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Differences In Stress Biomarkers In Women With High And Low Stress Appraisal

Clarissa Sara Gomez
University of Texas at El Paso, csgomez@miners.utep.edu

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CLARISSA SARA GOMEZ

Interdisciplinary Health Sciences Ph.D. Program

APPROVED:

Christina Sobin, Ph.D., Chair

David Wittenburg, Ph.D.

Sudip Bajpeyi, PhD.

Herbert Janssen, Ph.D.

Charles Ambler, Ph.D.
Dean of the Graduate School

DIFFERENCES IN STRESS BIOMARKERS IN WOMEN WITH HIGH AND LOW STRESS APPRAISAL

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CLARISSA SARA GOMEZ, M.S., B.S.

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Abstract

Measures of physiological biomarkers have been widely used in the field of stress research to explain how stress negatively impacts health outcomes. Women in particular have been shown to be more at risk for developing physiological and psychological stress-induced conditions (e.g., hypertension, depression) due to hormonal differences (Kirschbaum et al., 1992), but more importantly, because of their appraisal of stressful events (Schamus et al., 2008). Few studies however have examined whether women's stress appraisal is predictive of stress reactivity, as measured by stress biomarkers, during stressful events. The goal of this study was to examine whether stress appraisal predicted stress biomarker activity level. The Appraisal of Challenge or Threat Scale (ACTS; Tomaka et al., 2002) was used to select college-age females for inclusion in one of two mutually exclusive groups including High Stress-Low Coping subjects (Group 1, N = 24) who perceived trigger events as very threatening and their ability to cope as low, and Low Stress-High Coping subjects (Group 2, N = 24) who perceived trigger events as challenging rather than threatening and their ability to cope as high. All subjects (N = 48) completed a standardized stress-induction procedure (Trier Social Stress Test, TSST) that included two stress inducing tasks. Heart rate was continuously measured and saliva samples were collected to determine alpha-amylase stress biomarker levels at 3 time points, including prior to the stress induction, after the completion of the first stress task, and after completion of the second stress task. It was hypothesized that as compared to women with low stress-high coping appraisal, women with high stress-low coping appraisal would have significantly greater reactivity (higher biomarker levels) following stress induction.

A 3 x 2 mixed model ANOVA with time as the within subjects factor and group as the between subjects factor was used for the analyses. The findings showed that there was a main effect of stress induction on sAA ($F_{(2,45)} = 15.09$, $p = .00$) and HR ($F_{(2,45)} = 68.99$, $p = .00$) levels. Post-hoc analyses showed that significant differences in sAA and HR levels occurred only between baseline and the speech task indicating that the speech task and not the arithmetic task

induced a physiological stress response. With regard to the central hypothesis, stress appraisal did not predict stress reactivity (biomarker levels) during stress induction. There was no effect of group on sAA ($F_{(1,46)}$ = 1.42, p = .24) or HR ($F_{(1,46)}$ = .00, p = .95). Furthermore, no significant interaction was seen between group and sAA ($F_{(2,45)}$ = .71, p = .49) or between group and HR ($F_{(2,45)}$ = .61, p = .55), suggesting that high stress-low coping women did not experience different amounts of sAA or HR changes as compared with low stress-high coping women. When pre- and post-test mood state (PSM-9) scores were compared by group, findings showed no significant interaction ($F(_{1,46})$ = 2.48, p = 0.12), however there was a significant group effect indicating that groups differed overall for PSM scores. Paradoxically, high stress-low coping individuals had significantly lower scores before and after the TSST procedure as compared to low stress-high coping individuals ($F(_{1,46})$ = 3.99, p = .05).

The current findings provided valuable additional evidence that sAA is a sensitive biomarker of psychological stress. Importantly, the findings suggested that self-reported stress is not an indicator of biological stress reactivity and that self-reported stress should not be used by health care workers to determine whether subjects are at risk of stress-related disease. Furthermore, the findings suggested that high stress-low coping subjects may be vulnerable to emotional blunting. The results require replication.

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Chapter 1: Introduction

Stress is a multifaceted concept that includes environmental, cognitive, affective, behavioral, and physiological components. Over the years, various definitions of stress have emphasized one or more of these different facets (Lumsden, 1981). Today, most researchers define stress according to the effects that are produced when individuals are faced with situations or events that exceed their coping abilities.

It is important to study stress, and in particular, biomarkers of the stress response because it is well established that stress negatively impacts health outcomes. Identifying biomarkers of the stress response could provide a means to identify at-risk individuals long before adverse health outcomes have occurred. In order to understand which biomarkers may be most valuable for identifying individuals who experience high levels of stress, it is important to know the physiological underpinnings of the stress response.

The stress response is founded in the activation of the autonomic nervous system, which includes two components, the sympathetic (SNS) and parasympathetic (PNS) nervous systems. The SNS and PNS systems work together to maintain the body's homeostatic state (Tsigos and Chrousos, 2002). While the sympathetic nervous system initiates the fight or flight responses, the parasympathetic nervous system governs "rest and digest states" (Tsigos and Chrousos, 2002) including regaining homeostasis following fight or flight reactions and initiating salivation, tear production, defecation, digestion, and/or urination. In these ways, the sympathetic and parasympathetic systems are complimentary. For example, while SNS increases pulse rate and blood pressure levels in response to stress, the PNS attempts to bring levels back to a homeostatic norm (Tsigos and Chrousos, 2002).

These complementary processes are associated with the production of key biomarkers. Two components of the SNS, the sympathetic adrenal medullary (SAM) axis and the hypothalamic pituitary adrenal (HPA) axis, are responsible for changes in levels of enzymes, hormones and/or heart rate. The SAM axis is responsible for biophysiological responses to the fight or flight response and stimulates increase of circulating epinephrine (i.e., adrenaline), which then increases heart rate and redirects blood away from the periphery and towards the lungs and large muscle beds in the legs and arms. In turn, the SAM axis increases muscular efficiency, releases energy storage, and increases arterial blood pressure and muscle blood flow. Blood flow is controlled via vasodilation within internal organs and vasoconstriction; these mechanisms allow an organism to become mobile and cope with the stressor.

The second component of the SNS system, the HPA axis, stimulates the pituitary gland, which is responsible for secreting adrenocorticotropic hormone (ACTH). ACTH stimulates the adrenal glands to produce corticosteroid, a hormone that allows the body to stabilize blood sugar. Maintaining steady supplies of blood sugar facilitates the return of the body to a normal state. The HPA axis system also stimulates the release of other hormones and enzymes that contribute to the return of a physiologically homeostatic state.

As a result of these processes, the SAM and HPA axes activate the release of various chemicals and proteins. These are valuable biomarkers of stress and measuring these can allow researchers to reliably quantify an individual's reactions to stressful situations.

Alpha-amylase, a well-established stress biomarker is an enzyme governed by HPA axis reactivity. Alpha-amylase is found in saliva and is responsible for the digestion of carbohydrates and starches. Interestingly, it has also been shown that psychological stress results in marked changes of salivary alpha-amylase levels (Rohleder, 2004). Another valuable biomarker used in

stress research is heart rate. Heart rate changes are under the influence of the SAM axis and reflect the body's automatic fight or flight response.

Although stress responses were selected through evolution to ensure greater odds of survival (Cannon, 1932) they can cause bodily damage when activated chronically in response to recurrent psychological stress. The SAM axis is involved in acute stress, while the HPA axis is involved in longer-term reactivity to both acute and chronic stress, remaining active long after the stress is no longer present. Prolonged stress reactivity and long-term elevation of cortisol levels, for example, have been shown to produce a wide range of psychological and physiological symptoms including decreased memory, decreased thyroid function, and accumulation of abdominal fat, which contributes to additional cardiovascular health risks (Blascovich and Katkin, 1993). Also, chronic cortisol release causes immune, digestive, and endocrine systems to down-regulate in response to sustained energy release. This in turn, generally compromises the organism's healthful state (Tsigos and Chrousos, 2002).

When considering possible effects of the stress response systems on physiological health, research has shown marked differences in reactivity in males and females. As compared with men, women report greater levels of chronic stress (McDonough and Walters, 2001; Turner et al., 1995; Nolen-Hoeksema et al., 1999). Furthermore, males and females have marked differences in their perceptions of whether a situation is stressful (Barnett et al., 1987). As compared with men, women perceive greater numbers of stressors in their daily lives and studies have shown that as compared with men, women more often report being in stressful circumstances (Almeida and Kessler, 1998; McDonough and Walters, 2001). Research has also suggested that males and females differ with regard to their coping resources during stressful events. Importantly, gender and specifically the hormonal differences associated with being

male or female, are likely to alter the effects of stress on health (Kirschbaum et al., 1992). Because studies have suggested that women are both more psychologically and physiologically reactive to stress than men (Schamus et al., 2008), this study will focus exclusively on biomarker reactivity in females with differing levels of stress appraisal.

In order to fully assess differences in perceived stress, Tomaka et al. (2012) developed a unique stress appraisal scale using a theoretical framework suggested by Lazarus and Folkman (1984). The ACTS is a 24-item scale that describes potentially stressful life events in six content domains – conflict situations, unexpected events, public speaking, transportation, social anxiety, and financial concerns. Unlike other scales, the ACTS separately quantifies the perception of threat, that is, the extent to which a given trigger event is perceived as threatening, and one's perceived ability to cope with the given trigger event. The measure has been shown to have good factor structure, reliability, and validity (Tomaka et al., 2012).

Studies have not yet examined the extent to which individuals who differ with regard to their cognitive appraisals of threat as measured by the ACTS, differ with regard to their stress biomarker reactivity following a stressful event. Determining whether stress appraisal patterns predict biomarker reactivity following a stressful state could additionally guide our understanding in determining the extent to which an individual's perception influences physiological health risks.

It was hypothesized that alpha-amylase levels and heart rate, would differ in individuals according to their perceptions of situational demands as threatening or challenging. The ACTS was used to select women for inclusion in one of the two mutually exclusive groups; two groups of subjects included High Stress-Low Coping subjects (Group 1) who perceive trigger events as very threatening and their ability to cope as low and Low Stress-High Coping (Group 2) who

perceive trigger events as challenging rather than threatening and their ability to cope as high. Informed consents were completed and salivary samples and heart rate were measured to assess baseline biomarker levels of alpha-amylase and heart rate upon arrival. Subjects completed a widely-used laboratory stress test (Kirschbaum et al., 2010) that involved two stress-inducing activities (e.g., speech and arithmetic task). Biomarker samples were collected upon arrival and at the completion of the first and second stress task (e.g., speech and arithmetic task).

Chapter 2: Review of the Literature

2.1 A History of Stress Concepts

The concept of stress has a long history. In the 14th century, stress was most commonly used to describe the experience of hardship and adversity, for example, incidents involving the death of a loved one or the diagnosis of a chronic illness (Lumsden, 1981). In the 17th century, the term “stress” was used in the context of the physical sciences. “Load” was defined as an external force, “stress” was that which created the load, and “strain” was used to describe the distortion that resulted from load and stress (Hinkle, 1977). It was not until the 19th century that stress was first used to describe a source of ill health. During the 20th century, researchers such as Cannon, Wolff, and Selye (Cannon, 1914, 1932; Wolff, 1953; Selye, 1956, 1964, 1974, 1976) would continue to refine the health-related aspects of stress.

