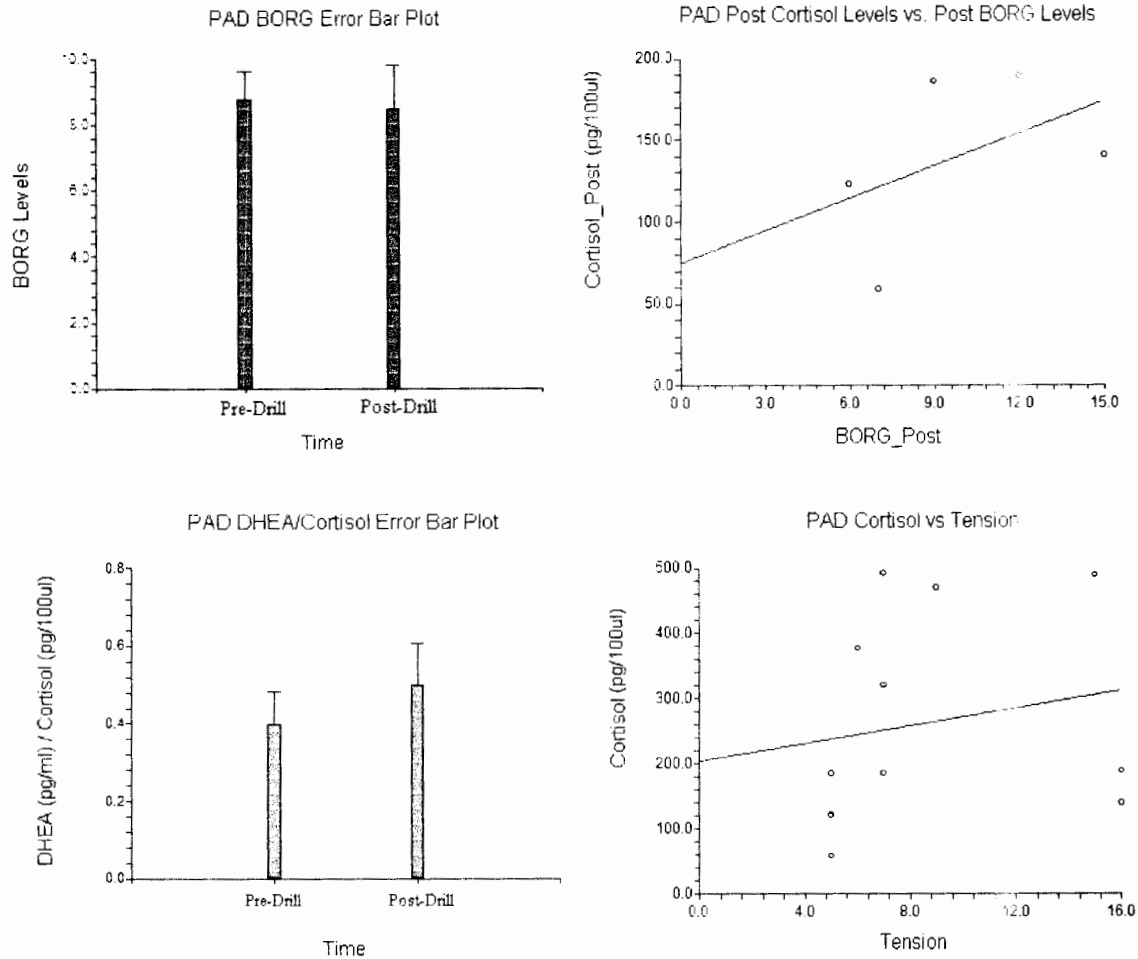


Figure 7 (cont'd). PAD Disaster Drill Group. Asterisks (*) indicate statistical significance.

Military Training Facility

Again, using a paired t-test, the FIG group (Figure 8) showed a significant increase in both DHEA [T=-4.013, P=0.001*] and cortisol [T=-3.296, P=0.005*] after the obstacle course, compared to before the obstacle course. Cortisol peaked around 330pg/100 μ l and DHEA peaked around 300pg/ml. Again, for the BORG RPE, the Wilcoxon Signed-Rank Test was used. A significant change was seen in this group's BORG RPE levels [Z=2.517, P=0.012*], rising from approximately 12 on the RPE scale before the course, to about 15 after the course. A linear regression between cortisol and DHEA showed a significant positive correlation [R²=0.306,

F(1,33)=14.123, P=0.001*], indicating a rise in DHEA coinciding with the rise in cortisol. Linear regression performed between cortisol and BORG RPE ratings showed no significant correlation [R²=0.003, F(1,33)=0.031, P=0.864] indicating that the cortisol levels and the BORG RPE ratings did not rise together. However, a linear regression performed between cortisol and perceived tension (derived from Thayer anxiety survey) showed a significant positive correlation [R²=0.236, F(1,33)=9.596, P=0.004*] indicating cortisol and the perceived level of tension rose simultaneously. A paired t-test was used to analyze the D/C ratios; the test yielded a significant increase in the D/C ratio in the Post-task sample [T=-2.530, P=0.022*].

