THE EFFECTS OF EXOGENOUS TESTOSTERONE ON CARDIOVASCULAR STRESS

by

KEVIN SOON-SIAN LAI

A THESIS

Presented to the Department of Psychology
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

June 2016

An Abstract of the Thesis of

Kevin Soon-Sian Lai for the degree of Bachelor of Science
in the Department of Psychology to be taken June 2016

Title: The Effects of Exogenous Testosterone on Cardiovascular Stress

Approved: _____

Pranjal Mehta

Although testosterone has recently been discovered to play a role in the biological stress response, its exact function is unclear. With the increased number of annual deaths around the world being attributed to heart disease, it is important to discern how hormones like testosterone influence the magnitude of the cardiovascular stress response. It is also critical to acknowledge the possibility of biological and psychological factors that may moderate these differences, such as anxiety: a state of inner worry or uneasiness that may be present prior to stressor exposure. This experiment examined cardiovascular and autonomic responses to psychosocial stress in healthy males aged 18-45 (n = 120) that were either given exogenous testosterone or placebo before being subjected to a social-evaluative stressor. Individual levels of trait anxiety were used as a moderator of testosterone's effects to the stress response. Results showed no significant effects of exogenous testosterone on heart rate and heart rate variability, suggesting that testosterone does not play a direct role in modulating the cardiovascular response to stress.

Acknowledgements

I would like to deeply thank Professor Pranjal Mehta for helping me to fully examine the specific topic and consider the various contexts related to this subject matter. I would also like to express my sincerest gratitude to Erik Knight for introducing me to this fascinating realm of psychological research as well as his dedicated supervision of my thesis work. I am additionally grateful to the numerous other research assistants and doctoral students who assisted me in the experimental and data gathering process. I would like to express my sincere gratitude to CHC faculty Professor Mai-Lin Cheng and Academic Coordinator Miriam Jordan in providing constant feedback so that I could successfully meet deadlines. Last, but not least, I thank my family and friends for their utmost support and faith in my undergraduate endeavors.

Table of Contents

List of Figures

List of Tables

Introduction

The Heart Problem

Heart disease remains the leading cause of death for both men and women worldwide (CDC, 2015). In the United States alone, approximately 610,000 individuals die of heart disease each year – accounting for about 1 in every 4 deaths annually (CDC, 2015). It is theorized that psychological stress is a key mechanism in poor long-term cardiovascular health including long-term, stressful experiences such as high-effort/low-reward conditions in the workplace (Siegrist, 1996), as well as acute, emotionally stressful situations, as in the case of the recently discovered Takotsubo cardiomyopathy, also known as "broken-heart syndrome" (Virani et al., 2007). Thus, it is important to study the various factors that influence cardiovascular reactivity in stressful contexts – in other words, what affords human resistance to stress in certain situations, and what exacerbates it in others.

Stress

Stress can be defined as "the generalized, nonspecific response of the body to any factor that overwhelms, or threatens to overwhelm, the body's compensatory abilities to maintain homeostasis" (Sherwood, 2012). Because such onsets of extraneous pressure are continuously present in everyday life, the human body has developed complex mechanisms to deal with the strain. In the case of the cardiovascular system, stress typically results in an increase in heart rate (HR) in order to provide the body with needed energy, oxygen, and nutrients to combat the stressor or run away from it. A key component of the physiological management of this stress response lies in the interplay

between the sympathetic and parasympathetic pathways of the nervous system (Appelhans & Luecken, 2006). Both the sympathetic and parasympathetic components play an antagonistic role towards one another: when at rest, HR is kept at a slower, but more variable rate under strong parasympathetic (vagal) influence. On the other hand, the parasympathetic nervous system will actively withdraw during stress to allow the sympathetic nervous system to take control and stimulate the heart, resulting in a faster, less variable HR (Sherwood, 2012). This measure of heart rate variability (HRV) indicates overall parasympathetic nervous system activity and acts as a proxy for the relative strength of vagal influence over the heart and the rest of the viscera.

Repeated and prolonged activation of the stress response may in fact correspond to overall detriment of the heart itself, seen in increased HR and decreased HRV (Brosschot et al., 2005; Krantz & McCeney, 2002). Furthermore, the amount of parasympathetic activity may link to the tendency to externalize or internalize health problems related to stressful situations such as martial conflict (El-Sheikh et al., 2001), immune system functioning (Thayer & Brosschot, 2005), and mood and anxiety disorders (Thayer & Lane, 2000). Therefore, studying HRV as a component of the stress response allows examination of a reliable predictor for overall health.

Hormones

In addition to the autonomic nervous system, endocrine systems are also fundamental components of the body's response to stressors. Cortisol is released as part of the hypothalamic-pituitary-adrenal (HPA) axis response to stress in order to metabolize amino acids, carbohydrates, and lipids in the body to sustain the brain during exposure to stressors (Sherwood, 2012). While cortisol is generally studied as

the principal stress hormone, recent primary literature has indicated that other hormones may modulate stress responses as well. Testosterone, a hormone usually associated with both physical and behavioral traits of masculinity, may in fact play a role in the regulation of the stress response, either by modulating the endocrine response to stress directly (Rubinow et al., 2005) or by altering the psychological response to the stressor (Josephs et al., 2003). However, previous studies have not tested to what extent testosterone impacts cardiovascular responses to social stress, and if so, whether testosterone will increase or decrease these responses.

There is evidence that basal testosterone is related to cortisol increases after a stressor related to status threat, for example after experiencing defeat in a dominance contest. High testosterone men who lost in a dog agility competition rose in cortisol, and low testosterone men experienced no difference in cortisol change regardless of winning or losing (Mehta et al., 2008). In a rigged videogame competition, post-contest increased cortisol levels were associated with high levels of basal testosterone among losers (Zilioli et al., 2013). At least in regards to competition stress, these studies point to a relationship between testosterone and increased stress reactivity.

On the other hand, there is evidence in both non-human animals and humans for the opposite pattern. Recent research on acute, social-evaluative stress revealed that basal levels of testosterone predicted reduced cortisol response (Stephens et al., 2015). Similarly, testing acoustic startle reflex and skin conductance responses in women after administration of testosterone or placebo showed that exogenous testosterone buffers the stress response, rather than boosts it (Hermans et al., 2007). Stimulated cortisol levels and peak cortisol were also reported to be significantly lower during testosterone

replacement in corticotropin-releasing hormone (CRH) stimulation tests during gonadal suppression (Rubinow et al., 2005). As it stands, classifying testosterone as a buffer or a promoter to the stress response remains open for debate.

Anxiety

Despite this present ambiguity, it may be possible that various psychological components of stress moderate testosterone's functional relationship to stress. A strong contender for this role is anxiety. According to the DSM-V, anxiety is "the anticipation of future threat" (American Psychiatric Association, 2013), resulting in general nervousness and unease. The degree to which an individual perceives an incoming situation as stressful – due in part to one's anxiety levels – may moderate testosterone's influence on the cardiovascular stress response.

On a biological level, neurons in certain parts of the brain are highly attuned to changes in the environment that the individual may or may not need to fear. Of note, the amygdala – the part of the brain often associated with fear – and the hippocampus – the part of the brain associated with processing environmental information as well as memory – are particularly sensitive to these anxiety fluctuations (Leuner & Shors, 2013). Furthermore, testosterone may interact with these brain areas that underlie anxiety, such as modulating amygdala reactivity (van Wingen et al., 2009). During an onset of anxiety, changes in synaptic plasticity at these regions facilitate the traits generally coupled with an anxiety attack. In other words, anxiety can be perceived as a preemptive response to a stressful event. This form of anticipation may then play a role in how the future stressor is managed upon its onset.

Furthermore, the vagus nerve may play a role in anxiety due to its substantial role in feeding information back into the brain. Eighty percent of the vagus nerve fibers are afferent (Berthoud & Neuhuber, 2000), feeding critical information on the current physiological state of the body back to brain. The vagal mediated feedback results then in a cascade of mechanisms and subsequent physiological changes built to cope until the stress subsidies. As the vagus nerve feeds information to the brain about a possible stressor, an individual may take register of the physiological changes produced in response, such as increased HR (Bechara & Naqvi, 2004). Thus the vagus nerve may underlie the conscious experience of one's internal physiological state – called *interoceptive awareness* – that can be a component of clinically-significant anxiety (Thayer & Ellis, 2010; Dunn et al., 2010). By studying the HRV responses as it relates to anxiety, the results obtained may prove useful for both medical and psychological clinicians.