Walter Cannon (1914) was the first researcher to equate stress with emergency, and elaborated its effect by coining the term, “fight or flight response.” He was also the first to use the term “homeostasis” which referred to the ability to maintain a stable, constant condition of the body’s internal environment such as temperature, pH level, and blood sugar level. His conception of ”fight or flight” included the view that stress was a necessity for overcoming threatening events and situations. Cannon believed that stress could be induced by stimuli that were either physiologically or emotionally challenging or threatening to one’s survival (Cannon, 1932).

Along with physiologist Philip Bard, Cannon developed the Cannon-Bard Theory (Bard, 1934), which explains why emotions were experienced before a physiological reaction occurred. Specifically, he proposed that the thalamus received a signal via the autonomic nervous system

to the amygdala, which then caused physiological reactions such as muscle tension and sweating. His most notable work (1932) focused on the effect of frequent acute stress on an individual's health and showed that frequent acute stress increased the risk of developing heart conditions and disrupted levels of insulin, triglycerides, cholesterol, and other hormones.

Cannon's groundbreaking findings led other scientists such as Harold Wolff, to use his work as a platform for conducting research. Wolff used a different approach to define and conceptualize stress and as a result introduced the concept of medicine in stress research by describing it as a state of the body (Wolff, 1953). Wolff's research focused on demonstrating the relationship between stressful environments such as domestic difficulties, strenuous working conditions, and unforeseen medical diagnoses; and cardiovascular disorders such as coronary heart disease, hypertension, and atrial fibrillation. He believed that because the cardiovascular system maintained the body's reactions and its state of well-being, anything that disrupted that rhythm caused the organism to undergo disturbances, which could eventually lead to diminished health and/or disease. His research revealed that stress responses such as, tension, frustration, conflict, anxiety, and depression affected the cardiovascular system by inducing irregular heart rates, contractions, and a decrease in the heart's potential to respond. He also suggested that stress experienced in day-to-day living eventually compromised the body's health depending on its frequency and duration.

Like Wolff, Hans Selye used Cannon's work to explain his notions of stress. Specifically, Selye (1956) incorporated Cannon's concept of "homeostasis." Selye was the first to define stress as the effects and symptoms seen and experienced by living organisms when presented with environmental challenges. He explained that stress followed a general pattern of responses, regardless of the type of stimulus. He subcategorized the pattern into three stages –

alarm reaction, resistance, and exhaustion, and referred to this sequence of stages as the General Adaptation Syndrome or GAS (1956). In his model, stress-induced responses activated the sympathetic nervous system and released hormones including cortisol and adrenaline into the bloodstream. This led to a coping response that caused large amounts of energy to be expelled so that a homeostatic state could be obtained. If stress was persistent and caused energy reservoirs to become depleted, a state of exhaustion was reached. This was considered highly detrimental to the health of the organism because tissue damage was probable and in severe cases could result in death. In addition to the GAS model, Selye's work prompted later investigations that helped to explain the progression of physiological stress responses, more specifically, the development from acute to chronic stress.

Adding to his research findings, Selye also distinguished between good and bad stress, otherwise referred to as eustress and distress. Both lead to the activation of the General Adaptation Syndrome (Selye, 1964) and are not defined by the type of stressor, but rather how the stressor was perceived, that is, as a threat versus a challenge. For example, eustress was defined as a positive stress when the psychological demand is perceived as a positive challenge and therefore led to a healthy outlook. In contrast, distress was the negative form of stress. Distress tends to have a negative effect because the situation from which it arose is unresolvable, for example, the death of a loved one, losing one's job, or injury. Thus, distress can be chronic and debilitating if unresolvable situations occur frequently and/or the associated distress persists. Importantly, Selye (1964) demonstrated that the stress response can be lessened or eliminated depending on how it is perceived and dealt with which led him to study the appraisal of stress and its effect on the activation and release of physiological biomarkers (e.g., corticosteroids) (Selye, 1975a, 1975b).

Prior to the 1960s, stress was largely a physiological concept and little to no attention was paid to the role of perception in the stress response. This focus shifted when Magda Arnold developed a cognitive theory of stress (1960). Arnold's theory was based on the idea that all emotions, including stress, resulted from the cognitive appraisal of a situation. For the first time, Arnold suggested that cognitive appraisal was the initial cause of psychological changes and subjective emotional experiences (Arnold, 1960). Interestingly, this theory was poorly received because many believed that the study of cognitive processes was fundamentally unscientific, and that such an approach was too general to explain emotional experience and behavior. It was not until later that her theories gained approval and provided insight for stress research.

Shortly after Arnold's theories were rejected by the scientific community, Richard Lazarus and his colleagues conducted a series of laboratory studies to test a "scientific" approach for measuring stress appraisal. They attempted to experimentally manipulate how subjects would appraise or interpret a potentially stressful situation (Lazarus, 1966, 1991, 1993; Lazarus and Folkman, 1984; Lazarus and Launier, 1978). For example, some of their studies were designed to create psychological stress in the laboratory (e.g., watching stressful films, anticipating an electric shock), allowing them to measure autonomic nervous system activity (e.g., heart rate and skin conductance). Findings showed that appraisal and coping processes shaped the stress reaction and were influenced by environmental stimuli (Lazarus, 1993). Also, their studies showed the potential impact of appraisal in the stress process by explaining the dynamics of taxing experiences. They explained that the way in which a stressful demand is perceived affects coping abilities and physiological responses. In other words, a taxing experience can either be perceived as a challenge or threat, depending on coping abilities/resources. A demand appraised as challenging suggests that an individual feels capable

of coping with a stressful situation; whereas a threat suggests that there is an inability to cope.

Advances in stress research have continued to the present time. For example, “allostasis” was introduced by Sterling and Eyer (1998) to describe the body’s ability to adjust and adapt to environmental changes that do not necessarily challenge survival. In order to describe the effects of the loss of allostasis, McEwen and colleagues (McEwen et al., 1998) conceptualized the notions of “allostatic load” and “allostatic overload.” Allostatic load described the effects the body endured from stress and allostatic overload was the period in which the body endured persistent stress or the inability to cope (2003). McEwen further delineated four different types of situations that could create allostatic overload. These included, 1) brief exposure to one or more environmental demands; 2) failure to adjust physiologically due to repeated stressful demands; 3) delay of physiological recovery even if the frequency or magnitude of the stress is normal; and 4) inability to cope and respond to the stress due to a state of poor well-being. Their research on animal models revealed that allostatic overload, regardless of the type, had potential to cause tissue damage if frequently experienced (McEwen and Stellar, 1993).

In summary, the concept of stress is centuries old. Throughout its long history stress has been recognized as a phenomenon that adversely impacts human functioning and health depending on its frequency, chronicity, and or how it is coped with. Particularly important for the proposed studies, modern researchers have shown that the perception of stress significantly alter its effects on humans.

2.2 Transactional Model of Stress

The stress model proposed by Lazarus and colleagues (1984) is particularly valuable for understanding the cognitive processes involved in stress appraisal. The model of Lazarus et al.

was referred to as the "transactional model" of stress because it viewed the stress response as the result of the relationship of a person to her environment. It has become one of the most widely used models in research on psychological coping (Lazarus and Folkman, 1984; Duhachek and Kelting, 2009).

Cognitive-relational stress theory (Lazarus and Folkman, 1987) defined appraisals as the process of categorizing situations based on their significance for well-being. In the transactional model of stress, both primary and secondary appraisals together determine the emotional, physiological, and behavioral responses to stress.

2.2.1 Primary Stress Appraisal

According to Lazarus and Folkman's model (1984), primary appraisals are judgments about the importance or significance of a particular "transaction" with respect to the well-being of the individual, referred to as a "person-environment transaction." There are three categories of primary appraisals of a "person-environment transaction" including, 1) irrelevant; 2) benign-positive; 3) stressful (Lazarus and Folkman, 1984). Events or transactions appraised as irrelevant occur when the individual has no stake or investment in the encounter (i.e., nothing can be gained or lost in the person-environment transaction). Transactions appraised as benign-positive or stressful have greater weight and are more likely to elicit an emotional response. (For the purpose of this study, irrelevant and benign-positive appraisals will not be discussed further because of their inability to affect or produce significant stress-inducing responses. Instead, primary appraisals of stress that carry threat and/or challenge will be discussed.)

The transactional model of stress suggested that an encounter began with the evaluation of a situation/event followed by the perception of its demands as threatening and/or challenging,

as illustrated in Figure 2.1. For example, if an individual appraised an encounter or situation as threatening, he or she anticipates that the outcome will produce negative feelings and a potential for loss. Appraisals of threat are associated with emotions such as fear, anger, and sadness.

While threat appraisals have negative implications, appraisals of challenge, reflect a potential for gain. Challenge appraisals are also anticipatory in nature but produce feelings of eagerness, motivation, and excitement. When an encounter is perceived as challenging, it poses an opportunity for growth and development (Lazarus and Folkman, 1984).

2.2.2 Secondary Stress Appraisal

Once a situation/event has been appraised as threatening, challenging, or irrelevant, coping resources, constraints, and options are assessed to determine the prospects for successful coping (Lazarus and Folkman, 1984). When evaluating coping options, the individual chooses those that he or she believes will accomplish a particular goal or those that provide the most effective outcome. Furthermore, an individuals' perception of coping abilities lead to experiencing positive stress (e.g., eustress) or negative stress (e.g., distress). Both positive and negative stress is determined according to whether or not the individual feels that they possess the necessary resources to cope with the stressor.

Considered together, primary and secondary appraisal processes shape the degree of stress and the strength of the emotional reaction. Both appraisal processes are integrated within Lazarus and Folkman's transactional model of stress and lead to emotional and behavioral outcomes and produce action tendencies that awaken the emotional system.

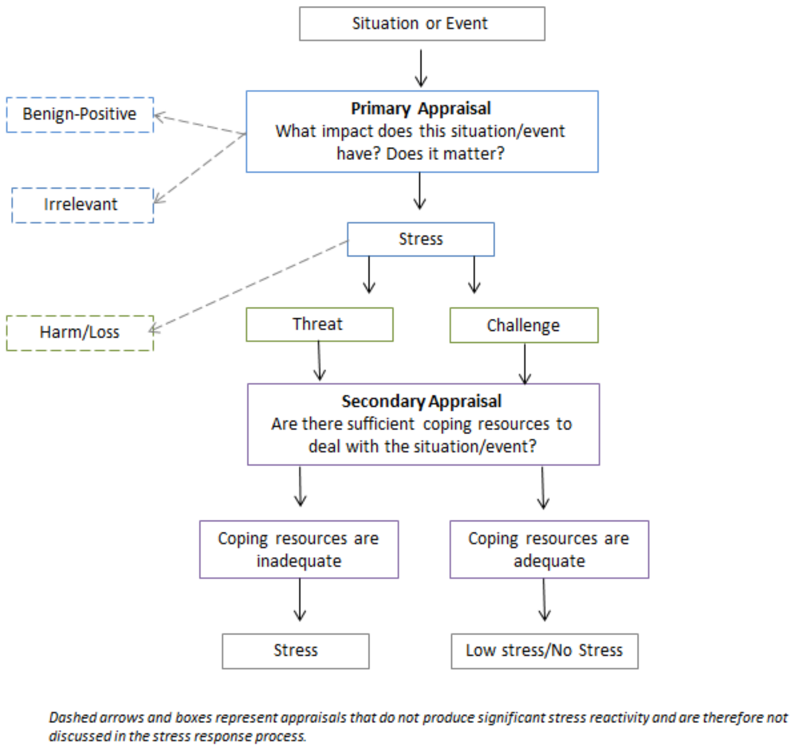

Figure 2.1 Appraisal process of challenge or threat.