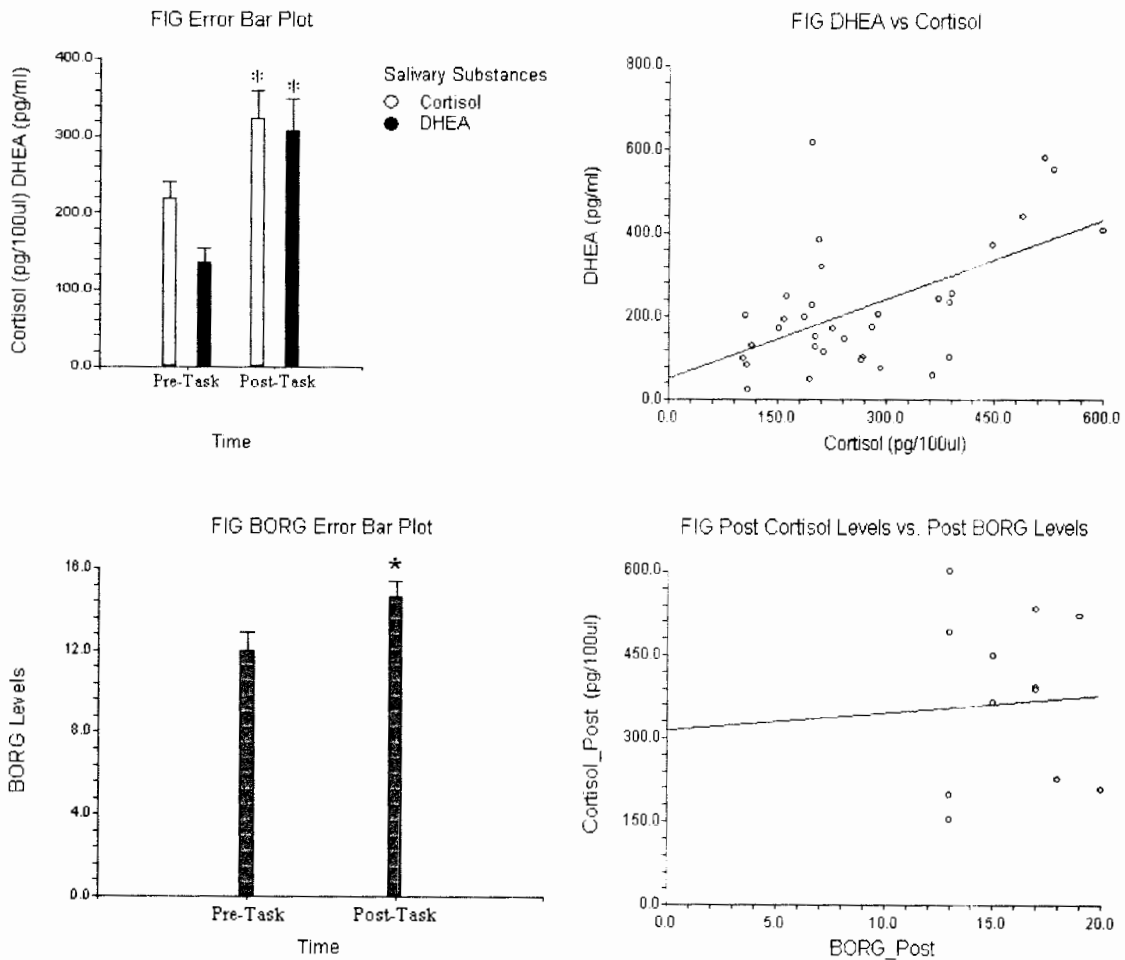


Figure 8. FIG Military Training Group. Asterisks (*) indicate statistical significance.

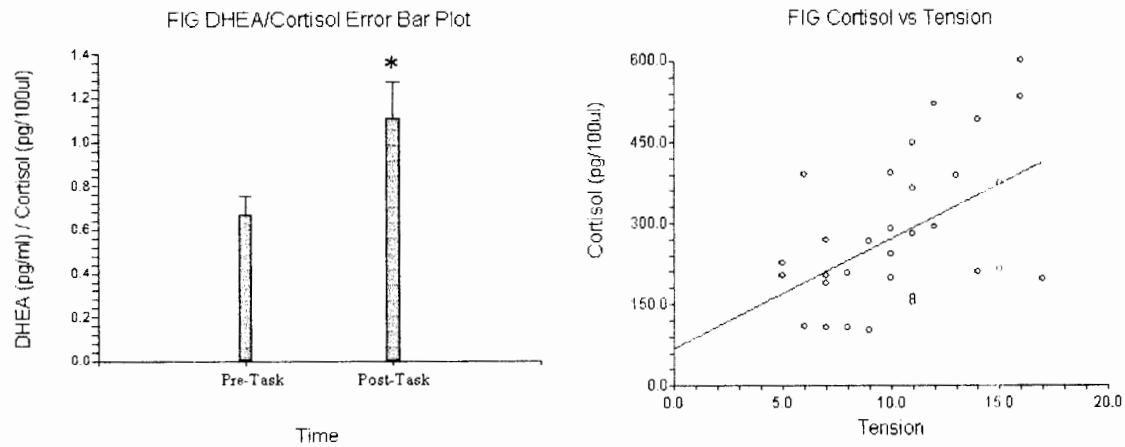


Figure 8 (cont'd). FIG Military Training Group. Asterisks (*) indicate statistical significance.