Although previous studies have not found anxiety to directly predict cardiovascular stress responses (Filová et al., 2015; Girlder, et al., 1997), testosterone could moderate this effect due to its own relation to the cardiovascular system (Norman et al., 2015). Experimental data suggests that self-reported levels of anxiety may in fact play a role in the interactions between stress hormones (like cortisol) and the cardiovascular system. Upon performing mental math tasks, student state anxiety scores were significantly correlated with recovery from the stressor, whereas their trait anxiety scores were significantly correlated with their resting period (Dimitriev & Saperova, 2012). Thus, it is important to explore whether or not anxiety levels act to moderate this perceived relationship between testosterone and the cardiovascular stress response.

The Cardiovascular System & ECG

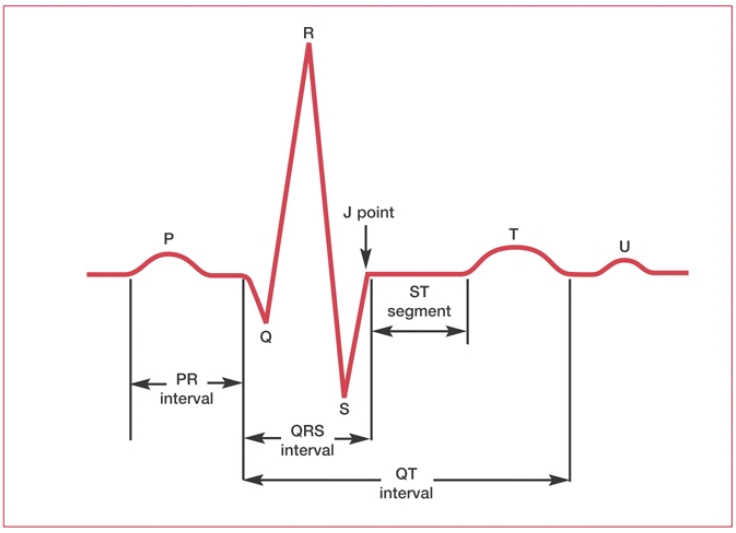

Figure 1. ECG Waveform.

The three major forms of note are the P wave, QRS complex, and the T wave. The P wave shows atrial depolarization of the subject heart, the QRS complex shows ventricular depolarization, and the T wave shows ventricular repolarization (Sherwood, 2012). Image was taken from Ashley E.A., & Niebauer J. (2004). Chapter 3: Conquering the ECG. *Cardiology Explained*. London: Remedica.

Whether or not testosterone or anxiety holds direct relevance in the stress response, natural resistance to stress can be greatly attributed to the nature of the human cardiovascular system itself. The cardiovascular system is a vital component of the human body: the pumping of blood distributes oxygen and removes carbon dioxide while allowing other nutrients to be delivered to where they are needed (Sherwood, 2012). In addition, the heart also assists in maintaining homeostasis through regulation of blood temperature and salinity (Sherwood, 2012). Overall activity of the heart can be measured by an electrocardiogram (ECG), in which electrical currents generated by heart tissue depolarization and repolarization can be surmised in a few simple waveforms (Fig 1). The number of beats generated per unit time describes HR. By

comparing time between each waveform as well as the frequency of each consecutive complex to normal rates and rhythms, a trained medical expert can identify any abnormalities that may lead to heart complications.

Purpose

Despite these correlations between hormones, stress, and cardiovascular responses to stress, whether or not testosterone acts almost exclusively as an enhancer or buffer of the cardiovascular stress response remains to be seen. Thus, this experiment investigated causal routes for exogenous testosterone's negative or positive effect on stress-induced situations, in addition to anxiety levels as a possible biopsychosocial moderator of the cardiovascular response to stress. Experimental results will elucidate possible interactions between hormonal and cardiovascular systems in stressful contexts, which may have clinical significance for stress resilience.

Methods

Participants

The sample pool consisted of 120 healthy male participants between the ages of 18 and 45 years old, as part of the "Effects of Testosterone on Human Social Behavior and Decision-Making" Protocol. Before enrollment, each participant was screened for physical or mental health issues. Informed consent was obtained at screening and at the time of their experimental session.

Protocol

The experimental protocol for each participant lasted approximately six hours, and was broken into two major sections: the morning and afternoon section, which was separated by a short lunch break. During the first section, consent forms were signed and baseline cardiovascular activity was recorded for five minutes while the participant rested in an individual testing room.

Androgel Application

The participant was then treated with either testosterone or placebo. Testosterone was administered in the form of Androgel, an FDA-approved trans-dermal topical gel used for treatment of male hypogonadism. The 162-mg dose was chosen by consultation with collaborators, under approval from the study's medical advisor[1]. The placebo consists of a gel composed of the same Androgel ingredients with exception of the active testosterone ingredient. Application of the gel was carried out by the gloved

[1] Androgel has yet to be approved for female usage, thus restricting the sample exclusively to males.

participant on two sites: the shoulders and upper arms, with a drying time of between one and five minutes (Fig 2).

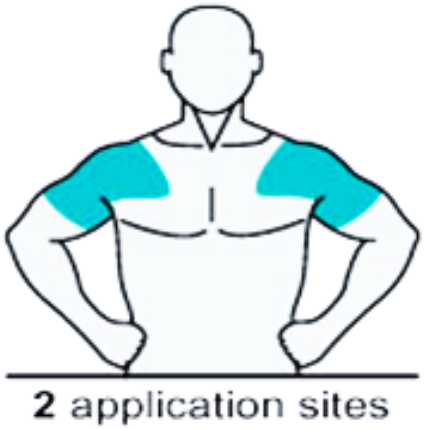

Figure 2. Androgel Application Model.

Blue shaded areas represent sites of Androgel/Placebo application to be administered by the participant with assistance.

Approximately half of the participants received Androgel, whereas the other half received placebo. Within each of these two groups, half were informed as to which product they received, whereas the remaining half were only told they had an equal chance to be given either the Androgel or the placebo. However, in all situations, experimenters did not know which group the participant has been placed into until the conclusion of the entire study. These single- and double-blind conditions were implemented to stringently control experimenter bias during data analysis and to be able to control for the participants' expectancy effects – the expectation of a given result unconsciously affecting the outcome.

The Trier Social Stress Test

Approximately 4 hours and 40 minutes after baseline recording, participants were introduced to the Trier Social Stress Test (TSST), a laboratory procedure that induces an acute stress response in the participant (Kirschbaum et al., 1993). The TSST consists of a mock job interview speech and performing mental math in front of a panel of two observers posing as specialists. Participants were given ten minutes alone to prepare a five-minute speech as to why he was the most qualified individual for a managerial job on campus (Appendix A).

After the preparation period, participants gave their speech in front of the panel. The panel was instructed to act as neutral to the participant as possible in order to keep the situation constantly stressful across all participants. After the five-minute period, the participant immediately proceeded to a serial subtraction math task in which participants repeatedly subtracted 13 from 6233. If they said an incorrect number, they had to start over from the beginning. A script was given to each member of the panel for procedure uniformity (Appendix B). After the participant completed the TSST, the participant was escorted back to his initial testing room, where he sat passively for a 10-minute recovery period.

Cardiovascular Data

Cardiovascular activity was measured in the morning at baseline (ten minutes before the gel was applied) and for the entire duration of the TSST, with data being recorded during the ten-minute preparation, ten-minute stressor, and ten-minute recovery periods.

Cardiovascular functioning for the entire experiment was recorded with a BioPac MP150 Data Acquisition system non-invasively through the use of seven disposable biosensors attached to the participant. Three of the biosensors were used for ECG recording: placed on the right clavicle, the lower left abdominopelvic quadrant and the lower right abdominopelvic quadrant (Fig 3).