2.3 Rationale for the Development of the Appraisal of Challenge or Threat Scale (ACTS)

Instruments that have measured the perception of stress appraisal confound the assessment of stress-related threats and challenges. Current measures of individual differences in stress-related cognitive appraisals include the Stress Appraisal Measure (SAM; Peacock and Wong, 1990), Stress Appraisal Measure for Adolescents (SAMA; Rowley et al., 2005), Appraisal of Life Events (ALE; Ferguson, Matthews, and Cox, 1999), Stress Appraisal Inventory for Life Situations (SAILS; 2002), and the Cognitive Appraisal Scale (CAS; Skinner and Brewer, 2002). Although these measures may assess distinct stress-related states (e.g., threat and challenge), each has multiple items assessing stress constructs that are distinct from the cognitive

appraisal process, including items that assess affective reactions. Statements such as “I feel anxious” (CAS) and “This situation would be [stressful]” (SAILS) measure constructs other than cognitive appraisal. The SAM for example includes items that assess behavioral reactions and stress consequences (e.g., “Stressful events impact me greatly” and “The outcome of stressful events is negative”).

Coping is another construct that confounds stress appraisal. This is apparent in items from the CAS and SAILS, which include statements such as “I tend to focus on the positive aspects of any situation” and “I would expect to gain something positive from this situation.” Such scales create a situation where measure of cognitive appraisal correlates with other stress constructs and adaptational outcomes (e.g., life satisfaction, social living).

Existing stress appraisal instruments suffer from conceptual and methodological problems and have been criticized for confounding the assessment of stress reactions and coping responses. The Appraisal of Challenge or Threat Scale (ACTS) was designed to address the need for an instrument that separately assessed threat and challenge aspects of stressful life events.

2.4 Appraisal of Challenge or Threat Scale (ACTS)

Lazarus and Folkman (1984) developed the transactional model of stress and coping and argued that stress was neither a part of the situation, nor a reaction of the body, but instead reflected a relationship of the individual with the environment. Appraisal, defined as an individual’s interpretation of a situation, was a key aspect of this relationship. They categorized stress appraisals on two dimensions: primary appraisal and secondary appraisal.

In the development of the stress appraisal scale that is used in the current research study,

Tomaka, Blascovich, and colleagues (1993) further refined the notions of threat and challenge appraisal and their measurement. In the study, the researchers used stressors that required behavioral performance, such as giving a short speech or performing mental arithmetic in order to examine threat and challenge appraisals and consequent reactions. Their results suggested that threat and challenge appraisals predict affective (relating to feelings and attitudes), behavioral (actions or reactions), and physiological (function in organisms) responses (Tomaka et al., 1993; Tomaka et al., 1997; Seery, 2011). Furthermore, challenge appraisals led to less negative affect and better task performance, whereas threat appraisals led to greater negative affective reactions and poor task performance.

To develop a comprehensive approach for the assessment of stress, Tomaka et al. (2012) used Lazarus and Folkman's transactional model of stress and coping and their empirical research findings (2012) to create the Appraisal of Threat or Challenge Scale (ACTS). Specifically, the ACTS assesses individual differences in the tendency to appraise events as threatening or challenging while evaluating primary and secondary stress appraisals.

The scale asks participants to appraise twenty-four potentially stressful life events across six content domains – conflict situations, unexpected events, public speaking, transportation, social anxiety, and financial concerns. Four items assess each of the six stress domains by presenting potentially stressful situations (e.g., you have an argument with boss or supervisor). Additionally, each item asks for two appraisals – (1) how demanding the event is (primary) and (2) how able a person is to cope (secondary). Total appraisal represents the addition of all items together. Whereas high scores suggest that a person appraises situations as threatening (i.e., less able to cope), low scores suggest that a person appraises situations as challenging (i.e., better able to cope). The more consistent a person is at appraising events as threatening, the higher the

ratings of perceived stress.

The ACTS was first analyzed using exploratory factor analyses. Results from an initial factor analysis (N = 166) suggested the presence of the six subscales, each with acceptable internal consistency (i.e., $\alpha = .74 - .85$) (Monks et al., 2010; Tomaka et al., 2012). Correlational analyses suggested good convergent and discriminant validity with the measures of perceived stress and dimensions of coping. Results of confirmatory factor analysis indicated that six distinct constructs for appraisal of stressful events did indeed exist. Results supported the six-factor model for the ACTS and confirmed reliability with total appraisal ($\alpha = .94$), as well as for each of the factors (i.e., $\alpha = .77 - .86$) (Tomaka et al., 2012; Monks et al., 2010). This data confirmed the factor structure and reliability of the scale, as well as provide additional evidence for its validity and ability to assess individual differences in cognitive appraisal without confounding other stress-related structures (e.g., affective and behavioral reactions, stress consequences, coping, and personality).

2.5 Stress Appraisal and Experienced Stress

A stress appraisal questionnaire can become a valuable tool for preventive medicine if the relationships between self-reported stress appraisal and an individual's physiological response to a stressful event can be established. To study this experimentally, the induction of stress in the laboratory and the measurement of established biomarkers of the stress response are required before and after stress induction.

There have been many approaches to inducing stress in the laboratory; a commonly used laboratory stress-inducer is an arithmetic task. Tomaka and colleagues (1993) used this technique to measure cardiovascular responses before and after task instructions were given and

throughout the task performance. Participants' self-reported demand and resource appraisals were assessed after the instructions but before beginning the task. The demand in relation to the task was calculated between the two assessments. Results showed that challenge participants displayed increased cardiac performance coupled with total peripheral resistance (i.e., vasodilation), while threatened participants displayed increased cardiac performance coupled with slightly increased total peripheral resistance (i.e., vasoconstriction). These patterns indicated higher levels of blood pressure and stress in threatened participants as opposed to challenged participants.

Rohrmann and colleagues (1999) also used a stress-inducing approach when measuring psychobiological reactions. Study participants were randomly assigned to a control group or an arousing and assuring manipulation group. Those who were in the arousing manipulation condition were told that during the performance of their speech task they were physiologically highly aroused and afraid. Those in the reassuring manipulation group were told that they were not physiologically and emotionally aroused. Findings showed that heart rate, systolic blood pressure, cortisol, and electrodermal responses were highest in the reassuring manipulation group and lowest in the no manipulation group. In order to explain these findings, Rohrmann hypothesized that participants may have had interference in internal cognitive coping processes while being told that they were not aroused. In addition, they may have been irritated or nervous when being informed of their lack of arousal because in actuality they were experiencing symptoms of arousal. As expected, results showed that stress reactions were highest in the condition with arousing manipulation and lower in the condition with reassuring manipulation, therefore suggesting once again that demands of threat and challenge affect levels of physiological biomarkers.

Similar findings to that of Tomaka and colleagues (1993) showed that significant associations existed between both threat and challenge and greater diastolic blood pressure (DBP) reactivity when exposing participants to a mental arithmetic task in the laboratory (Maier et al., 2003). DBP increased in those participants reporting a high degree of threat appraisal and a low degree of challenge appraisal. Adding to the literature, Maier and colleagues also found that threat appraisal significantly predicted enhanced negative affect, whereas challenge appraisals predicted greater positive affect and task management. Their results supported the cognitive appraisal model and partially supported models of cardiovascular reactivity. Furthermore, a relationship between DBP reactivity to greater risk of cardiovascular disease suggested the importance of continued investigation to verify the association between cognitive appraisal and DBP reactivity.

2.6 Gender and its Effects on the Stress Process

Researchers using stress inducing laboratory tasks have also shown that there are significant gender differences in the appraisal of stressful demands. For example, findings have showed that women as compared to men are more prone to increased stress reactivity and psychological conditions (e.g., depression, anxiety) (Schmaus et al., 2008). These differences have been related to women's perception of stressful demands (threatening vs. challenging) and HPA activity. Specifically, measures of HPA activity on stress-induced tasks have showed greater HPA and autonomic responses in women as compared to men (Kajantie and Phillips, 2006; Kudielka and Kirschbaum, 2005).

A study conducted by Schmaus and colleagues (2008) showed that stress responses and coping abilities are affected by differences in appraisal. Participants were instructed to view a

stressful video on two occasions with a 2-day interval between sessions. Findings showed that women exhibited significantly greater heart rate reactivity to the repeated laboratory stressor as compared to men. This indicated that the women's perceptions of the videos and therefore coping abilities affected their physiological stress responses; findings also suggested that unlike men, women appeared to be more vulnerable to the effects of repeated stress exposures, which in turn, also made it more difficult to cope with the psychological stress (Schmaus et al., 2008).

Researchers have also assessed gender differences in stress experienced in work-related settings. Herrero and colleagues (2012) showed that men and women exhibited different stress and coping processes, adopting distinct techniques when encountering a stressful situation at work. It was found that work-related mental fatigue induced by task demands (e.g., tight deadlines) had more negative influence in women than on men. It is believed that the women's appraisal of the task demand greatly affected their coping abilities. More specifically, findings suggested that women were more prone to psychological stress responses as compared to men when work situations involved pressure of deadlines, intellectually and complex tasks, and high levels of attention (Herrero et al., 2012).

In summary, researchers have shown that the appraisal process greatly affects physiological and psychological stress responses. Individual perceptions can dramatically increase or reduce stress reactivity, therefore causing changes in autonomic responses (e.g., heart rate, blood pressure). As seen in previous research, studies measuring biomarkers of the autonomic nervous system in response to stress are widely used because of their sensitivity to stress reactivity. More specifically, measuring hormones, enzymes, and heart rate have shown to be a promising approach when assessing individual differences in cognitive stress appraisal.

2.7 Biomarkers of Experienced Stress

When an individual perceives a situation as stressful, as described in the introduction, the SAM and HPA axes stimulate the release of hormones and enzymes and affect physiological responses. Recognized biomarkers include alpha-amylase and heart rate. Overall, findings have consistently shown that after being exposed to stress-inducing and psychologically demanding situations changes in these biomarkers occurred. As a result, these biomarkers can indicate a person's reactivity to stress and thus their risk of stress-induced diseases (Dickerson and Kemeny, 2004; Jacobs et al., 2001; Owen and Steptoe, 2003).