Exam Groups

The E1pm (Figure 9) group showed no significant increase in either cortisol [$T=-0.087$, $P=0.934$] or DHEA [$T=0.618$, $P=0.564$] between the two time points using the paired t-test. Cortisol levels remained constant around 140pg/100 μ l and DHEA only varied slightly between 300 and 320pg/ml, though the change is not statistically significant. A linear regression between cortisol and DHEA showed no significant correlation [$R^2=0.179$, $F(1,11)=2.180$, $P=0.171$]. A paired t-test analyzed the D/C ratio, yielding no significant change between time points [$T=0.244$, $P=0.817$].

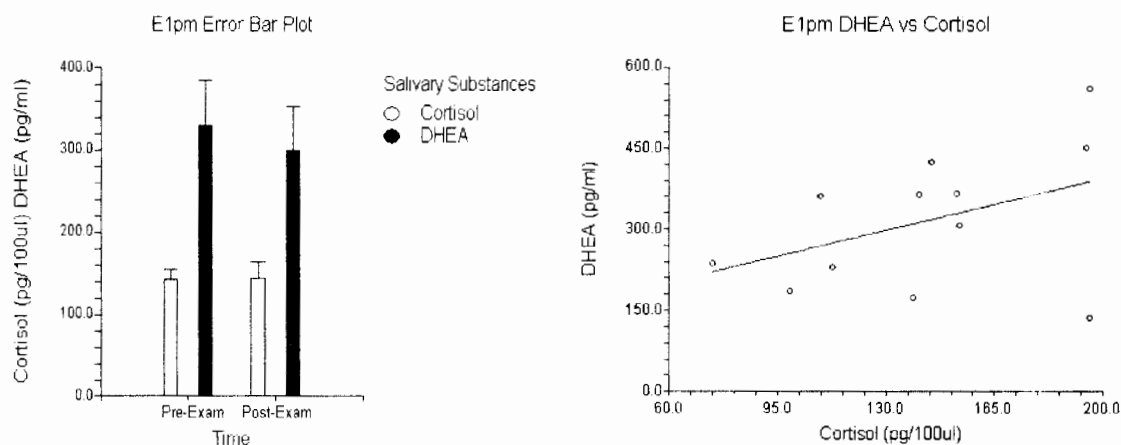


Figure 9. E1pm Exam Group. Asterisks (*) indicate statistical significance.

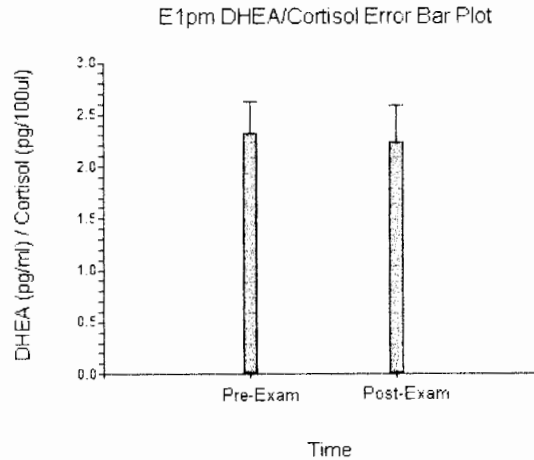


Figure 9 (cont'd). E1pm Exam Group. Asterisks (*) indicate statistical significance.

Using rmANOVA, the E2 group (Figure 10) showed a significant increase in cortisol in the pre-exam sample, compared to those collect after the exam (post-exam) and the post-control sample. [F(3,39)=5.280, P=0.005*], with highest levels around 350pg/ μ l. There was no significant change in DHEA levels across the four time points [F(3,39)=2.320, P=0.098]. The linear regression between cortisol and DHEA showed a significant positive correlation [R²=0.197, F(3,39)=9.321, P=0.004*], showing a simultaneous rise and fall with cortisol and DHEA. Again, the rmANOVA yielded no significant change in D/C ratios across time points [F(3,39)= 1.200, P=0.328].

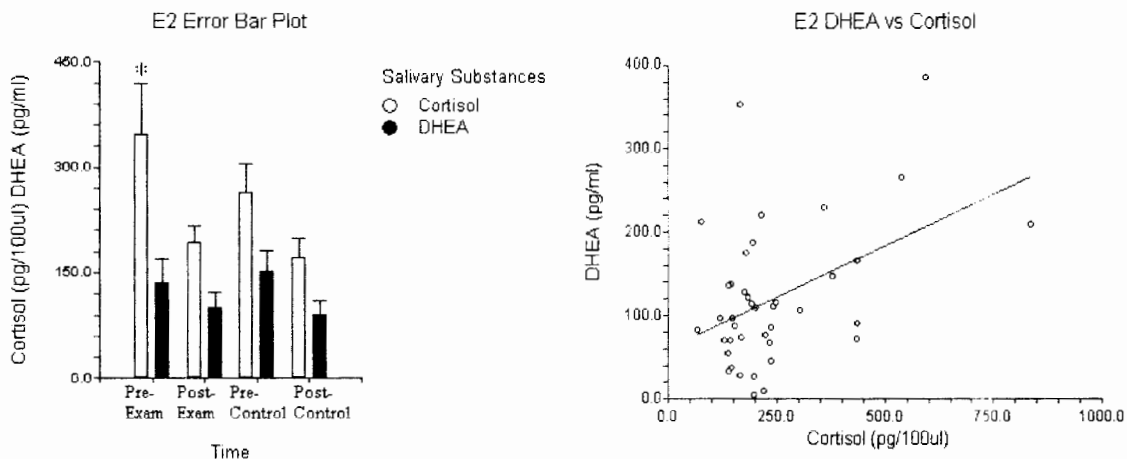


Figure 10. E2 Exam Group. Asterisks (*) indicate statistical significance.