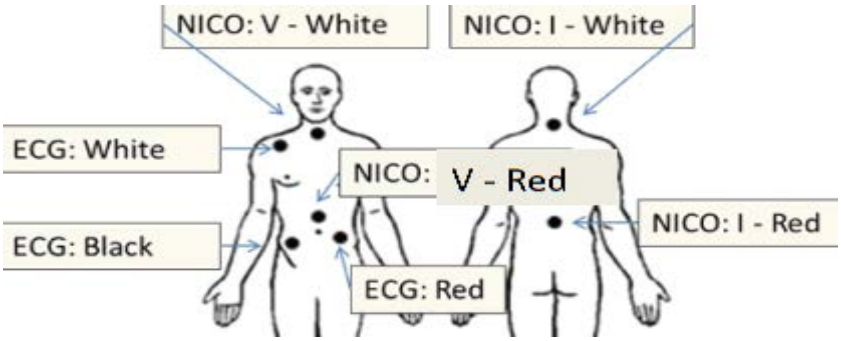

Figure 3. BioPac Sensor Placement.

Used for in-lab physiological measures.

During measuring periods, HR and high frequency HRV were measured continuously with the BioPac MP150 wireless unit, then averaged across 1-minute increments – this was done to make sure an equal amount of values were obtained for each participant. HR was recorded as the number of beats per minute (bpm). HRV was characterized by the high-frequency (HF) component (0.15-0.4 Hz) of the auto-regressive, power-spectral analysis of the participant's cardiovascular waveform. This HF output of HRV is strongly correlated with vagal input (Kamath & Fallen, 1993), making it a useful index of parasympathetic activity. This data was reported using normalized units, allowing for statistical analyses that assume normal distribution of experimental results.

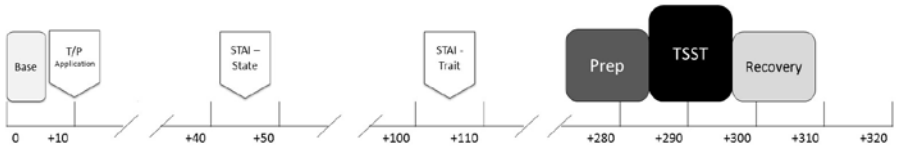

Figure 4. Timeline of Experimental Procedure.

Duration denotes ECG recording time. Bottom units are in minutes, with + denoting minutes after recording of baseline HR and HRV.

Anxiety

We examined the results of the Spielberger State-Trait Anxiety Inventory (STAI) (1983). Experimental medians were used to assign both high and low state/trait anxiety groups. The numeral results were then used to test the extent to which trait anxiety scores moderated the impact of the testosterone manipulation on cardiovascular stress responses. Based on a 4-point Likert scale, the STAI uses 40 self-reported questions to measure state anxiety (Appendix C) – levels of anxiety at a given moment – as well as trait anxiety (Appendix D) – personal levels of overall anxiety.

Data Analysis

Data was cut using ACQKnowledge to the specific durations for all four epochs: Baseline, Prep, TSST, and Recovery (Fig 4). Kubios HRV was then used to clean artifacts and separate each file into one-minute samples for all epochs. SPSS Statistics (Version 23, 2015) was used for analyses. Averages for HR and HRV as well as standard error were derived for each individual epoch.

HR and HF HRV data were subjected to separate 4 (Epoch) × 2 (Testosterone vs. Placebo) repeated measures general linear model (GLM), which controlled for

blinding assignment. To test interactions between testosterone/placebo and anxiety, the self-reported state and trait anxiety measures were entered as a continuous variable in repeated measures analyses, producing separate 4 (Epoch) × 2 (T/P) × continuous (anxiety) repeated measures GLMs for HR and HF HRV.

In order to control for departures from sphericity, a Huynh-Feldt correction was applied when $\varepsilon > 0.75$ and a Greenhouse-Geisser correction was applied when $\varepsilon < 0.75$ (Girden, 1992). Within-subject effects were subsequently analyzed. Degrees of freedom were reported for all interactions, and statistical significance was assigned as $\alpha = 0.05$, with $p < \alpha$ considered significant. Lastly, eta-squared values (η^2) were used to measure effect size – the strength of relationship between two selected variables.

Results

Measure	N	1	2	3	4	5
1. Testosterone (1) vs. Placebo (0)	120	1.000				
2. Baseline HR	115	-0.097	1.000			
3. Baseline HRV	115	0.114	-0.445**	1.000		
4. Trait Anxiety	119	0.005	0.084	-0.042	1.000	
5. State Anxiety	120	0.040	0.000	-0.015	0.594**	1.000

Table 1. Correlations between Measures.

Numeral values represent Pearson Correlation coefficients r, with positive/negative values representing a positive/negative correlation. The closer r = 0, the less closely the measures are related. ** indicates a significant correlation.

Preliminary Results

Several participants did not have baseline cardiovascular activity recorded (n = 3) due to equipment failure or because the data was too noisy to be scored (n = 2). Also, one participant neglected to answer the trait anxiety portion of the STAI (n = 1).

One hundred twenty male participants (Mean age = 21.55, SD = 3.49, range = 18-39) were used in total for the study. Significant correlations were observed between Baseline HR and HRV (Table 1), which is consistent with prior research (Mangin et al., 1998). Additionally, a significant positive relationship was observed between state and trait anxiety levels (Table 1), also in line with previous research that utilized the STAI (Fadaei et al., 2011). Thus, the experimental procedure was confirmed to have a strong working measure for obtaining accurate cardiovascular data as well as individual anxiety measures, in line with previous research.

Effect of Testosterone on Heart Rate

A main effect of time was found on HR, indicating that the onset of the social-evaluative stressor related to an expected increase in HR ($F(1.83,184.98) = 170.280$, $p < 0.001$, $\eta^2 = 0.628$). Examining the Time × Androgel/Placebo coefficient revealed no significant differences in HR response to TSST between administration of Androgel or placebo ($F(1.83,184.98) = 1.250$, $p = 0.287$, $\eta^2 = 0.012$) (Fig 5A). This indicates that testosterone does not increase or decrease the cardiovascular stress response. Participants in the testosterone group on average experienced an increase in average HR from Baseline ($M = 68.651$, $SD = \pm 9.360$) to TSST ($M = 87.504$, $SD = \pm 13.817$) with a drop to near-baseline levels during Recovery ($M = 69.103$, $SD = \pm 9.801$) (Fig 5A). This trend was also observed in the placebo group, with average HR increasing from Baseline ($M = 72.152$, $SD = \pm 9.342$) to TSST ($M = 87.853$, $SD = \pm 13.558$) followed by a decrease during Recovery ($M = 70.429$, $SD = \pm 9.705$) (Fig 5A). These outcomes suggest that testosterone does not play a significant role in moderating HR changes during exposure to a stressor.

Effect of Testosterone on Heart Rate Variability

A main effect of time was found on HRV, indicating that the onset of the social-evaluative stressor related to an expected increase in HRV ($F(2.79,282.32) = 19.127$, $p < 0.001$, $\eta^2 = 0.159$). A significant difference was observed in HF HRV response to TSST between Androgel and placebo ($F(2.80,282.31) = 2.911$, $p = 0.038$, $\eta^2 = 0.028$), though this is likely a spurious result as Androgel was found to increase HF HRV to the greatest degree during Baseline ($M = 38.414$, $SD = \pm 13.618$) in comparison to placebo ($M = 33.280$, $SD = \pm 14.918$) (Fig 5B). To further clarify these results, a follow-up GLM

was run with the latter three epochs controlling for individual baseline HRV. In this model, the Time × Androgel/Placebo was reported to be null ($F(2,200) = 0.997$, $p = 0.371$, $\eta^2 = 0.01$) (Fig 5B). Given that the testosterone vs. placebo gel had not been applied prior to collecting this data, it is unlikely that this effect extends causally from increased testosterone concentration. Additionally, these elevated levels of HF HRV were only seen during Baseline and Prep, as both TSST and Recovery yielded lower average levels of HF HRV in the testosterone group (TSST: M = 28.791, SD = ±10.344; Recovery: M = 38.220, SD = ±13.984) compared to placebo (TSST: M = 30.809, SD = ±10.010; Recovery: M = 38.408, SD = ±12.897) (Fig 5B). Thus, although a significant relationship was observed between testosterone and HF HRV, since the differences are most evident during pre-stressor exposure, results make it hesitant to interpret this as a significant effect of testosterone's moderating influence on the parasympathetic response to social stress.