2.7.1 Alpha-amylase as a Biomarker in Stress

Salivary alpha-amylase (sAA) is a widely used stress biomarker. sAA is an enzyme that is produced by activity of the sympathetic nervous system, specifically the SAM axis mechanisms. Interest in sAA as a stress biomarker greatly increased when Chatterton and colleagues (1996) published findings of increased sAA in response to a variety of stressful conditions. Their findings led other researchers to use sAA as an indicator of physiological changes caused by psychological stressors. Since that time, several other studies have confirmed the first findings. Although it is normal for alpha-amylase to steadily increase with daily activity, studies have shown that psychological stress significantly increases sAA through the effects of psychological stress on the autonomic nervous system (ANS) (Nater and Rohleder, 2009; van Stegeren et al., 2006; Proctor and Carpenter, 2007). Research has shown that levels of sAA increase with physiological stress exposure (Li and Gleeson, 2004; Walsh et al., 1999). More specifically, a study conducted by Rohleder and colleagues (2004) subjecting participants to a psychosocial stress test (Trier Social Stress Test, TSST) showed that exposure to the TSST

induced significant increases of sAA and norepinephrine; both parameters were positively associated with stress.

Few studies have found no changes in sAA in response to stressful stimuli (Nater and Rohleder, 2009). Thus, sAA has become regarded as a reliable biomarker of ANS activity in response to psychological stress in various fields of biobehavioral research (Keller et al., 2009; Gordis et al., 2010; Gordis et al., 2008; Granger, 2007), and investigators using the TSST have shown that this stress induction protocol produces changes in sAA.

2.7.2 Heart Rate as a Biomarker in Stress

Along with enzymes, cardiac autonomic responses such as heart rate (HR) have been studied in response to mental stress. Heart rate refers to the speed of the heartbeat and is typically expressed as beats per minute (bpm). The time between beats is called the inter-beat-interval (IBM) and is used to analyze hear rate variations. HR varies according to the body's physical needs and its reaction to physical and psychological stimuli.

Heart rate has been increasingly used when assessing reactivity to behavioral or psychological stress because of its potential to predict and identify individual differences in cardiovascular risk and health (Treiber et al., 2003). Extensive research on the cardiovascular system led researchers to determine that its main role is to maintain homeostasis and adequate blood flow to bodily tissues (Cohen et al., 1995). Changes in the system caused by stress evoke physiological autonomic responses such as increases in HR, blood pressure, among others.

In order to understand how the heart responds to stress, it is important to know the physiology of the heart. The heart is able to beat due to pacemaker cells, more specifically, the sinoatrial (SA) and atrioventricular (AV) nodes. These nodes contract heart muscles and when

the sympathetic nerve fibers are activated, the SA node produces an increase in HR and ventricular contraction. The increase in HR is activated by the fight or flight response and cause the release of epinephrine and norepinephrine into the bloodstream from the adrenal medulla (Cohen et al., 1995). As a result, blood is diverted from areas that are not required in an emergency (e.g., gastric tract) to areas that need the additional blood flow (e.g., skeletal muscles). Once the stress or threat has dissipated, the parasympathetic system brings the HR back to a normal state and re-diverts the blood back to all areas (i.e., to the gastrointestinal tract from the skeletal muscles).

Taking an interdisciplinary approach may help researchers to better understand the cognitive appraisal of stress. By integrating different approaches and using multiple measurements (e.g., appraisal scale, biomarkers), a more reliable method for assessing stress appraisal can be developed. Incorporating more than one technique can also lead to new and innovative findings that contribute to our understanding of the relationship between cognitive appraisal of stress and physiological reactivity to stress. While many studies have examined whether biomarkers are associated with physical and psychological stress, to the best of our knowledge, no studies have yet examined the possibility that cognitive stress appraisal habits predict biomarker reactivity following a stressful experience. If cognitive stress appraisal style predicts biomarker reactivity following completion of a stressful event, it might suggest that altering one's perception of stress might be used to alter their biological risk of harmful stress reactions. This could guide our understanding of which types of appraisals pose the greatest physiological stress for the individual.

Because studies have suggested that women are both more psychologically and physiologically reactive to stress than men (Schamus et al., 2008), this study focused exclusively

on biomarker reactivity in females with differing levels of stress appraisal.

In order to fully assess differences in perceived stress, the newly developed Appraisal of Challenge or Threat Scale (ACTS) (Tomaka et al., 2012) was used to quantify the perception of threat, that is, the extent to which a given situation poses threat, from the perception of coping abilities in a variety of life situations. Scores on the Primary Threat Appraisal and Secondary Threat Appraisal subscales were used to select subjects for inclusion in one of two groups.

Baseline salivary alpha-amylase and heart rate measurements were collected. Challenge samples were collected upon completion of the first and second stress task (e.g., speech and arithmetic task).

The general hypothesis was that the biophysiological biomarker levels would differ in individuals according to their perceptions of situational demands as threatening and/or challenging. It was anticipated that greater perceived stress would predict higher levels of stress-induced biomarkers.

Chapter 3: Method

3.1 Power Analysis

A power analysis was calculated for power (.90) and effect size (.25). The analysis determined that 46 subjects were needed. A total of 48 participants, 24 participants in each group, were enrolled in the study to protect against attrition.

3.2 Participant Recruitment

Participants were recruited from undergraduate classes in the College of Health Sciences. Faculty members were contacted and asked if a brief explanation of the study could be presented by the researcher during one of their lectures. Female students who were interested in participating in the study completed a demographic and health questionnaire and the Appraisal of Challenge or Threat Scale (ACTS). Participants who qualified and successfully completed all phases of the study were given extra credit in their respective course.

3.3 Participants

Approximately 120 participants were screened for inclusion in the study. Examination of health questionnaire data resulted in the exclusion of approximately 40 participants. The Appraisal of Challenge or Threat Scale (ACTS) further eliminated participants from qualifying in the study and selected approximately 60 for inclusion in the study.

Data was collected from 48 participants – 24 from the "High Stress-Low Coping" group and 24 from the "Low Stress-High Coping" group. Inclusion criteria for this study were female, ages 18-30 and enrolled at the University of Texas at El Paso and completion of the Appraisal of

Challenge or Threat Scale (ACTS). Exclusion criteria included prior diagnosis of a psychological stress disorder (e.g., depression, anxiety, PTSD), cardiovascular disorder (e.g., hypertension, high blood pressure), active inflammatory disease and/or allergies, thyroid condition, diabetes condition, and/or any chronic disease. In addition, participants were excluded if they recently or were presently receiving treatment that contains beta-blockers and/or steroids.

3.4 Blood Pressure

Blood pressure measurements were taken for all participants prior to the TSST stress induction. Participants were allowed to rest for 5 minutes prior to taking the readings. Blood pressure measurements were taken three times at two minute intervals using a digital sphygmomanometer cuff. Mean blood pressure readings were calculated.

3.5 Scale/Survey Measurements

Participants were administered three scales – the Appraisal of Challenge or Threat Scale (ACTS), the Social Readjustment Rating Scale (SRRS), and the Psychological Stress Measure (PSM-9). The SRRS was administered to participants prior to beginning the stress tasks and the PSM-9 was administered twice, before and after the stress tasks.

3.5.1 Appraisal of Challenge or Threat Scale (ACTS)

Stress appraisal was assessed using the Appraisal of Challenge or Threat Scale (ACTS). The 24-item scale was created to quantify individual differences in the appraisal of threat and challenge. The scale lists potentially stressful life events across six content domains including

conflict situations, unexpected events, public speaking, transportation, social anxiety, and financial concerns. Participants were asked to rate each item on a 5-point scale ("not at all" to "very much") indicating their appraisal of 1) how demanding the situation is (primary appraisal) and 2) how able she is to cope with the situation (secondary appraisal). Two subscale scores (primary and secondary appraisal) and one total score were calculated for each participant.

3.5.2 Social Readjustment Rating Scale (SRRS)

A life event questionnaire was administered to participants in order to determine if scores on the questionnaire influenced and/or predicted changes in biomarker levels.

The Social Readjustment Rating Scale (SRRS) is an inventory of common stressors experienced during the past year (Holmes and Rahe, 1967). It is a 42-item short-phrase questionnaire that has about a 5-minute response time. Each life event listed on the SRRS has an assigned "life changing unit" that is used to reflect the amount of stress that that event causes. The values are added and the total is reflective of the amount of stress experienced within the past year.

For the purposes of this study, the scale was modified to query events that may have occurred in the past week. The time frame was modified in order to control for these events in the analysis. The rationale is that stress inducing events that occurred in the past seven days may have a greater effect on biomarker levels than those that have occurred over the past year.

3.5.3 Psychological Stress Measure (PSM-9)

The third scale administered to participants was the Psychological Stress Measure (PSM-9). The survey is an abridged version of the original 49-item PSM and was created to allow for a

shorter testing period (Lemyre and Lalande-Markon, 2009). Participants were asked to read 9 items that described a particular mood and rate them on a scale from 1-8 (e.g., "not at all" to "extremely") depending on their current state. The measure was administered to participants before and after the study in order to collect pre- and post-test data.

3.6 Stress-Induction Method – Trier Social Stress Test (TSST)

The study used the Trier Social Stress Test (TSST) for the induction of psychosocial stress. The TSST was chosen because it has proven to be an effective research tool for inducing HPA activity and changes of endocrine and cardiovascular parameters (Kudielka et al., 2007). In addition, it has been used in over 4,000 laboratory study sessions worldwide (Kudielka et al., 2007) and has been used to investigate a wide range of different outcome variables. More importantly, it has shown to have a profound effect on numerous physiological biomarkers including cortisol (Kirschbaum et al., 1993) and alpha-amylase (Maruyama et al., 2012).

For example, Kirschbaum and colleagues (1993) witnessed a 2- to 4- fold increase in salivary cortisol levels in different population samples when using the TSST protocol. Additionally, their findings showed that more than 70% of participants responded with increased cortisol levels as compared to baseline measurements after the TSST procedure. Similar findings have found that 70-80% of all participants experienced a rise of salivary cortisol levels of 100-200% with peak levels at approximately 30 minutes after initiation of the TSST protocol (Hellhammer, 2011). Again, indicating that the TSST is a highly sensitive instrument in the assessment of stress effects.

Researchers have shown that because the TSST contains two stress-inducing motivated performance tasks, it has been associated with the largest HPA axis stress responses and longest

recovery times (Dickerson and Kemeny, 2004). It is highly effective in inducing stress reactivity because it is a motivated performance task that combines elements of uncontrollability and high levels of social-evaluative threat.

The TSST has shown to induce psychological, endocrine, and cardiovascular stress responses reliably in healthy subjects as well as in clinical populations (Goodyer et al., 2000; Goodyer et al., 2001; Harris et al., 2000; Kirschbaum et al., 1996). Thus, the Trier Social Stress Test is a reliable, valid, and standardized protocol for inducing stress in laboratory settings and has become one of the most widely used psychosocial stress protocols in the field of basic, applied, and clinical psychobiological research.

As briefly described above, the TSST is a laboratory procedure that involves two types of stress-inducing activities (e.g., speech and arithmetic task) and is generally composed of five phases (explained below). Unlike other stress-induced tasks (e.g., Stroop color word test, computerized game such as Minesweeper), the Trier Social Stress Test does not require extensive setup or the use of additional programs/software.

3.6.1 Pilot Study Manipulation

In order to assure that the Trier Social Stress Test (TSST) would indeed induce changes in biomarker levels in the current study, a pilot study was conducted. Ten women were recruited and asked to complete the TSST. Baseline heart rate and blood pressure was determined upon arrival; heart rate was monitored throughout the stress-induction tasks. Following on the results of past studies, the TSST produced changes in biomarker levels within individuals.