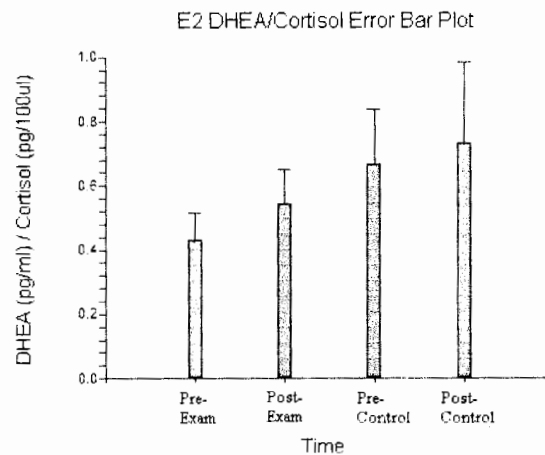


Figure 10 (cont'd). E2 Exam Group. Asterisks (*) indicate statistical significance.

The E3 group (Figure 11) should no significant change in cortisol [$F(3,19)=1.020$, $P=0.418$] or DHEA [$F(3,19)=0.900$, $P=0.468$] across the time points. Cortisol varied only slightly between 160 and 190 pg/100 μ l, while DHEA varied between 260 and 360pg/ml. A linear regression showed a positive correlation between the two [$R^2=0.269$, $F(3,19)=6.631$, $P=0.019^*$] indicative of cortisol and DHEA varying together. An rmANOVA yielded no significant changes in D/C ratios across time points [$F(3,19)=0.890$, $P=0.476$].

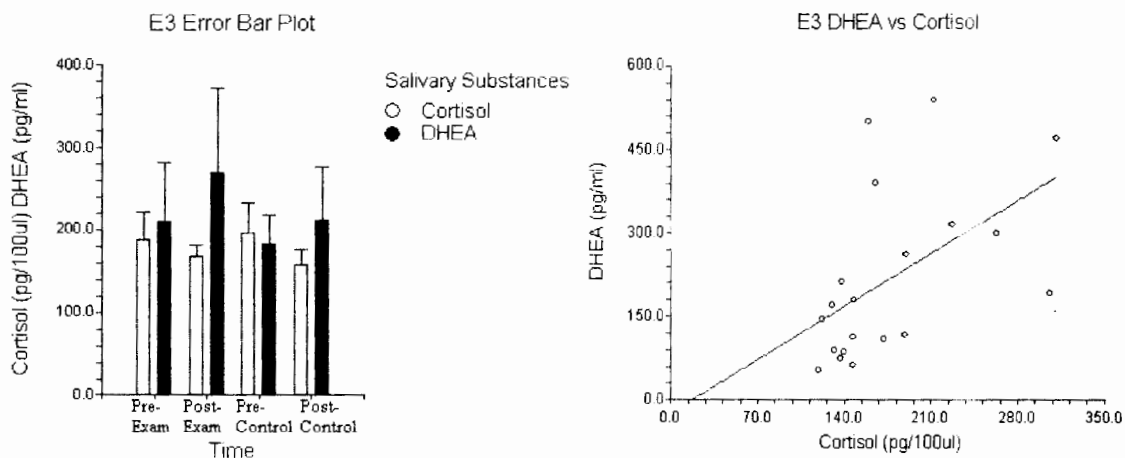


Figure 11. E3 Exam Group. Asterisks (*) indicate statistical significance.

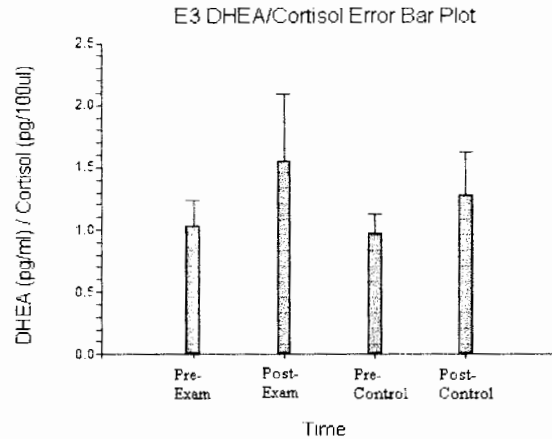


Figure 11 (cont'd). E3 Exam Group. Asterisks (*) indicate statistical significance.

The E4 group (Figure 12) – using rmANOVA – displayed a significant increase in cortisol in the pre-control sample compared to the post-exam and post-control samples, but not the pre-exam sample [$F(3,15)= 7.190, P=0.009^*$], peaking around 270pg/100 μ l. There was no significant change in DHEA levels across the time points [$F(3,15)= 1.690, P=0.238$]. DHEA levels varied between 260 and 360pg/ml. Once again, linear regression showed a positive correlation between cortisol and DHEA [$R^2=0.291, F(3,15)= 5.742, P=0.031^*$], indicating cortisol and DHEA levels varied together. The D/C ratios were analyzed using rmANOVA; the results showed no significant changes across the time points [$F(3,15)= 1.660, P=0.215$].