State Anxiety

State anxiety scores were recorded by the participants with the STAI, with the self-report recorded in the morning session. First, statistical analysis tested whether or not state anxiety predicted differences in physiological response to the stressor. Examination of the 4 (Time) × continuous (STAI – State) interaction term revealed no main effect ($F(1.85,187.08) = 2.805$, $p = 0.067$, $\eta^2 = 0.027$). Again employing a repeated-measures GLM – this time a 4 (Time) × 2 (T/P) × continuous (STAI – State) – once again showed no significant effects on either the HR ($F(1.90,180.92) = 0.189$, $p = 0.818$, $\eta^2 = 0.002$) or HF HRV ($F(2.94,279.32) = 0.195$, $p = 0.896$, $\eta^2 = 0.002$)

interaction (Fig 6A; 6B). Based on these outcomes, individual state anxiety played no role in moderating testosterone action on HR or HF HRV.

In order to confirm the observed results, interactions between different anxiety levels across each participant was examined. High and low state anxiety was assigned using the median STAI score (= 31), with scores > 31 signifying high levels of state anxiety. State anxiety during the morning session was not found to be a significant moderator of the HR response to social stress ($F(1.90,188.38) = 0.608$, $p = 0.538$, $\eta^2 = 0.006$) (Fig 6A). Similarly, interactions between average HF HRV, in each epoch, testosterone vs. placebo, and levels of state anxiety were not statistically insignificant ($F(2.85,282.13) = 0.176$, $p = 0.905$, $\eta^2 = 0.002$) (Fig 6B). As such, results suggested that the effects of testosterone on HRV were not moderated by individuals' state anxiety.

Trait Anxiety

Trait anxiety scores were also recorded by the participants with the STAI during the morning session. No significant main effect between 4 (Time) × continuous (STAI – Trait) was observed ($F(1.82,187.48) = 0.395$, $p = 0.655$, $\eta^2 = 0.004$), suggesting that trait anxiety did not predict any differences in cardiovascular response upon exposure to the stressor. Additional GLMs on 4 (Time) × 2 (T/P) × continuous (STAI – Trait) effect on HR also yielded nonsignificant moderation ($F(1.89,177.68) = 0.325$, $p = 0.711$, $\eta^2 = 0.003$) (Fig 7A), a result also observed in HF HRV ($F(2.68,251.90) = 0.272$, $p = 0.824$, $\eta^2 = 0.003$) (Fig 7B). Similar to state anxiety results, this suggests that personal levels of anxiety do not moderate HR or HF HRV in regards to the effects of testosterone.

As with state anxiety measures, high and low trait anxiety was assigned using the median STAI score (= 38) with scores > 38 signifying high levels of trait anxiety.

Examining the HR interaction term for epoch, trait anxiety, and T/P groups revealed statistically insignificant results ($F(1.85,181.38) = 0.874$, $p = .412$, $\eta^2 = 0.009$) (Fig 7A), suggesting no link between trait anxiety and any testosterone-HR interaction. Similarly, the Time × T/P × Trait anxiety interaction was also determined to be statistically insignificant ($F(2.85, 279.74) = 0.253$, $p = .850$, $\eta^2 = 0.003$) (Fig 7B). Thus, underlying, long-term anxiety does not seem to significantly moderate cardiovascular activity, ruling out anxiety as a psychological moderator of testosterone's effects on the HF HRV responses to social stress.

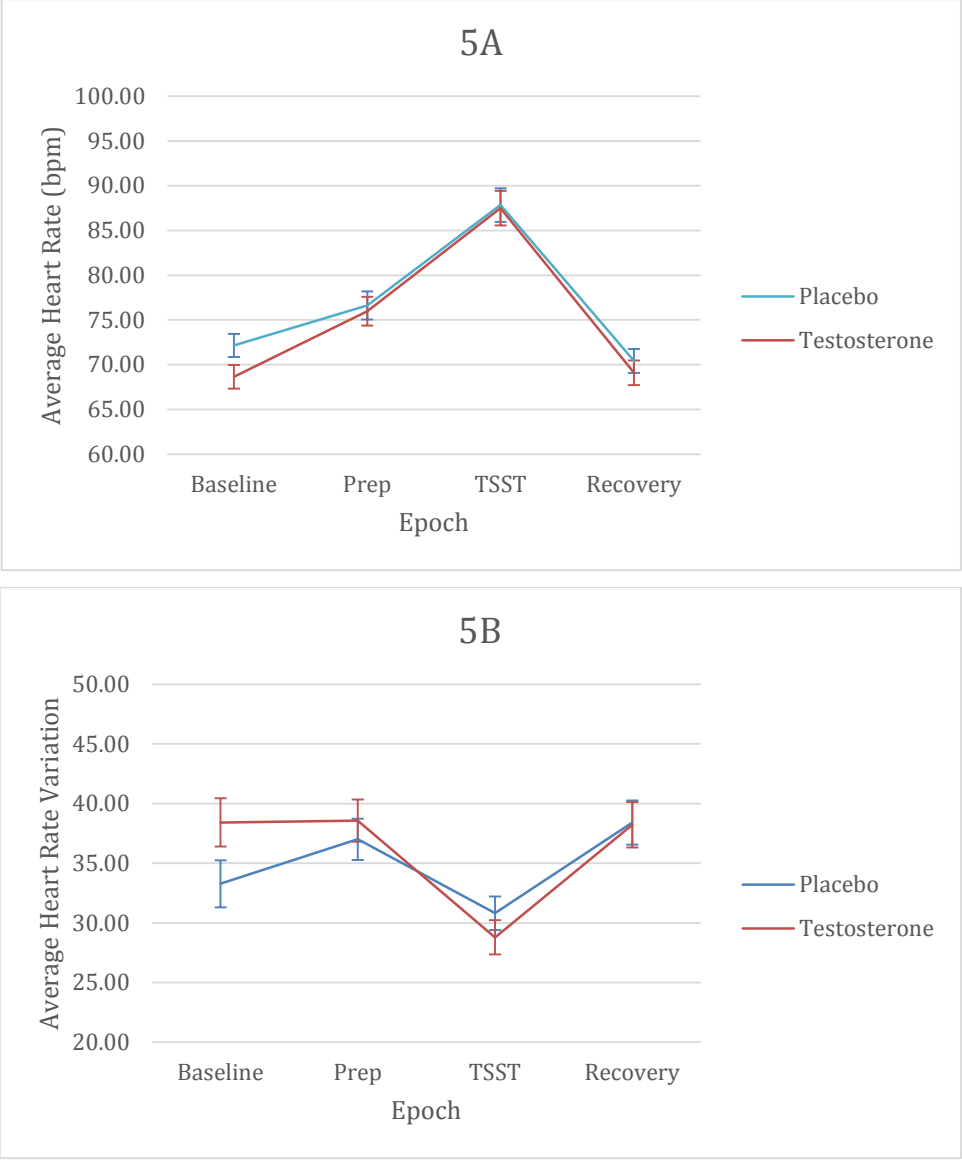

Figure 5. Average HR & HF HRV.

Heart data recorded with BioPac MP150 and processed with Kubios HRV. **A.** Average HR (bpm) recorded across all four epochs for both Placebo and Testosterone groups. **B.** Average HRV recorded across all four epochs for both Placebo and Testosterone groups. Error bars represent standard error with 95% confidence interval.