3.6.2 Pre-stress Period

The first phase of the TSST procedure is referred to as the pre-stress period. On arrival, participants were unaware that they would be asked to participate in a series of stress-inducing tasks. Participants were asked to confirm that they did not consume food or any beverage (other than water) in the 8 hours prior to testing. Baseline biomarker levels and blood pressure were determined after the participants rested for 5 minutes.

Participants were asked to complete the PSM-9 pre-test and the SRRS questionnaire. The first saliva sample was collected after questionnaires were completed.

3.6.3 Preparation Period

Participants were escorted to a second room for the remainder of the study. Participants were asked to lie on a bed and relax as six electrodes were placed on their chest and abdominal area. Baseline heart rate was measured for a total of five minutes prior to beginning the stress-induction tasks. To initiate the first stress task a pre-recorded video was played. The video featured an individual providing instructions to the participants and informing them that their presentation will be audio recorded. A researcher was present in the room at all times throughout the session. (The researcher maintained a neutral expression, made eye contact with the participant, and made no comments throughout the tasks.). To begin the TSST speech preparation period, the following script was read to the participants by the individual in the video: "*Your task in this experiment is the following: please imagine that you have applied for a job and have been invited for an interview. In contrast to a real interview, however, you are supposed to give a talk, in which you are to convince me in five minutes why you think that you would be the best candidate for this position. Please note that you will be audio recorded for*

subsequent voice analysis. You should try to leave the best possible impression, and assume the role of the applicant for the duration of the talk as best as you can. A researcher will reserve the right to ask follow-up questions in case of uncertainties to receive all necessary information from you. Following your talk, you will be given a second task, which will only be explained to you in a second video clip. You will have some time to organize your thoughts and mentally prepare. Do you have any questions?" After seeing the video, participants were allowed to ask the researcher additional questions or clarification on the speech task. Participants were left alone in the room to prepare. (It is important to note that any conversation about the situation was avoided.)

3.6.4 Speech Task

After two minutes, participants were asked to lie as still as possible during the tasks in order to avoid removing all attached heart rate monitoring electrodes and to minimize noise caused by movement in the readings. The prop audio recorder was turned on to increase performance stress. The researcher began the session by saying, *"Please begin your talk."*

If the participants finished before the 5-minute time period, they were allowed to formulate additional elaborations. The researcher remained silent for 20 seconds while the participants did so. If they did not continue speaking, the researcher prompted them to do so by saying, "*You still have time, please continue..."* If the participants did not continue with their speech after 10 seconds, the researcher asked questions until the end of the time period.

Examples of questions were:

- *Why do you think you are especially well-qualified for this task?*
- *Why do you think you are better qualified than the other applicants?*

- *What new skills/knowledge can you offer in this position?*

At the end of the 5-minute speech task, a second sample of saliva was collected using the saliva collection kit.

If at any time a participant appeared to have an adverse reaction (i.e., begin to cry, become agitated) the researcher asked, *"Are you okay to continue?"* Any participant indicating that they did not wish to continue, would be debriefed and escorted out of the laboratory.

3.6.5 Arithmetic Task

At the end of the 5-minute speech task, the video was played once more and explained the second stress task and clarified that it was not related to the interview presentation. The individual in the video addressed the participant by reading a script that stated: *"I now want you to solve a calculation task. Please count aloud backwards from 1,022 to zero in 13-step sequences. Please calculate as quickly and correctly as possible. Should you miscalculate, the researcher who is present in the room will point out your mistake and you will have to start all over again. Do you have any questions?"*

Once again the participant was able to ask questions and receive clarification on the arithmetic task instructions. The researcher was provided with correct responses. A digital clock set for 5 minutes was visible to the participants. If the participants made a mathematical mistake, the researcher responded with, *"That is incorrect, please start over from 1,022."*

As in the speech task, a participant experiencing an adverse reaction and who wished to stop would be debriefed and escorted out of the laboratory.

3.5.6 Recovery and Debriefing Period

At the end of the 5-minute arithmetic task, a third and final sample of saliva was collected. Heart rate was monitored for an additional five minutes following the arithmetic task. Once the five minutes elapsed, participants were asked to complete the PSM-9 post-test and the researcher removed all electrodes from the participants' abdominal and chest area. Participants were thanked for their participation and were debriefed as to the goals and objectives of the experiment. They were informed that their performance was not audio recorded and that no analysis on their speech or math performance would be conducted.

3.7 Salivary Alpha-amylase Measures

To assess alpha-amylase, passive drool saliva kits were used (Salimetrics, Inc., etc.). This method was chosen due to the convenient and minimally-invasive methods necessary for measuring alpha-amylase levels.

Participants were instructed to rinse their mouth with water 10 minutes prior to collection. When preparing to collect the sample, participants were instructed to allow saliva to pool in the mouth. With head tilted forward, participants drooled down a 2-inch straw attachment and collected saliva in the cryovial provided to them. Because saliva tends to foam, a cryovial with twice the capacity of the sample was used. Approximately one mL (excluding foam) was collected for testing. The samples were kept cold after collection (4°C) and frozen (-20° to -80°C) soon after.

3.7.1 Salivary Alpha-amylase Kinetic Enzyme Assay Kit

Saliva samples were removed from freezer and were allowed to thaw to room

temperature. Reagents were also removed from the refrigerator and were allowed to reach room temperature.

Once thawed, all saliva samples were vortexed for 3-5 seconds at a high setting. Samples were centrifuged at 1500xg (3000 rpm) for 15 minutes. Centrifuging removes mucins and other particulate matter from the whole saliva, forming a pellet at the bottom of the vial. All labeled centrifuged samples were set aside.

Once the plate layout was determined the plate reader was incubated at 37°C and set to read in center measurement kinetic mode at 60 second increments for 3 minutes. A 405 nm filter with no reference filter was chosen.

Saliva samples were diluted with the alpha-amylase diluent. A 1:10 dilution of the saliva was prepared by pipetting 10uL of saliva into 90uL alpha-amylase diluent. The solution was mixed well and was further diluted by pipetting 10uL of the 1:10 dilution into 190uL alpha-amylase diluent (1:200). The final dilution was 1:200. The remainder of the 1:10 dilution was set aside in case a different dilution was necessary.

The alpha-amylase substrate solution was heated to 37°C in a trough that was provided using a preheated microtiter plate incubator. The reagent was warmed and mixed before use.

Six strips were tested at a time. 8uL of controls (prediluted) and/or diluted saliva samples were added to individual wells. 320uL of preheated (37°C) alpha amylase solution were added to each well simultaneously using a multichannel pipette. The plate was transferred to a reader to read optical density (change in absorbance) at exactly 60 second increments for three minutes. The plate was programmed to continuously mix in slow mode between readings.

The enzymatic activity (U/mL) was calculated using the following formula: (ΔAbs./min x TV x DF)/(MMA x SV x LP) (where ΔAbs/min = absorbance difference per minute, TV = total

assay volume (0.164 mL), DF = Dilution Factor (200), MMA = millimolar absorptivity of 2-chloro-p-nitrophenol (12.9), SV = Sample volume (0.08 mL), LP = Light path (0.97)). Calculations were conducted by subtracting the one minute reading from the three minute reading and multiplying by the conversion factor. The conversion factor took the 1:200 sample dilutions into account for the prediluted controls and samples.

3.8 Heart Rate Measures

Participants were asked to lie down on a bed. HR was examined by utilizing a 6 lead electrode placement across the chest using the Physioflow pulse wave analysis system. HR was measured continuously throughout the study and markers were entered signifying the start and completion of each stress task. Upon the completion of the baseline measure the subjects were introduced to a stress task phase. Upon completion of the stress task phase the HR was monitored for another 5 minutes to determine HR recovery.

A PhysioFlow device was used because it noninvasively measures cardiac output and other cardiac parameters such as heart rate. The PhysioFlow has the capability to continuously present heart rate, stroke volume, ejection faction (estimate), cardiac output/index, contractility index and early diastolic filling ratio. For the purposes of this study heart rate was the only vital sign used in the analyses.

3.9 Procedure

Participants who met the inclusion criteria for this study were screened for participation using the Appraisal of Challenge or Threat Scale (ACTS). Scores on the Primary Threat Appraisal and Secondary Threat Appraisal subscales were used to select subjects for inclusion in

one of two groups according to the following criteria. Group 1, High Stress-Low Coping group included women whose Primary Threat Appraisal scores were in the $\geq 75^{th}$ percentile (upper quartile) of scores possible (Primary Appraisal subscale score ≥ 72) and a Secondary Threat Appraisal score in the bottom 25^{th} percentile (bottom quartile) of scores possible (Secondary Appraisal subscale score ≤ 24); Group 2, Low Stress-High Coping group included women whose Primary Threat Appraisal scores were in the bottom 25^{th} percentile (bottom quartile) of scores possible (Primary Appraisal subscale score ≤ 24) and Secondary Threat Appraisal scores in the $\geq 75^{th}$ percentile (upper quartile) of scores possible (Secondary Appraisal subscale score ≥ 72).

Participants whose scores did not fit into one of the two groups did not continue in the study. Participants whose ACTS scores did fall into one of the two groups were asked to return for continued participation. Participants were asked to schedule their testing appointment within one-week post menses. In preparation for the testing day, continuing participants were asked to return to the laboratory between 7:00 a.m. and 10:00 a.m. on the day of testing, fast for the 8 hours prior to testing, and to refrain from taking over-the-counter anti-histamines and anti-inflammatories for 48 hours prior to the testing.

Testing was carried out in the morning in a controlled laboratory setting. Upon arriving at the laboratory, participants were asked to rest for a period of 5 minutes prior to beginning the study. The SRRS and the PSM-9 questionnaires were administered to participants during the resting period. Samples were then collected to determine baseline heart rate and alpha-amylase. Instructions for the TSST test (described above) were provided to participants. Challenge samples were collected following the speech and arithmetic task.

3.10 Analyses

A 3 x 2 mixed model analysis of variance with time as the within subject factor and group as the between subject factor was used to compare biomarker outcomes (heart rate and salivary alpha-amylase). Participants' demographics were examined for differences among groups in order to determine whether any demographic variables needed to be controlled in the analysis. Clinical characteristics were also statistically tested for differences among groups. Additionally, multiple regression analyses were conducted to explore whether domains of the ACTS scale might predict HR and sAA biomarkers.

Chapter 4: Results

4.1 Purposes of Study

The purpose of this study was to determine whether the level of self-reported perceived stress predicted the level of stress-induced biomarkers. Prior to enrollment in the study, potential participants completed demographics and health questionnaires and the Appraisal of Challenge or Threat Scale (ACTS). Scores on the ACTS were used to quantify participants' levels of perceived threat and levels of perceived ability to cope in six types of common life situations. Participants' ACTS scores were used to select subjects for inclusion in the study. Participants were chosen to participate if the ACTS scores indicated either High Stress-Low Coping (Group 1) or Low Stress-High Coping (Group 2) (see Method section, page 24, paragraph 1 for complete description of selection rationale).

Selected participants returned to the study and completed a life inventory of stressful events questionnaire (SRRS) and the Trier Social Stress Test (TSST), a standardized stress-induction procedure that included a speech and arithmetic task. Pre- and post-stress induction measures were obtained for current mood state (PSM-9). Measures of two stress biomarkers, including heart rate (HR) and salivary alpha-amylase (sAA), were collected before, during, and after the stress induction. Blood pressure was measured prior to the beginning of the TSST procedure.