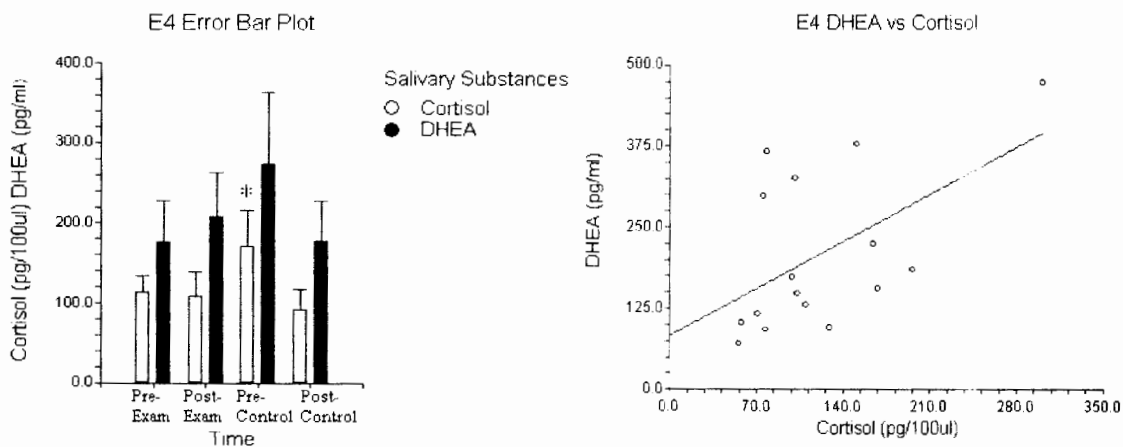


Figure 12. E4 Exam Group. Asterisks (*) indicate statistical significance.

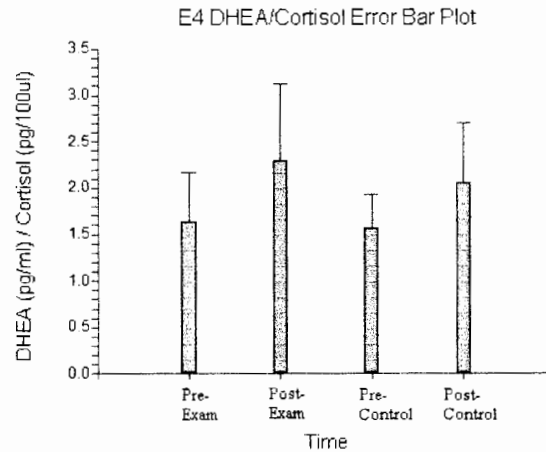


Figure 12 (cont'd). E4 Exam Group. Asterisks (*) indicate statistical significance.

Group Analysis

In addition, the groups were analyzed together, using rmANOVA to ascertain if D/C ratios were significantly higher in any one particular study group over another. The test yielded a significantly higher D/C ratio in the FIG and Exam Groups compared to the Taser and PAD groups [F(3,307) (3, 11)=9.400, P=0.004*].

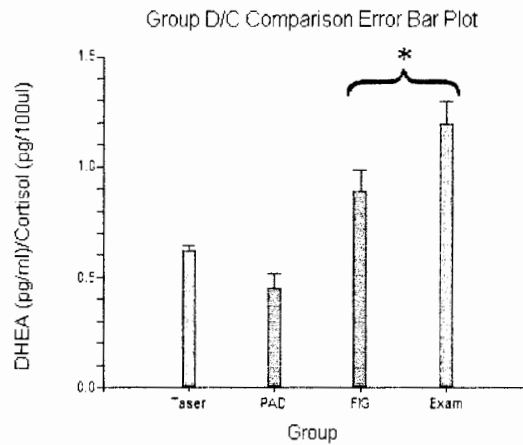


Figure 13. Group D /C Comparison. Asterisks (*) indicate statistical significance.

Discussion

Taser (FC, CH, SF, NB, FFX, WH)

The majority of the Taser groups behaved in a similar fashion. All groups, except the SF group, had a peak in both cortisol and DHEA during the second time point, 20 minutes after the tasing exercise. This 20 minute mark, as mentioned earlier, is the peaking point of cortisol after termination of a stressor (Petrides, 1994), indicating the time at which cortisol levels are highest in the body. This may also mark the peaking point for DHEA since they are on the same synthetic pathway (Netherton, et al., 2004) so levels may also be highest in the body at this point. For all groups, this rise in cortisol was a significant increase compared to the baseline sample and the sample collected the following morning. This also applies to DHEA levels in all the groups, save the SF group, which did not see a particularly noticeable change throughout the study.