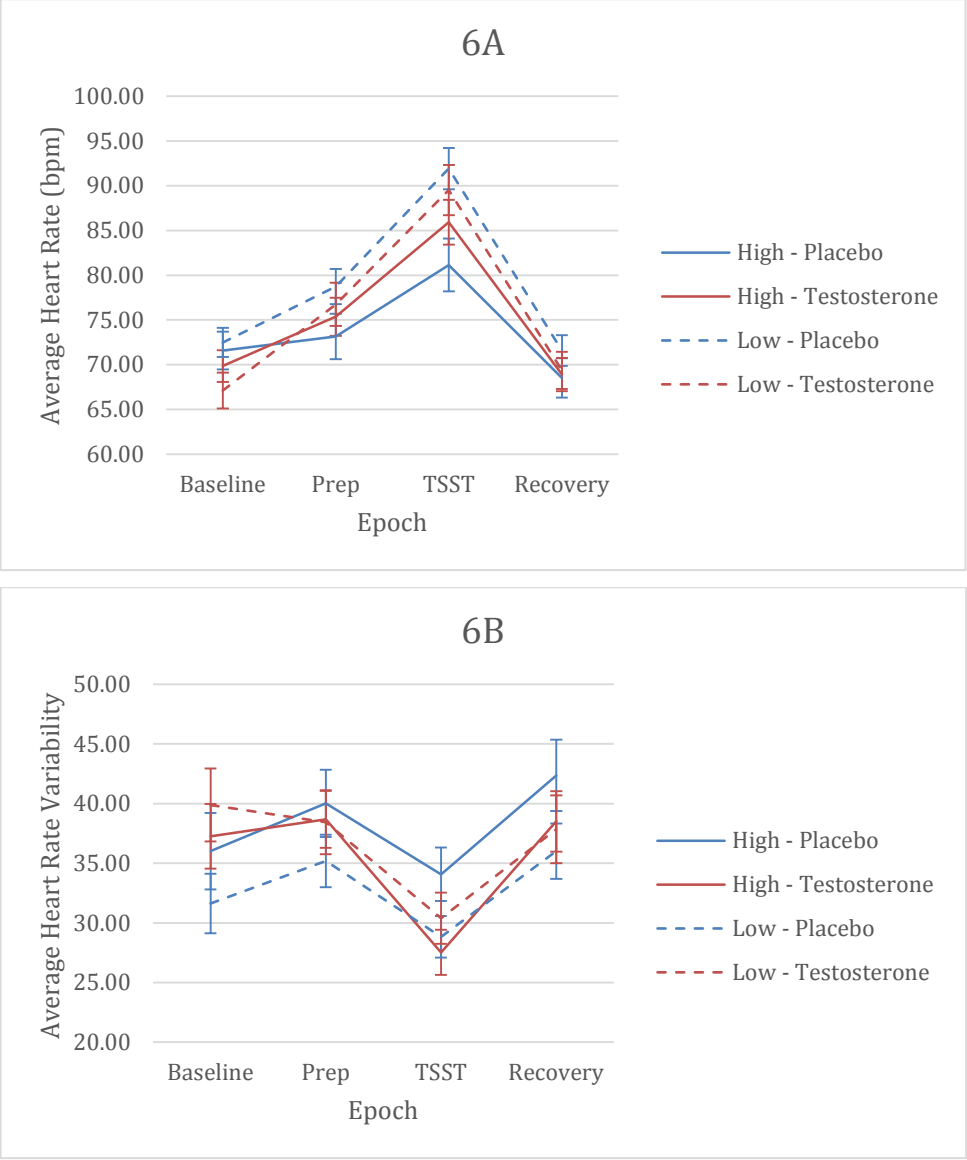

Figure 6. State Anxiety.

Heart data recorded with BioPac MP150 and processed with Kubios HRV. Low state anxiety determined by STAI state score < 31. **A.** Average HR (bpm) recorded across all four epochs for both Placebo and Testosterone groups. **B.** Average HRV recorded across all four epochs for both Placebo and Testosterone groups. Error bars represent standard error with 95% confidence interval.

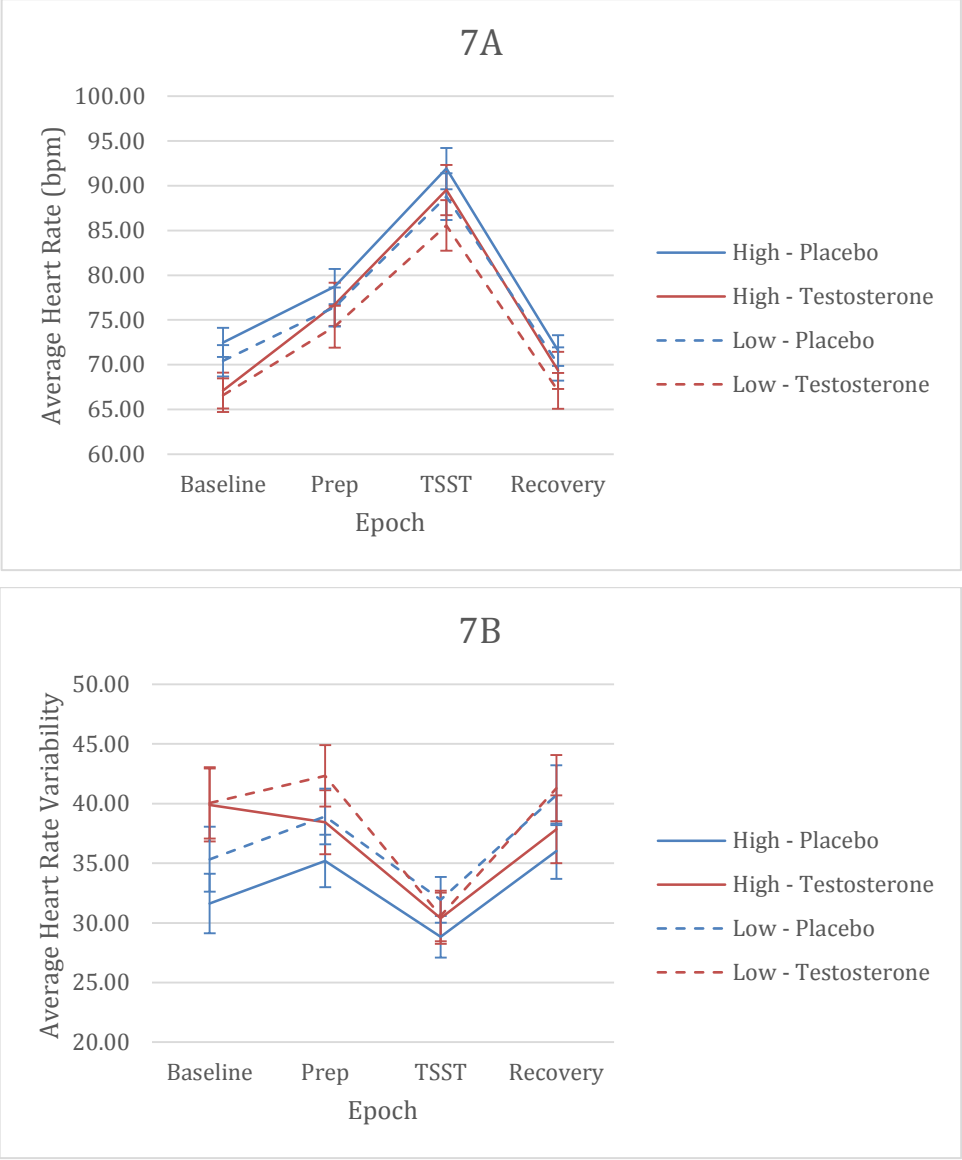

Figure 7. Trait Anxiety.

Heart data recorded with BioPac MP150 and processed with Kubios HRV. Low trait anxiety determined by STAI state score < 38. **A.** Average HR (bpm) recorded across all four epochs for both Placebo and Testosterone groups. **B.** Average HRV recorded across all four epochs for both Placebo and Testosterone groups. Error bars represent standard error with 95% confidence interval.