4.2 Participant Demographic Characteristics

Data were collected from 48 undergraduate women, ranging in age between 18 and 30 years (M = 21.46, SD = 2.95) and enrolled at the University of Texas at El Paso. Participant

demographics are presented in Table 4.1. The majority of participants were single. Over three-fourths of the participants identified themselves as Hispanic/Latino; the remaining self-identified as Non-Hispanic white/Caucasian or "other" (Asian/Pacific Islander, Black, Native American). Approximately half of the participants were from the College of Health Sciences, less than one-third were from the College of Sciences, and the remaining participants were from the College of Education, Business, or Liberal Arts.

Participant demographics were examined for differences among groups in order to determine whether participant college should be controlled in the analysis. As seen in Table 4.1, demographic characteristics that might be expected to influence outcomes were similarly distributed in Group 1 and 2.

Tale 4.1 Participant Demographic Characteristics

		Group 1 24/24 (100%)	Group 2 24/24 (100%)	Total Group
Age		21.88 (3.23)	21.0 (2.60)	21.46 (2.95)
Marital Status	Single	20/24 (83.3%)	21/23 (87.5%)	41/47 (97.9%)
	Married	4/24 (16.7%)	1/23 (4.2%)	5/47 (10.4%)
	Separated	0/24 (0.00%)	1/23 (4.2%)	1/47 (2.1 %)
Ethnicity	Hispanic/Latino	20/24 (83.3%)	18/23 (75.0%)	38/47 (80.9%)
	Non-Hispanic	3/24 (12.5%)	3/23 (12.5%)	6/47 (12.8%)
	Other	1/24 (4.2%)	2/23 (8.3%)	3/47 (6.4%)
College	Health Sciences	13/24 (54.2%)	12/24 (50.0%)	25/48 (52.1%)
	Sciences	8/24 (33.3%)	9/24 (37.5%)	17/48 (35.4%)
	Other	3/24 (12.5%)	3/24 (12.5%)	6/48 (12.5%)

4.3 Participant Clinical Characteristics

Participant clinical characteristics are presented in Tables 4.2-4.4. As Table 4.4 shows Group 1 had slightly higher systolic and diastolic blood pressure readings as compared to Group 2. Blood pressure measurements were examined for differences among groups; if measurements suggested that groups differed substantially, differences in systolic and diastolic readings would have been controlled in the analysis. Responses from the SRRS showed that Group 2 either experienced more stressful events or events that were more severe in the past week prior to the study (Table 4.3). According to the PSM-9 measure both groups experienced a difference in

current mood after the study (Tale 4.3). Group 1 participants reported an improvement of current state/mood, whereas Group 2 participants reported a worsening of current state/mood (e.g., became more nervous, stressed). Scores on the individual ACTS domains showed that both groups perceived Unexpected Medical Events as being the most stressful, whereas Social Anxiety Events were the least stressful when compared to the other domains (Table 4.2). Furthermore, Group 1 had higher average primary and secondary appraisal subscale scores on the ACTS as compared to Group 2. In terms of biomarkers, HR was highest during the speech task in both groups. Group 1 experienced peak alpha-amylase levels during the speech task whereas Group 2 experienced peak alpha-amylase levels during the arithmetic task.

Clinical characteristics were also statistically tested for differences among the groups. Considering the means and SDs of the variables that differed, only differences in SRRS scores were large enough to warrant a statistical test of significance. A one way analysis of variance showed that the difference was not statistically different ($F_{(1, 47)} = 1.10$, $p=.30$). The experience of stressful events prior to the study did not differ between the two groups.

Table 4.2 Participant Clinical Characteristics – Appraisal of Challenge or Threat Scale (ACTS) Domains and Subscales

		Group 1 24/24 (100%)	Group 2 24/24 (100%)	Total Group
ACTS Domains	Public Speaking Average	0.89 (0.80)	0.98 (0.53)	0.94 (0.67)
	Unexpected Medical Events Average	1.53 (0.74)	1.04 (0.58)	1.29 (0.70)
	Financial Concerns Average	1.28 (0.80)	1.01 (0.54)	1.15 (0.69)
	Conflict Situation Average	0.93 (0.58)	0.87 (0.62)	0.90 (0.60)
	Transportation & Automotive Average	1.23 (0.77)	0.89 (0.56)	1.08 (0.69)
	Social Anxiety Average	0.78 (0.69)	0.71 (0.54)	0.74 (0.61)
ACTS Subscales	Average Primary Appraisal Score	98.29 (7.70)	77.92 (8.66)	88.10 (13.10)
	Average Secondary Appraisal Score	77.54 (6.10)	53.79 (9.62)	65.67 (14.41)

Table 4.3 Participant Clinical Characteristics – Social Readjustment Rating Scale (SRRS) and Psychological Stress Measure (PSM-9)

		Group 1 24/24 (100%)	Group 2 24/24 (100%)	Total Group
SRRS		33.83 (57.05)	53.25 (64.30)	44.08 (60.65)
PSM-9	Average Pre-test Score	24.17 (11.06)	27.21 (10.45)	25.69 (10.75)
	Average Post-test Score	21.75 (8.53)	29.25 (11.23)	25.50 (10.57)

Table 4.4 Participant Clinical Characteristics – Blood Pressure (BP), Heart Rate (HR), and Alpha-amylase

		Group 1 24/24 (100%)	Group 2 24/24 (100%)	Total Group
Blood Pressure	Average Systolic	108.38 (10.24)	105.67 (10.79)	107.02 (10.50)
	Average Diastolic	71.08 (9.88)	69.25 (10.07)	70.17 (9.91)
Heart Rate	Average Baseline	79.35 (9.88)	78.41 (16.62)	78.88 (13.53)
	Average Speech – 1st minute	90.79 (13.63)	92.53 (14.23)	91.66 (13.81)
	Average Arithmetic – 1st minute	79.42 (9.75)	79.31 (18.20)	79.37 (14.45)
Alpha-amylase	Average Baseline	29.14 (21.90)	23.93 (17.86)	26.53 (19.94)
	Peak Speech	50.66 (41.07)	37.56 (38.34)	44.11 (39.86)
	Peak Arithmetic	32.16 (22.45)	25.28 (17.18)	28.72 (20.08)

4.3.1 Blood Pressure (BP)

Clinical characteristics were also statistically tested for differences among the groups. The blood pressure measurements (taken at two-minute intervals preceding the TSST) are shown in Figures 4.2 and 4.3 below. The mean of systolic readings ranged from 90-137; mean diastolic readings ranged from 49-100. Systolic and diastolic readings for Group 1 participants were greater when compared to Group 2 (Figure 4.2 and 4.3). One way analysis of variance showed however that the differences in systolic ($F_{(1,47)} = .80$, $p = .38$) and diastolic ($F_{(1,47)} = .41$, $p = .53$) readings were not statistically significant, therefore the differences in measurements did not have to be controlled for in the analysis (Figure 4.1).

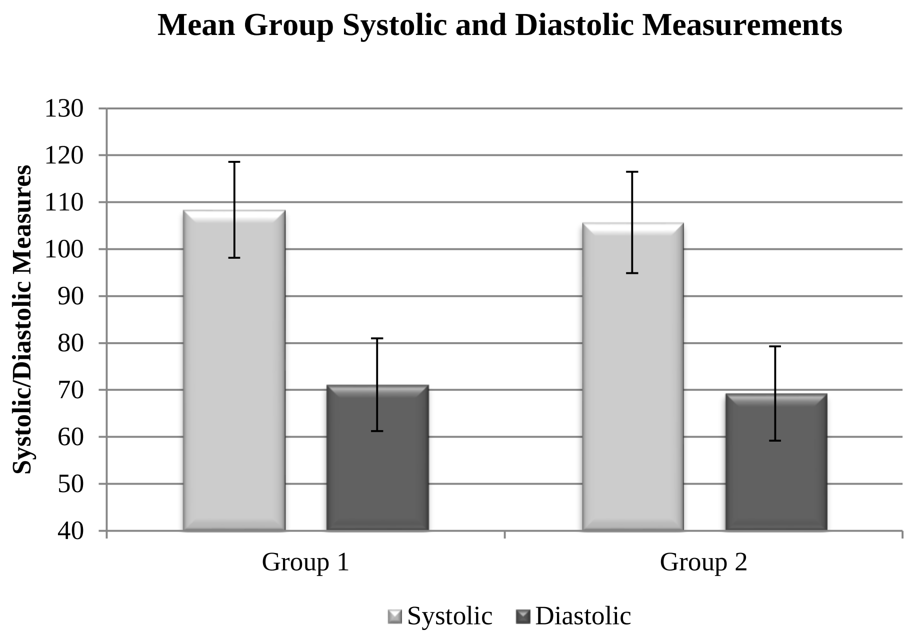

Figure 4.1 Mean systolic and diastolic blood pressure measurements were measured before the stress induction and calculated by group (Group 1, N = 24; Group 2, N = 24). An ANOVA showed that the differences in systolic ($F(1,47) = .80$, $p = .38$) and diastolic ($F(1,47) = .41$, $p = .53$) readings were not statistically significant between the two groups of women.

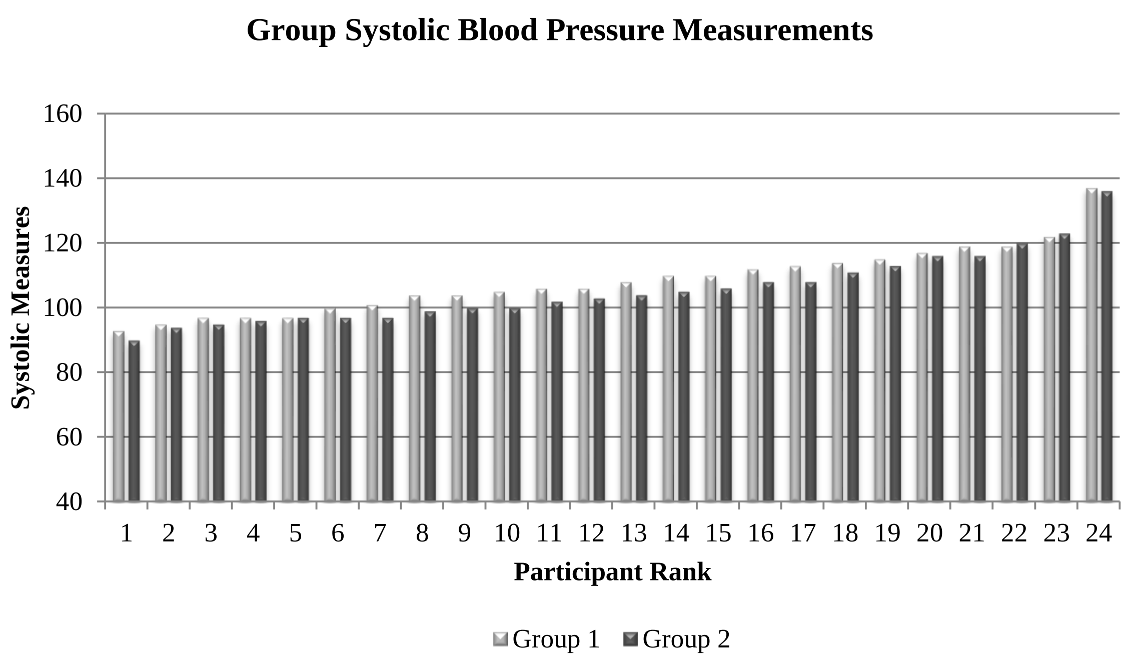

Figure 4.2 Systolic blood pressure measurements for Group 1 (N = 24) and Group 2 (N = 24) taken prior to the stress induction. Systolic measurements were taken three times at 2 minute intervals and a mean measurement was calculated.