The peak at the post-20 minute mark allows for supposition that cortisol levels rose in reaction to the tasing event, as opposed to anticipation of the tasing exercise. If anticipation were the cause of rise in cortisol, then a peak would have been seen at the first time point when the baseline sample was taken, which was not the case. If cortisol levels rose in both anticipation of and in reaction to the tasing exercise, a peak would have appeared during both the baseline samples and the post-20 minute samples, which was also not seen here. The post-20 minute peak could be due to a combination of anticipation and the taser; however, the FC group gave baseline samples immediately before the tasing. If anticipation were the cause, a peak in cortisol would be seen in the baseline sample of this group; however, the lack of a cortisol peak in these samples mitigates anticipation as a cause for increased cortisol. This suggests that Tasing with a salivary cortisol biomarker can be used as a positive control for a physical stress response. Other studies, such as

the one performed by Petrides et al. used treadmill exercise to induce physical stress upon the body (1994) and were able to see a significant increase in blood plasma cortisol levels, with an average peak as high as 900nmol/L ($3 \cdot 10^4$ pg/100 μ L) (Dubai ML, 2002; Maguire, et al., 2007). Salivary cortisol levels represent about five percent of blood cortisol levels (Netherton, et al., 2004) so a quick conversion of the average levels gathered in the taser study yields an approximate plasma cortisol level of approximately $2 \cdot 10^4$ pg/100 μ L. Another study using skydiving preparation as the psychological stress (Chatterton, et al., 1997) showed significant increases in salivary cortisol, peaking around 20nmol/L (about 724pg/100 μ L), in response to the stressor. The study performed by Morgan, et al. (2004) also showed an increase in salivary cortisol in response to a survival training/interrogation stressor, with an average peaking concentration of 0.87ug/dL (870pg/100 μ L). These values are similar, factoring in standard deviation, validating taser as an acceptable positive control for cortisol stress response.

The rise in DHEA seems to be in response to the tasing event, as well. Studies have shown DHEA can act as an anxiolytic hormone (Morales, et al., 1994) and can have antagonistic effects against glucocorticoids, such as cortisol (May, et al., 1994). Levels may increase to combat the possible negative effects of high corticosteroid concentrations in the body, such as negative effects on cognition (Bender, et al. 1988).

The D/C ratios analyzed yielded similar results across groups, with the exception of the SF and WH groups. For all the others, there was not a significant change in D/C ratios across the time points. This means throughout the study the levels of DHEA and cortisol rose and fell in the same proportions at each point a sample was taken. There was no particular point at which the DHEA

increase was dwarfed by the cortisol increase or vice versa. One exception, the SF group saw a significant increase in the D/C ratio in the baseline sample compared to the other samples. The other exception was the WH group who had a significant increase in D/C ratio in the third sample, taken the morning after the tasing. Despite this, it may hold no physiological significance, although further testing should be done.

The WH group data underwent additional testing to determine if cortisol levels were correlated with the duration of exposure to the taser. The results indicated no correlation, meaning an increase in cortisol did not coincide with a longer or shorter exposure. While one cannot draw causal implication from correlation data, it seems safe to assume cortisol levels are not dependent upon the length of time the subject is exposed to the taser.

Disaster Drill (PAD)

The disaster drill used to study cortisol levels in a relatively psychological setting. The subjects were those in charge of the drill, rather than the drill participants; these subjects reported feeling very little, if any, physical exertion throughout the study, which is supported by the analysis of BORG RPE data. Both the cortisol and DHEA levels fell from the beginning of the drill to the end which reveals the time of day was responsible for the cortisol response. Cortisol follows a circadian rhythm with the highest levels in the early morning and dropping off through the day (Ice, et al., 2004, Weitzman, et al., 1971). The pre-drill samples are slightly lower than normal morning values at approximately 400pg/100ul (Ahn, et al., 2007; Wüst, et al., 2000); the post-drill samples coincide with normal noon/early afternoon values at approximately 150-250pg/100ul

(Ahn, et al., 2007). DHEA also follows a circadian rhythm (Guagnano, et al., 2001) which account for the change in DHEA levels.

There was a significant correlation between cortisol and DHEA; however both hormones follow circadian rhythms so this is expected. There was no correlation between the post-drill cortisol levels and the post-drill BORG ratings, although this was expected. The BORG levels stayed consistent throughout the study while cortisol levels dropped so correlation between the two is not likely. Again, an attempted correlation between cortisol and tension yielded no correlation. Any changes in the levels of tension the subjects felt did not coincide with changes in cortisol levels. This agrees with a psychological stress study performed by Noto, et al., who studied the relationship between salivary biomarkers, including cortisol, and the STAI scores using mental arithmetic as the stressor. Their study found no correlation between the two (2005). Analysis of the D/C ratios yielded no significant change between time points. This follows along the fact that both cortisol and DHEA follow a circadian rhythm and levels are in the process of dropping.

Military Training Facility (FIG)

A significant rise in cortisol and DHEA were seen in the post-task samples, which is indicative of a stress response. If no stress response were present, cortisol and DHEA levels would drop in accordance with their circadian rhythms. However, the stress response seen is not characteristic of a high stress response since is not dramatic. This could be due to the constraints of the study. Due to the high heat and highly physical nature of the task, the post sample was collected immediately after the task, as opposed to the standard 20 minutes. Twenty minutes after the termination of the task would have marked the point at which cortisol peaks in the body, but the subjects would not

have been able to wait an additional 20 minutes without water to provide a clean sample. Presently, studies are being conducted to determine if water is a confounding variable in salivary cortisol and DHEA samples. Again, cortisol and DHEA levels correlated in the set of subjects.