Discussion

HR & HRV

The present experiment aimed to clarify whether or not heightened levels of testosterone acted as buffer or enhancer of the cardiovascular stress response. In contrast to prior evidence suggesting that testosterone may either buffer or boost stress responses, there were nonsignificant effects of testosterone compared to placebo treatment on HR and HRV responses to the social-evaluative stressor. There were also nonsignificant interactions between self-reported anxiety and testosterone treatment on cardiovascular stress responses. Although these null effects of testosterone on the cardiovascular stress response was not anticipated, they cannot be due to a failure of the stressor to induce cardiovascular changes. Indeed, there were robust increases in HR (and reductions in HRV) during the stressor. Other analyses from the overarching study – which were outside the scope of this experiment – revealed that testosterone treatment led to increased cortisol changes during the stressor compared to placebo treatment (Data not shown). Thus, it seems that exogenous testosterone selectively impacted one component of the stress response – cortisol – but had no direct influence on cardiovascular responses to the stressor.

It is possible that the usage of Androgel limited the pool of participants solely to males. Other independent research concerning stress has used both males and females. This is of particular importance since prior social-evaluative stress experiments revealed that females exhibited greater stress-related HR increases compared to males (Kudielka et al., 2004). It is possible that these increases are caused by gender differences when

faced with a laboratory stressor: women were observed to act more as “cardiac” reactors in comparison to men, who were considered “vascular” reactors (Allen et al., 1993). Because only males were subjected to the TSST in this study, null effects may have been due to neglecting the changes in systolic and diastolic blood pressure responses in favor for cardiovascular responses. Thus, given these sex-based differences in laboratory stress responding, it may be of interest for future research to include both men and women when studying the causal effects of testosterone on the cardiovascular responses to stress. Inclusion of blood pressure measurements would also provide an additional variable to check for possible interactions.

Another possibility that could account for the null results in the present experiment deals with the age group. Aggregated TSST results revealed a significant age-related decline in HR upon psychosocial stressor exposure, with greater increases correlating with children and younger adults (Kudielka et al., 2004). Conversely, responses to daily stressors were not different between younger adults (M_{age} = 20) and older adults (M_{age} = 80) (Stawski et al., 2008). Because the majority of participants in the study were younger adults, this could have explained the similar increased HR readings across epochs. Further experimentation using cardiovascular activity from a narrower age sample or across different age groups will help to confirm whether or not age actually plays a role in the cardiovascular stress response and any testosterone interactions.

Lastly, experimental results were strictly derived from whether or not testosterone levels were heightened with Androgel, or unchanged with placebo. Although baseline HR and HRV was measured at the start of each procedure, basal

testosterone levels – individual inherent amounts of testosterone in the body – were collected but were not considered between each participant in the present report. Basal testosterone levels have been shown to correlate with other forms of stress physiology, including cortisol under competition conditions (Mehta et al., 2008; Zilioli et al., 2013) and cortisol reactivity to social-evaluative stress (Stephens et al., 2015). If basal testosterone levels between each individual in this study were greatly different, experimental results may have been skewed.

Looking at the experimental design, the procedures were part of a far larger procedure that lasted a total of around 6 hours per participant. It is possible that the large gaps of time before, between, and after each cardiovascular recording period had a significant impact on how the acute stressors were handled by each participant. In addition, the nature of the various tasks carried out by the participant before the TSST necessitates consideration. Several tasks were carried out by the participant before the TSST, in addition to a lunch-break and instruction-giving periods. These tasks could have either heightened or decreased the observed individual stress response during the Prep and TSST epochs, as the participant may have been tired out by the day's prior tasks. This, in turn, may have left subjects more vulnerable to a social stressor such as the TSST, or possibly even less responsive due to exhaustion. An independent, focused experiment on the relationship between exogenous testosterone and the cardiovascular stress response may provide cleaner experimental setting to test the nature of the theorized pathway linking testosterone to stress.

Effects of Anxiety

In this experiment, anxiety was used as a psychological moderator to check for any intermediate controllers between testosterone and cardiovascular activity. Using self-reported measures of state and trait anxiety did not moderate the effect of testosterone vs. placebo on cardiovascular reactivity to stress. Similarly, varying levels of state anxiety yielded no statistically significant results. This was consistent with previously observed studies (Filová et al., 2015; Girlder, et al., 1997). The present work extends these findings by showing that heightening individual levels of testosterone exogenously does not interact with self-reported anxiety to alter the cardiovascular stress response.

These results raise the question of whether or not there are other, more important variables that may more clearly interact with testosterone levels to alter the cardiovascular stress response. One such variable is positive and negative affect – subjective experiences of pleasant or unpleasant emotions, respectively. Upon exposure to a speech task, adolescent females reported higher negative affect and increased baseline HR in comparison to adolescent male participants (Steiner et al., 2002). If an individual harbors a greater degree of general emotional negativity, they may be more prone to any effects testosterone has on HR and HF HRV. Again, the gender differences seen in this study suggest that the null results could be due to only males being examined. As such, focusing on a broader sense of positivity or negativity for a person's current emotional state may hold greater value than anxiety when examining relationships between testosterone levels and cardiovascular activity.

Another possibility that could link testosterone and cardiovascular reactivity is social status – one's standing in a group or society. Testosterone and social status have been shown to be linked in some way – in higher status positions, individuals with higher levels of testosterone performed better on spatial and verbal tests and simultaneous blood pressure drops compared to those in a low-status position (Newman et al., 2005). There is also evidence for mismatch: high testosterone levels in low status positions has been shown to predict increased stress reactivity and poorer performance (Josephs et al., 2003). Lower socioeconomic status has also been linked, increased risks for cardiovascular disease. In participants with a measured low grade of employment, exposure to performance tasks post-stress resulted in longer recovery times of cardiovascular activity to baseline (Steptoe et al., 2002). Based on these previous studies, it may be possible that low-status individuals are more prone to testosterone acting as an enhancer of the cardiovascular stress response. Because perceived levels of social status were not accounted for this experiment, any observed null relationships between testosterone and stress reactivity could have been due to this lack of consideration.

Finally, it is worth noting that many of the experiments conducted on testosterone and the human stress response use cortisol rather than cardiovascular activity in order to measure reactivity (Demyttenaere et al., 1989; Mehta et al., 2008; Rubinow et al., 2005; Stephens et al., 2015; Zilioli et al., 2013). Thus, this experiment demonstrates that although testosterone does indeed play a role in the human stress response by influencing cortisol levels, the cardiovascular component of stress reactivity may not be susceptible to testosterone's influence. Future work must continue

to explore multifaceted stress reactivity to be able to clearly state which aspects of the stress response are more and which are less dependent on testosterone.

Conclusion

The results of this experiment suggest that increasing levels of testosterone exogenously has no impact on the cardiovascular stress response, even though testosterone treatment increased cortisol responses to the stressor. Future research should focus on varying age groups and gender, in addition to exploring other possible biological or psychosocial moderators to see if any pathways still exist. Despite the lack of statistical significance, outcomes contained within this report will help encourage future important questions surrounding steroid hormones such as testosterone and their interplay with stress and cardiovascular health. In addition, the continued investigation of testosterone's effects on stress holds much clinical merit, particularly in observing adolescent or younger individuals when the human body starts production of testosterone: The role (or lack thereof) that testosterone plays in the stress response during puberty – a time of great social, psychological, and biological change – is largely unknown. Discerning the relationship between testosterone and cardiovascular stress in this experiment will open new avenues for clinical research and possibly new techniques involving individuals managing stress throughout puberty and beyond.

Glossary

Affect – The feelings of emotion; usually expressed in facial features and gestures.

Afferent – Conducted towards the central nervous system (brain and spinal cord).

Amygdala – Part of the brain that is responsible for the fear response.

Anxiety – A psychological disorder characterized by nervousness.

Buffer – Something that assists to lessen or moderate a given change.

Cardiomyopathy – "Heart Muscle Disease"; any condition that involves the slow degradation of the heart and impedance of function.

Cardiovascular – Pertaining to the heart.

Cortisol – A steroid hormone released in the body to control stress.

Degrees of Freedom – The amount of values from a reported calculation which are free to vary.

Double-Blind – Experimental condition in which critical information that may influence tester or subject behavior is withheld until the end of said experiment.

Effect Size – The strength of an observed relationship between two variables.

Electrocardiogram – Instrument that uses electrical signals generated by the heart in order to monitor its activity.