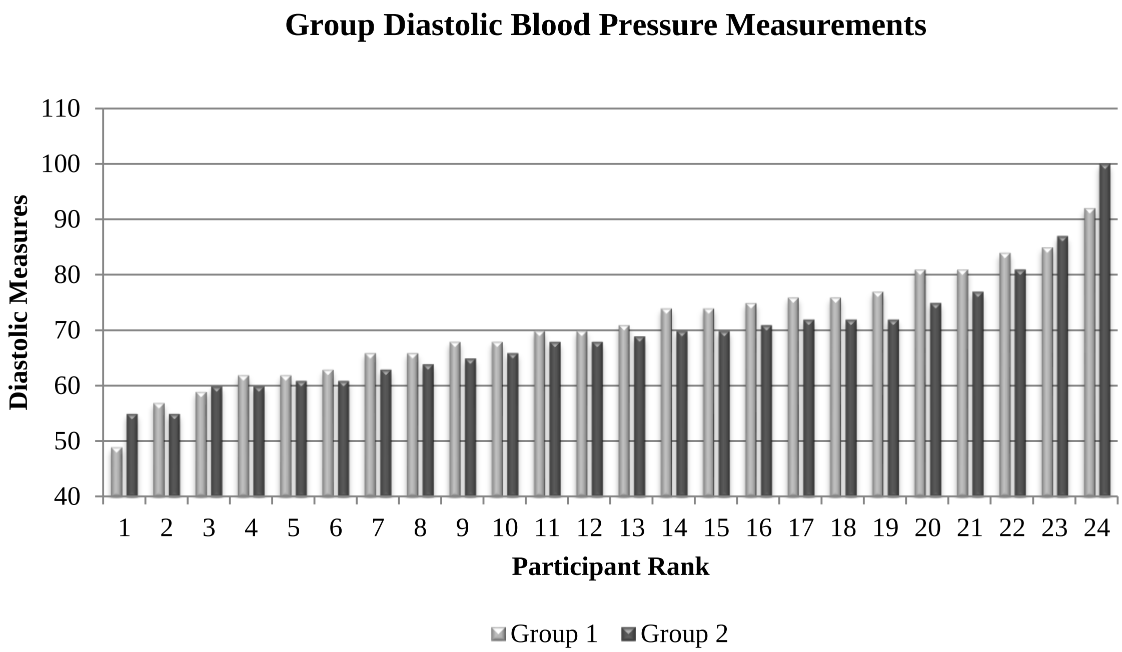

Figure 4.3 Diastolic blood pressure measurements for Group 1 (N = 24) and Group 2 (N = 24) taken prior to the stress induction. Diastolic measurements were taken three times at 2 minute intervals and a mean measurement was calculated.

4.3.2 Appraisal of Challenge or Threat Scale (ACTS)

The 24-item self-report ACTS measured two types of stress appraisal, including primary appraisal (perception of stress) and secondary appraisal (perception of ability to cope with stress). Participants were included if ACTS scores fell in the highest or lowest stress risk groups. High Stress-Low Coping participants (Group 1) had Primary Threat Appraisal scores $\geq 75^{th}$ percentile (Primary Appraisal subscale score ≥ 72) and Secondary Threat Appraisal score $\leq 25^{th}$ percentile of scores possible (Secondary Appraisal subscale score ≤ 24). Low Stress-High Coping participants (Group 2) had Primary Threat Appraisal scores $\leq 25^{th}$ percentile of scores possible (Primary Appraisal subscale score ≤ 24); Secondary Threat Appraisal scores in the $\geq 75^{th}$ percentile of scores possible (Secondary Appraisal subscale score ≥ 72).

Total scores for both the primary and secondary subscales were calculated. Figures 4.5

and 4.6 showed higher target scores for Group 1 in both primary and secondary appraisal subscales as compared to Group 2. A one way analysis of variance showed that the group differences in primary appraisal scores ($F_{(1,47)} = 74.29$, $p = .00$) and secondary appraisal scores ($F_{(1,47)} = 104.28$, $p = .00$) were significantly different substantiating the clinical difference between these groups with regard to stress appraisal (Figure 4.4).

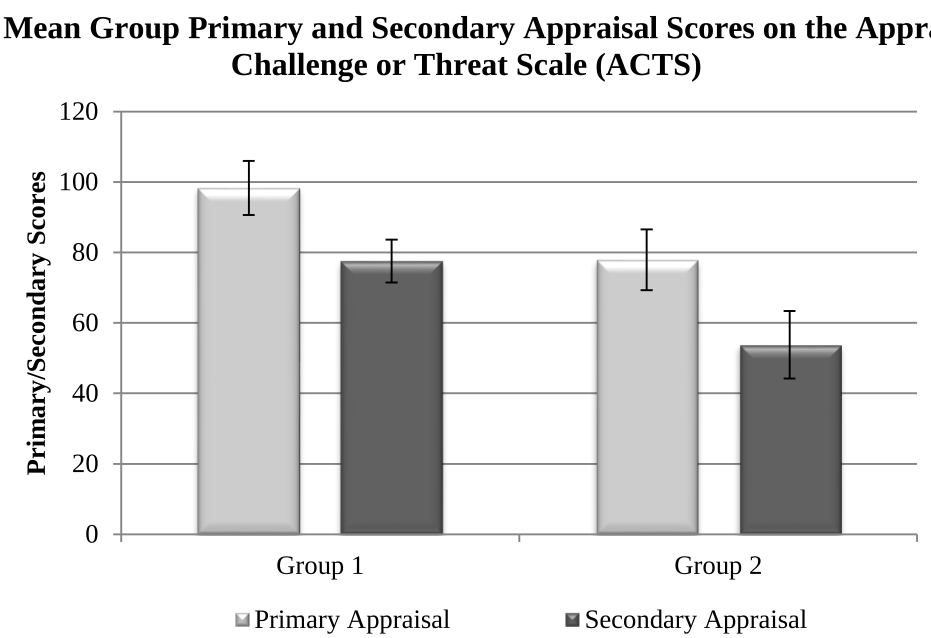

Figure 4.4 Mean primary appraisal scores and secondary appraisal scores were calculated by group (Group 1, N = 24; Group 2, N = 24). An ANOVA showed that the differences in primary appraisal scores (F(1,47) = 74.29, p = .00) and secondary appraisal scores (F(1,47) = 104.28, p = .00) were statistically significant between the two groups of women, substantiating the clinical difference between these groups with regard to stress appraisal.

Group Scores of Primary Appraisal on the Appraisal of Challenge or Threat Scale (ACTS)

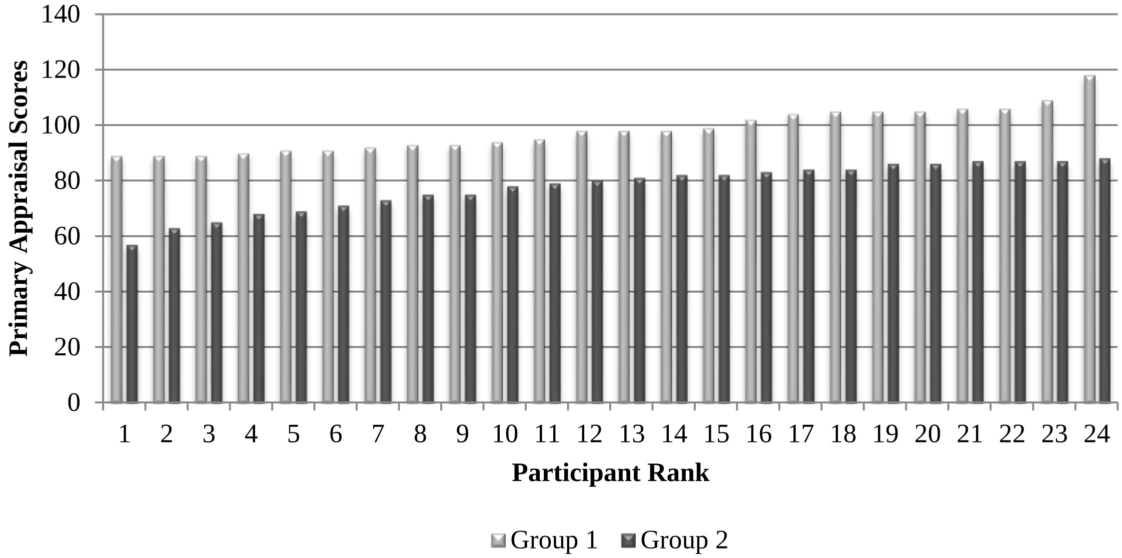

Figure 4.5 Primary appraisal subscale scores on the Appraisal of Challenge or Threat Scale (ACTS) were used for inclusion into one of the two mutually exclusive groups (Group1, High Stress-Low Coping; Group 2, Low Stress-High Coping). Participants with primary appraisal scores above the 75th percentile and secondary appraisal scores below the 25th percentile were selected for inclusion into Group 1. Participants with primary appraisal scores below the 25th percentile and secondary appraisal scores below the 75th percentile were selected for inclusion into Group 2.

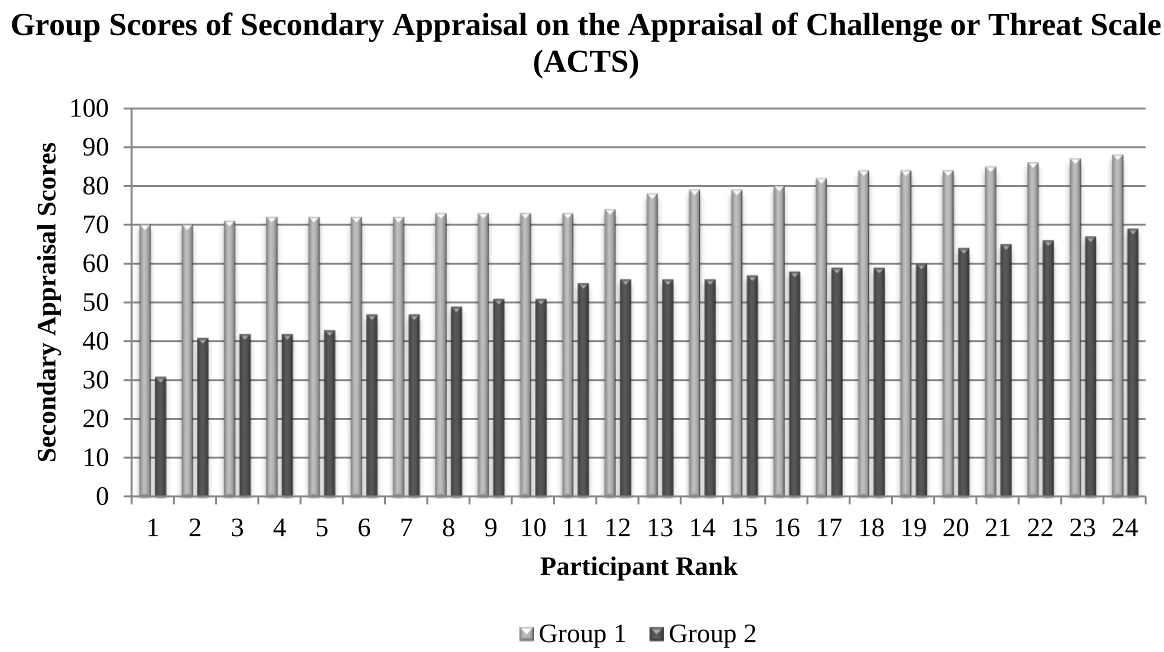

Figure 4.6 Secondary appraisal subscale scores on the Appraisal of Challenge or Threat Scale (ACTS) were used for inclusion into one of the two mutually exclusive groups (Group1, High Stress-Low Coping; Group 2, Low Stress-High Coping). Participants with primary appraisal scores above the 75th percentile and secondary appraisal scores below the 25th percentile were selected for inclusion into Group 1. Participants with primary appraisal scores below the 25th percentile and secondary appraisal scores below the 75th percentile were selected for inclusion into Group 2.