The BORG data analysis indicates the subjects felt as if they had exerted themselves. The levels changed from 12 on the BORG RPE scale (light exertion) to about 15, which is considered hard exertion (CDC, 2007). No correlation was found between cortisol and the BORG ratings, which was unexpected. Cortisol is hallmarked as the stress hormone, especially for physical stressors (Aryeh, et al., 1989; Deuster, et al., 2000) so the lack of correlation between the RPE ratings and cortisol levels goes against the grain. A correlation between cortisol and tension was found in this group. This contradicts a study that took place in Japan, whose results showed no correlation between the STAI scores and cortisol levels due to a psychological stress that consisted of mental arithmetic (Noto, et al., 2005). However, this is supported by Singh, et al., whose study showed a significant increase in plasma cortisol levels (500nmol/L (about 18,000pg/100µL) for high responders; 350nmol/L (about 1100pg/100µL) for low responders) after a psychological stressor (1999). Salivary cortisol tends to reflect approximately five percent of the plasma concentration (Netherton, et al., 2004), corresponding salivary levels would be about 900pg/100µL for high responders and 55pg/100µL for low responders. This particular study was focused on physical stressors, such as the obstacle course, but it is nearly impossible to remove the psychological component. The stress induced by the pressure to perform in front of one's superiors and peers suggests that this drill is a combination of psychological and physical components. In comparison, a study performed by Ben-Aryeh, et al. showed a non-significant increase in salivary cortisol after either a 30-second Wingate anaerobic test or a submaximal cycle exercise that lasted for nine

minutes. For the Wingate test, the pre-exercise levels were 6.54 ± 2.84 ng/ml (654 pg/ 100μ l) and the post levels were 6.68 ng/ml (668 pg/ 100μ l). The submaximal pre- and post- levels were 6.55 ± 1.75 ng/ml (655 pg/ 100μ L) and 8.32 ± 3.51 ng/ml (832 pg/ 100μ L), respectively (1989). It is possible the psychological component in the FIG study led to a significant increase, compared to the Ben-Aryeh, et al. study.

The analysis of D/C ratio yielded a significant increase in the post-task sample. There are many possible explanations for this. For one, DHEA may peak sooner than cortisol and could be peaking at the time the sample was taken. Secondly, it is possible that DHEA is released faster than cortisol due to the fewer number of steps necessary to synthesize it in the HPA axis (Ganong, 1989/2005), resulting in a quicker increase in DHEA level thus increasing the ratio.

Exam (E1pm, E2, E3, E4)

The exam groups showed the most discrepancy among all the groups. The E1 group showed no noticeable changes across the time points; however their DHEA levels were extremely high for the time of day (Ahn, et al., 2007) and cortisol levels were lower than normal, but not significantly so. One possible explanation is the subject demographics. Five of the six participants were females, who naturally have higher DHEA levels (Sulcova, et al., 1997) peaking in young women between the age of 15 and 19 (Wellman, 1999). Studies have shown that women taking oral contraceptives have significantly less active unbound cortisol available due to the increase production of corticosteroid-binding globulin in response to the contraceptive (Kirschbaum et al., 1999). This could be the cause of the slightly blunted salivary cortisol response seen here. In addition, the average age of the group was 19 years old, and DHEA levels are higher in younger people and

start to drop as one gets older (Ferrari, et al., 2001; Orentreich et al., 1984). Another explanation is the subject group is too small (n=6) to properly draw any conclusions. No correlation was seen between DHEA and cortisol, but again, the subject number may be too small to accurately analyze the data.

Examination of the E2 results shows a significant increase in cortisol in the pre-exam sample compared to the other samples, indicating an anticipatory stress response. These levels do not show a high stress response for this time of day; however if one were to deconstruct the data and look at individual subjects' responses (Figure 14), it is possible to see two groups of subjects with the study: responders and non-responders. For example, subjects 3 and 11 are clearly responding to a stressor due to the high cortisol levels, whereas subjects 4 and 7 are not responding, whose levels stay constant and within a normal range for the time of day across time points. This discrepancy shows the importance of deconstructing the data and determining how the subjects are really responding to the stressors presented.