Epoch – A set division of time.

Exogenous – On the surface.

GLM – Generalized Linear Model; a generalization used for statistical analysis of variables that do not have a normal distribution.

Gonads – Any gamete-producing organ; a testicle or an ovary.

Hippocampus – Part of the brain that is the center of emotion, memory, and the autonomic nervous system.

Homeostasis – A self-regulating process that involves keeping variables at an optimal level under different conditions.

Hormone – A class of signaling molecules that regulate physiology and behavior.

Hypothalamic-Pituitary-Adrenal (HPA) Axis – Endocrine gland complex in the body that controls various bodily processes, such as stress reactivity.

Impedance Cardiography – Measuring technique used to examine a number of cardiovascular parameters over time.

Interoceptive Awareness – Phenomenon in which an individual is conscious of the current inner state of his/her body.

Moderator – Any factor that can alter the direction or magnitude of a given relationship between a set of variables.

Normalized Units – Values that have been adjusted in order to bring distributions of measures into alignment for analysis.

Parasympathetic Nervous System – Division of the autonomic nervous system that deals with the body's "rest and digest" function.

Pearson Correlation Coefficient – A measure of the linear correlation between two variables by assigning a value from -1 to +1.

Placebo – A substance with no actual therapeutic benefit; often used in experiments to test the effectiveness of drugs.

Single-Blind – Experimental condition in which critical information that may influence the subject is withheld until the end of said experiment.

Social Status – The position an individual holds in society.

Sphericity – An assumption held by ANOVAs that assumes variance of difference between all independent variables are even.

State Anxiety – Anxiety of an individual based on current circumstances.

Steroid – A class of organic compound, many of whom act as signaling molecules.

Stress – The body's response to any threat that may disrupt homeostasis.

Stressor – Any agent that induces stress upon an individual.

Sympathetic Nervous System – Division of the autonomic nervous system that deals with the body's "fight or flight" function.

Testosterone – The male sex hormone.

Trait Anxiety – Anxiety of an individual based on personal levels.

Vagus Nerve – Cranial nerve that connects and moderates parasympathetic control of the heart.

Appendix

Appendix A. Job Listing

Seeking job candidate with managerial experience to supervise student-employees in an on-campus position at the University of Oregon, Division of Student Affairs Career Center.

About the Career Center: The Career Center provides career and job search services and resources to UO students and alumni. Our mission is to help develop long-term career goals and strategies, facilitate self-exploration and discovery, connect with potential employers, and empower and challenge students and alumni to fulfill their potential. We serve as advocates to help others pursue an inspired and fulfilling future.

Position: Parent Professional Network Manager

Salary Range: $36,000-$60,000 per year, commensurate with applicant qualifications

About the position: This is a managerial position that requires you to oversee up to twelve student-employees. You will make decisions regarding their daily tasks, evaluate your employees on their performance, and determine how to compensate the students for their work. You will report to the Career Center Director. The job additionally requires you to:

- Design and manage the Parent Professional Network (PPN) program in support of UO student and alumni career and professional development

- Recruit and provide outreach/support to parent and alumni professionals to engage them in the PPN program via participation in informational interviews, job shadowing, panels/ presentations, networking events, mentoring opportunities, recruitment and hiring, and/or other career related activities with UO students and alumni

- Assess learning and program outcomes for program enhancements and program/ROI reporting

- Collaborate widely with a diversity of program stakeholders for program development, implementation and coordination

Required Qualifications

- Demonstrated experience in advising, consulting or professional customer service experience
- Demonstrated supervision experience of paraprofessional or professional staff
- Demonstrated experience with and/or commitment to working effectively with students, parents, faculty and staff from diverse backgrounds in support of the office's goals

Additional Required Qualifications

- Demonstrated ability to work in a dynamic, highly collaborative, team-oriented environment
- Demonstrated time management, follow-through and organizational skills
- Demonstrated interpersonal, presentation skills as well as demonstrated ability to set and achieve goals

Appendix B. TSST Panel Instructions

The Speech

Experimenter introduces the participant to the panel and then leaves the room. The job listing is on the table. The panel then speaks to participant:

> As you know, you have 10 minutes to prepare a 5-minute speech on why you are the perfect applicant for the vacant position on campus. We are the panel in charge of hiring for this position; you will give your speech in front of us. The notepad and job listing is here to help you prepare for your speech but you will not be able to use them during the speech. Once we leave the room, you will have ten minutes to prepare.

Panel leaves, start time once door closes. After ten minutes, Panel reenters the room and says:

> Time is up. Please hand me your notes.

5 minute timing starts at end of these next instructions. Interviewer #1 states:

> Please stand on the X and begin when you are ready. We will tell you to stop after 5 minutes.

If participant pauses for 15-20 seconds during the first 3 minutes, Interviewer #1 will say:

> You still have some time left. Please continue.

If after the first three minutes the participant stops talking and does not begin again after prompting (as above), OR if he states specifically that he has nothing left to say, the panel may ask the following questions. Use them sparingly and rely on silence to encourage the participant to continue speaking:

> Tell us about a time you managed a project or a group of people.
> We are interviewing 120 candidates for this job: Why should we hire you?
> Tell me one thing you would change about your last job?
> Tell me about your strengths & weaknesses.
> Tell me about a time when old solutions didn't work.
> What's the biggest risk you've ever taken?
> Describe a time when a team you were part of did not agree.

If the participant doesn't talk for very long after a question, wait a 5-10 seconds after the end of their response, then use one of the following:

> Can you elaborate on that answer?
> What else can you tell us about XXX?

After 5 minutes, Interviewer #2 states the following:

> Please stop, your time is up.

The Math

Interview #1 takes over and says:

> Now we would like you to subtract 13 from 6233 out loud, and keep subtracting 13 from the remainder until we tell you to stop. You should do the subtraction as fast and as accurately as possible. Please begin.

If the participant doesn't realize they are supposed to keep subtracting, Interviewer #1 says:

> Please continue subtracting 13 from your answer.

Whenever the subject makes an error, Interviewer #1 says:

> That is incorrect. Please start again from the beginning. [*Provide the number 6233 again as necessary.*]

After 5 minutes, Interviewer #2:

Please stop, your time is up. You may leave the room now.

Appendix C. STAI-State Form Y-1

STAI-State Form Y-1

Instructions: A number of statements which people have used to describe themselves are given below. Read each statement and then choose the appropriate number to the right statement to indicate **how you feel right now**, that is, **at this moment**.

1 = Almost Never
2 = Sometimes
3 = Often
4 = Almost Always

	Almost Never	Sometimes	Often	Almost Always
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confidant	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

Appendix D. STAI-Trait Form Y-2

STAI-Trait Form Y-2

Instructions: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you **generally** feel.

1 = Almost Never
2 = Sometimes
3 = Often
4 = Almost Always

	Almost Never	Sometimes	Often	Almost Always
1. I feel pleasant	1	2	3	4
2. I feel nervous and restless	1	2	3	4
3. I feel satisfied with myself	1	2	3	4
4. I wish I could be as happy as others seem to be	1	2	3	4
5. I feel like a failure	1	2	3	4
6. I feel rested	1	2	3	4
7. I am "calm, cool and collected"	1	2	3	4
8. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
9. I worry too much over something that really doesn't matter	1	2	3	4
10. I am happy	1	2	3	4
11. I have disturbing thoughts	1	2	3	4
12. I lack self-confidence	1	2	3	4
13. I feel secure	1	2	3	4
14. I make decisions easily	1	2	3	4
15. I feel inadequate	1	2	3	4
16. I am content	1	2	3	4
17. Some unimportant thought runs through my mind and bothers me	1	2	3	4
18. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
19. I am a steady person	1	2	3	4
20. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Bibliography

Allen, M.T., Stoney, C.M., Owens, J.F., & Matthews, K.A. (1993). Hemodynamic adjustments to laboratory stress: the influence of gender and personality. *Psychosomatic medicine*, 55(6), 505-517.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders: DSM-5*. Washington, D.C: American Psychiatric Association.

Appelhans, B.M., & Luecken, L.J. (2006). Heart Rate Variability as an Index of Regulated Emotional Responding. *Review of General Psychology*, 10(3), 229-240.

Ashley E.A., & Niebauer J. (2004). Chapter 3: Conquering the ECG. *Cardiology Explained*. London: Remedica.

Bechara, A., & Naqvi, N. (2004). Listening to your heart: interoceptive awareness as a gateway to feeling. *Nature Neuroscience*, 7, 102-103.

Berthoud, H. R., & Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Autonomic Neuroscience*, 85(1), 1-17.

Brosschot, J.F., Pieper, S., & Thayer, J.F. (2005). Expanding stress theory: Prolonged activation and preservative cognition. *Psychoneuroendocrinology*, 30(10), 1043-1049.

Centers for Disease Control and Prevention. (2014, October 29). *Heart Disease Facts*. Retrieved May 4, 2015, from http://www.cdc.gov/heartdisease/facts.htm.

Demyttenaere, K., Nijs, P., Evers-Kiebooms, G., & Koninckx, P.R. (1989). The effect of a specific emotional stressor on prolactin, cortisol, and testosterone concentrations in women varies with their trait anxiety. *Fertility and Sterility*, 52(6), 942-948.

Dimitriev, D.A., & Saperova, E.V. (2010). Trait anxiety as a predictor of cardiovascular regulation during psychological stress. *International Journal of Psychophysiology*, 77, 270-271.

Dunn, B.D., Stefanovitch, I., Evans, D., Oliver, C., Hawkins, A., & Dalgleish, T. (2010). Can you feel the beat? Interoceptive awareness is an interactive function of anxiety- and depression-specific symptom dimensions. *Behaviour Research and Therapy*, 48(11), 1133-1138.

El-Sheikh, M., Harger, J. & Whitson, S. M. (2001). Exposure to Interparental Conflict and Children's Adjustment and Physical Health: The Moderating Role of Vagal Tone. *Child Development*, 72, 1617–1636.

Fadaei, M., Rusnani, A. K., Sidek, M. N., & Siti Aisha, H. (2011). The relationship between state and trait anxiety with career indecision of undergraduate students. *International Education Studies, 4*(3), 31–35.

Filová, B., Domonkos, E., Borbélyová, V., Bábíčková, J., Tóthová, L., Ostatníková, Celec, P., & Hodosy, J. (2015). Does the non-genomic effect of testosterone on social anxiety require the presence of a classical steroid receptor? *Acta Neurobiologiae Experimentalis*, 75: 457-461.

Girden, E. (1992). *ANOVA: Repeated measures*. Newbury Park, CA: Sage.

Girdler, S.S., Jamner, L.D., & Shapiro, D. (1997). Hostility, testosterone, and vascular reactivity to stress: Effects of sex. *International Journal of Behavioral Medicine*, 4(3), 242-263.

Hermans, E. J., Putman, P., Baas, J. M., Gecks, N. M., Kenemans, J. L., & Van Honk, J. (2007). Exogenous testosterone attenuates the integrated central stress response in healthy young women. *Psychoneuroendocrinology*, 32(8), 1052-1061.

Josephs, R. A., Newman, M. L., Brown, R. P., & Beer, J. M. (2003). Status, testosterone, and human intellectual performance stereotype threat as status concern. *Psychological Science*, 14(2), 158-163.

Kamath, M.V., & Fallen, E.L. (1993). Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. *Critical Reviews in Biomedical Engineering*, 21(3), 245-311.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D.H. (1993). The 'Trier Social Stress Test' – A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, 28(1-2), 76-81.

Krantz, D.S., & McCeney, M.K. (2002). Effects of psychological and social factors on organic disease: a critical assessment of research on coronary heart disease. *Annual Review of Psychology*, 53, 341-369.

Kudielka, B.M., Buske-Kirschbaum, A., Hellhammer, D.H., & Kirschbaum, C. (2004). Differential heart rate reactivity and recovery after psychosocial stress (TSST) in healthy children, younger adults, and elderly adults: the impact of age and gender. *International Journal of Behavioral Medicine*, 11(2), 116-121.

Leuner, B., & Shors, T.J. (2013). Stress, Anxiety, and Dendritic Spines: What are the Connections? *Neuroscience*, 251, 108-119.

Mangin, L., Swynghedauw, B., Benis, A., Thibault, N., Lerebours, G., and Carré, F. (1998). Relationships between heart rate and heart rate variability: study in conscious rats. *Journal of Cardiovascular Pharmacology*, 32(4), 601-607.

Mehta, P. H., Jones, A. C., & Josephs, R. A. (2008). The social endocrinology of dominance: basal testosterone predicts cortisol changes and behavior following victory and defeat. *Journal of personality and social psychology*, 94(6), 1078.

Newman, M.L., Seller, J.G., & Josephs, R.A. (2005). Testosterone, cognition, and social status. *Hormones and Behavior*, 47(2), 205-211.

Norman, R. E., Moreau, B. J., Welker, K. M., & Carré, J. M. (2015). Trait anxiety moderates the relationship between testosterone responses to competition and aggressive behavior. *Adaptive Human Behavior and Physiology*, *1*(3), 312-324.

Rohleder, N., Kudielka, B. M., Hellhammer, D. H., Wolf, J. M., & Kirschbaum, C. (2002). Age and sex steroid-related changes in glucocorticoid sensitivity of pro-inflammatory cytokine production after psychosocial stress. *Journal of neuroimmunology*, 126(1), 69-77.

Rubinow, D., Roca, C.A., Schmidt, P.J., Danaceau, M.A., Putnam, K., Cizza, G., Chrousos, G., & Nieman, L. (2005). Testosterone Suppression of CRH-Stimulated Cortisol in Men. *Neuropsychopharmacology*, 30, 1906-1912.

Siegrist, J. (1996). Adverse health effects of high-effort/low-reward conditions. *Journal of Occupational Health Psychology*, 1(1), 27-41.

Sherwood, L. (2012). *Fundamentals of Human Physiology* (4th Ed.). Belmont, CA: CENGAGE Learning.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Stawski, R.S., Sliwinski, M.J., Almeida, D.M., & Smyth, J.M. (2008). Reported exposure and emotional reactivity to daily stressors: The roles of adult age and global perceived stress. *Psychology and Aging*, 23, 52-61.

Steiner, H., Ryst, E., Berkowitz, J., Gschwendt, M.A., & Koopman, C. (2002). Boys' and girls' responses to stress: affect and heart rate during a speech task. *Journal of Adolescent Health*, 4, 14-21.

Stephens, M.A.C., Mahon, P.B., McCaul, M.E., & Wand, G.S. (2015). Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones. *Psychoneuroendocrinology*, 66, 47-55.

Steptoe, A., Feldman, P.J., Kunz, S., Owen, N., Willemsen, G. & Marmot, M. Stress responsivity and socioeconomic status. A mechanism for increased cardiovascular disease risk? *European Heart Journal*, 2002, 23(22), 1757-1763.

Thayer, J.F., & Brosschot, J.F. (2005). Psychosomatics and psychopathology: looking up and down from the brain. *Psychoneuroendocrinology*, 30(10), 1050-1058.

Thayer, J.F., & Ellis, R.J. (2010). Music and Autonomic Nervous System (Dys)function. *Music Perception*, 27(4), 317-326.

Thayer, J.F., & Esther, M.S. (2008). Neural Aspects of Immunomodulation: Focus on the Vagus Nerve. *Brain, behavior, and immunity,* 24(8), 1223–1228.

Thayer, J.F., & Lane, R.D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders*, 61(3), 201-216.

Virani, S.S., Khan, A.N., Mendoza, C.E., Ferreira, A.C., & Marchena, E. (2007). Takotsubo Cardiomyopathy, or Broken-Heart Syndrome. *Texas Heart Institute Journal*, 34(1), 76-79.

Van Wingen, G., Zylicz, S.A., Pieters, S. Mattern, C., Verkes, R.J., Buitelaar, J.K., & Fernández, G. (2009). Testosterone Increases Amygdala Reactivity in Middle-Aged Women to a Young Adulthood Level. *Neuropsychopharmacology*, 34, 539-547.