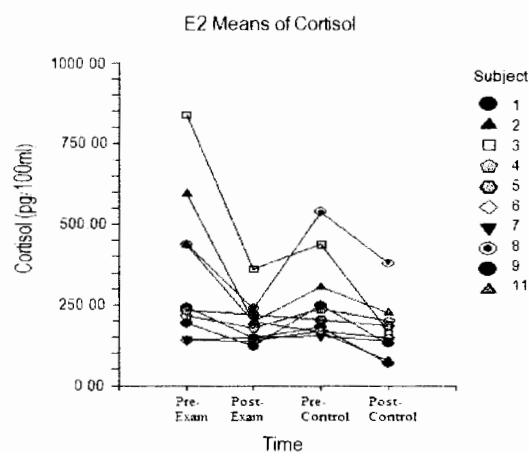


Figure 14. E2 Group. *Deconstruction of cortisol data.*

The subjects in E3 did not seem to undergo a stress response, judging by the low levels of cortisol in the pre-exam samples. High levels in this sample would have indicated anxiety or anticipation of the upcoming exam, but levels fell within the range of normal values for the time of day (around 1pm) (Ahn, et al., 2007). The E4 group saw a significant increase in cortisol levels in the pre-control sample; however, while this is statistically significant, it is highly unlikely physiologically significant since the levels were within the normal evening concentration range (100-150pg/100ul range) (Ahn, et al., 2007). These exam groups provided varying results, although a study by Cook, et al. found increases in cortisol immediately before sitting an exam, but not during the days beforehand or earlier the same day (1992), which could be indicative of acute anticipatory stress (Hill, et al., 2001). It is possible that cortisol only responds to psychological stressors when there is an actual risk of physical danger, which was seen in volunteer parachute jumpers immediately before jumping (Hill, et al., 2001). Despite this, the majority of the subjects in this study did not find the exams stressful, as indicated by the results. DHEA and cortisol were correlated in both groups, but again, both levels were normal for the time of day and are expected to be proportionate. D/C ratios in E3 and E4 were analyzed, and no particular time point was significantly different from the others. The post-levels (both exam and control) were slight higher, but not significantly so. It is possible that cortisol only responds to psychological stressors when there is an actual risk of physical danger, which was seen in volunteer parachute jumpers immediately before jumping (Hill, et al., 2001). Despite this, the majority of the subjects in this study did not seem to find the exams stressful, as indicated by the results.

Group Comparison

The D/C ratios from each major group were combined, averaged and then compared to the other major groups. The results indicated significantly higher D/C ratios in the FIG and Exam groups than the Taser and PAD groups (Figure 13). This leads to the possibility that age is more of a factor in D/C ratios than military training. The average ages of all the Taser subjects and the PAD subjects were 23.0 and 46.8 years old, respectively. The average ages for the FIG and Exam groups were 19.8 and 19.6, respectively. It has already been shown in publications that age is a major factor in DHEA levels in the body, with higher levels in younger people, and a marked decline as age increases (Ahn, et al., 2007; Orentreich et al., 1984). This contradicts the study done by Morgan, et al. where military trained personnel showed higher D/C ratios (Morgan, et al., 2004). However, Morgan's study compared DHEA-S (the sulfate derivative of DHEA) – which is found only in blood plasma – and salivary cortisol, which may yield different results than a ratio comparing two salivary substances. The two groups with lower D/C ratios had the higher average age; in addition, each of the low/high D/C ratio sets consisted of what would be considered a 'trained' group (Taser and FIG) and an 'untrained' group (PAD and Exam group). Granted, the trained Taser group had a higher D/C ratio than the untrained PAD group, it was not significantly different, and the Taser group still had a much lower age average than the PAD group. The high D/C ratio groups had approximately the same age average (19.8 versus 19.6) and the trained FIG group had lower –though not significantly – D/C ratio than the untrained Exam group, which further contradicts Morgan's study. However, gender must be accounted for in this comparison. The exam group had a much greater number of females volunteer for the study (20 in the exam group compared to 5 in the FIG group) and studies have been inconsistent in determining the reactivity of the HPA axis in males and females. Males

tend to have a stronger response to stressors (Kirschbaum, et al., 1992) but the different phases of the menstrual cycle may affect the reactivity of the HPA axis in females (Kirschbaum, et al., 1999) so this could be a confounding variable affecting the D/C ratio within the groups. Additionally, these groups underwent a combination of stressors, both psychological (PAD and Exam) and physical (Taser and FIG) while the subjects in Morgan's study had only undergone psychological stress, making it difficult to draw a straight comparison between the studies.

Conclusion

In conclusion, cortisol is excellent biomarker for stress, especially physical stressors such as tasing and extreme physical exertion. Inconsistent findings with regards psychological stress in this study indicate the exams taken by the subjects were not stressful for the majority of them; although many other studies (Chatterton, et al., 1997; Morgan, et al., 2004) have been successful in showing a significant cortisol response due to psychological stress. The possibility exists that psychological stressors such as parachuting, skydiving, etc. are the ideal scenarios for studying the stress response, but there is a chance the subject population would over-represent the types of people who would participate in this kind of activity – the thrill-seekers – and under-represent the more cautious people in the general population (Chatterton, et al., 1997). To compensate for this discrepancy, mental arithmetic or public speaking could be used as psychological stressors, both of which have been able to provide reliable, reproducible increases in salivary cortisol (Kirschbaum, et al., 1992).

DHEA as a reliable biomarker is still under consideration. Many confounding variables exist with this hormone that are not an issue with cortisol, such as concentration differences across

ages and gender, and possibly even within gender since the female menstrual cycle may have an effect on DHEA levels. Wide-scale studies on DHEA activity are needed to sort out any inconsistencies within the data collected so far.

In terms of collection methods, salivary samples seem to be the most efficient and non-invasive method available. It allows for fast, on-the-spot sampling, without the need for a trained professional to collect the samples; whereas blood samples require a trained phlebotomist on site to perform each collection and can be very time consuming. It is not without its flaws, however. Studies are needed to confirm if food and/or beverages (coffee, water, sports drinks, etc.) contaminate the sample and interfere with salivary hormone concentrations.

Lastly, studies have shown the separation of responders within sample populations, as seen with the E2 exam group here. One particular study showed that subjects classified as ‘high-responders’ to the physical stressors (those whose cortisol levels increased significantly over their baselines in response to exercise) were also high-responders to the psychological stressors. The same held true for low responders – they had low responses to both the physical and psychological stressors (Singh, et al., 1999). While additional studies should be done about this matter, this could be a major breakthrough in determining a person’s susceptibility to stress and allow for a more specific selection process for high-stress military and emergency positions. While it is only speculation, if a selection process for those more capable of handling stress is established, it has the potential to greatly reduce the number of military and emergency personnel who develop stress-related disorders, which would benefit everyone in the long-run.

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