4.3.3 Social Readjustment Rating Scale (SRRS)

The Social Readjustment Rating Scale (SRRS) was administered after BP measurements were recorded. This scale was administered to provide a means of controlling for recent stressful experiences if scores suggested groups differed substantially in this regard. The 42-item SRRS queries common stressors that may have occurred. The scale was modified to query events that may have occurred in the week prior to study participation. In both groups a substantial proportion of subjects reported no stress-inducing events in the week prior to the study. Group 2 appeared to have had more occurrences of stressful events or more severe stressful events (Figure 4.9) however an analysis of variance showed that the groups did not differ significantly with regard to SRRS scores ($F_{(1, 47)} = 1.10$, $p = .30$) (Figure 4.7).

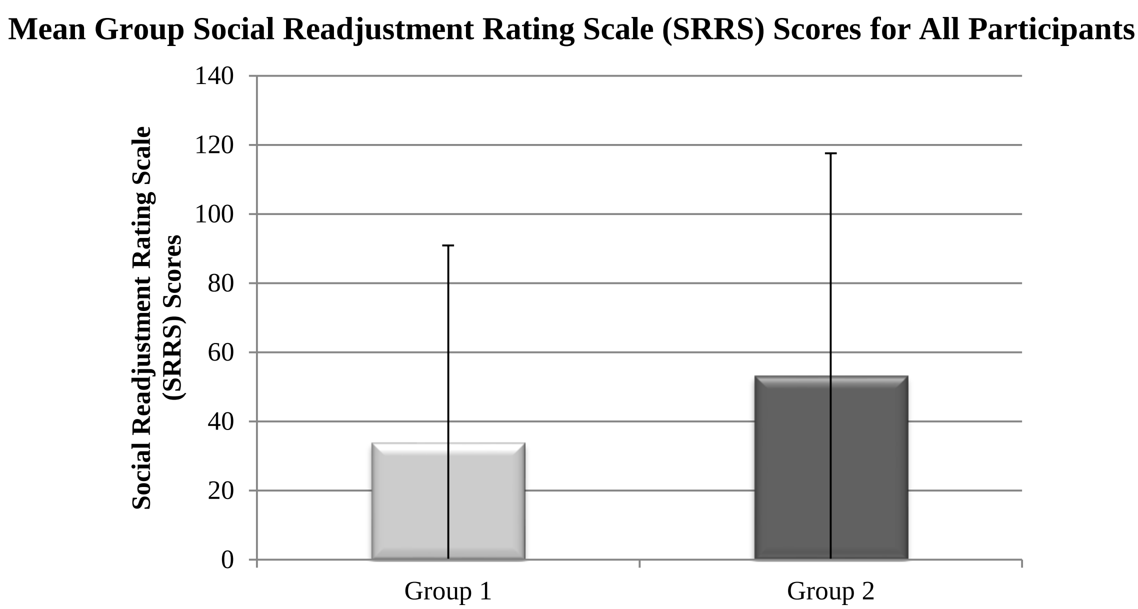

Figure 4.7 Mean Social Readjustment Rating Scale (SRRS) scores were calculated by group (Group 1, N = 24; Group 2, N = 24) for all participants including those that did not experience a stressful event the week prior to the study. An ANOVA showed that the groups did not differ significantly with regard to SRRS scores (F(1, 47) = 1.10, p = .30) likely due to the large standard deviations in both groups.

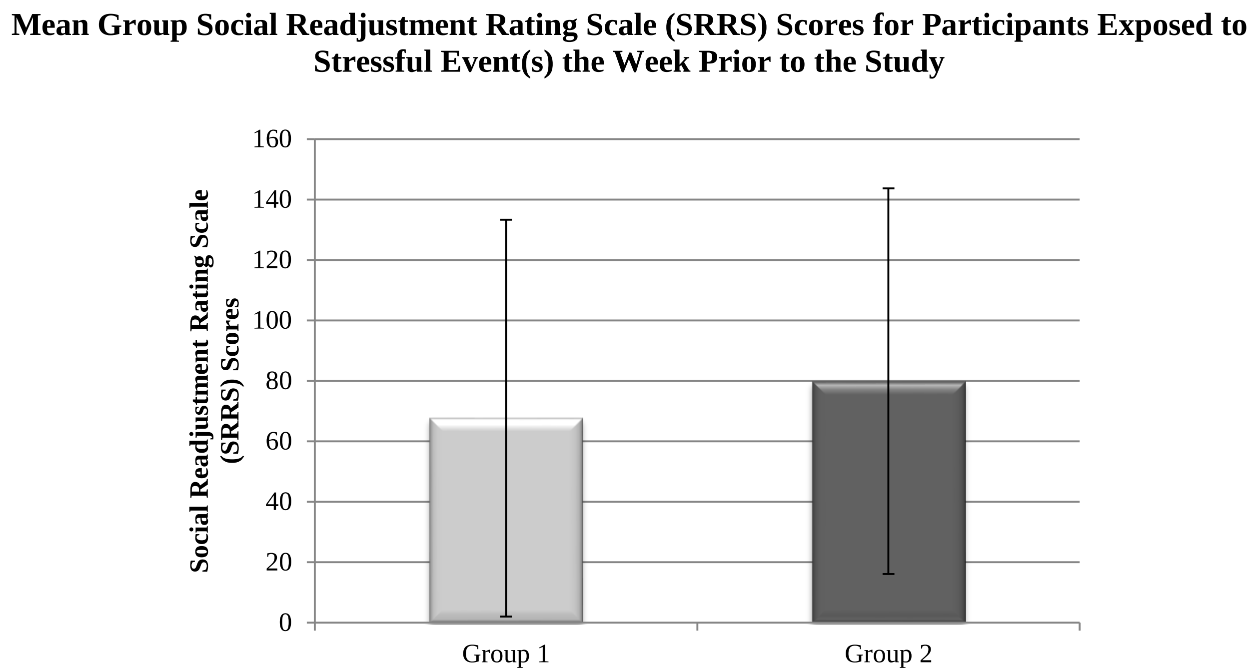

Figure 4.8 Mean Social Readjustment Rating Scale (SRRS) scores were calculated by group for only those participants that indicated that they experienced a stressful event the week prior to the study (Group 1, N = 12; Group 2, N = 16).

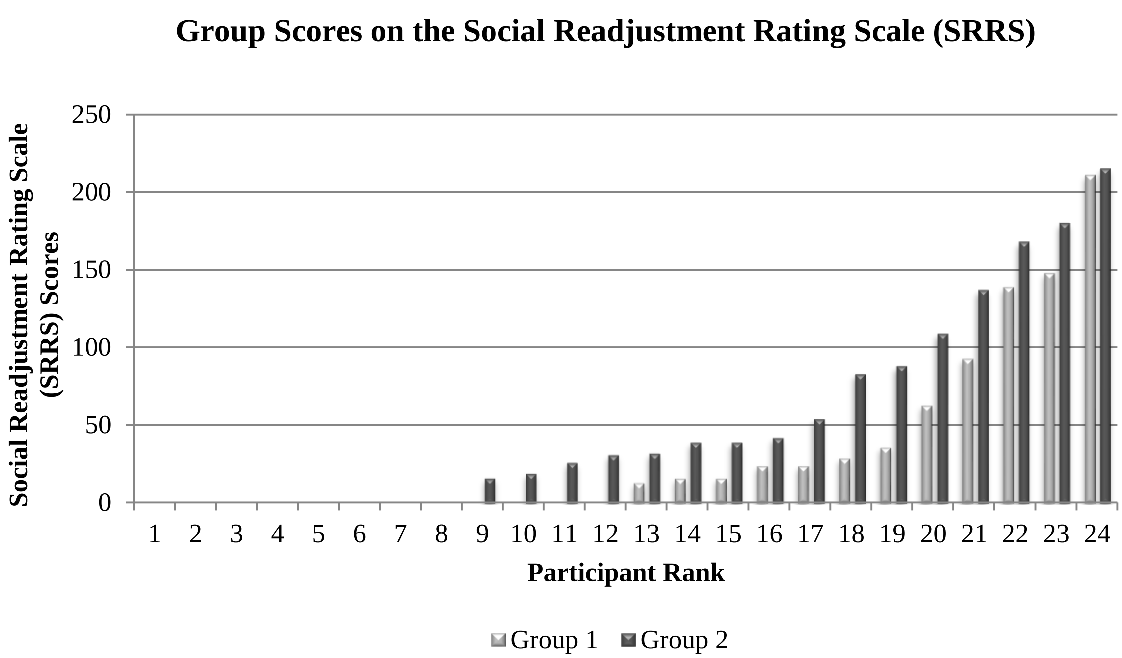

Figure 4.9 The Social Readjustment Rating Scale (SRRS) was administered during the pre-stress period of the TSST procedure. Both groups had participants indicate that they did not experience a stressful events the week prior to the study (Group 1, N = 12; Group 2, N = 8). The scale was administered to provide a means of controlling for recent stressful experiences if scores suggested groups differed substantially in this regard.

4.3.4 Psychological Stress Measures (PSM-9)

The Psychological Stress Measure (PSM-9) was administered to participants at two time points – after administering the SRRS and at completion of the 5 minute recovery phase following the arithmetic task. The goal of this measure was to assess the extent to which the participants' mood/state differed in response to the study. The 9-item survey asked participants to identify their current mood/state prior to beginning the TSST procedure and after completion of both tasks.

Pre- and post-test scores on the PSM-9 scale were summed separately and descriptive analyses showed that pre-test scores were similar to those of the post-test scores across all participants. Furthermore, descriptive analysis showed that pre-test scores were higher for Group 2 when compared to Group 1 (Figure 4.11); post-test scores for Group 2 were also higher when compared to Group 1 (Figure 4.12). A 2 x 2 mixed model ANOVA comparing pre- and post-test mood state (PSM-9) scores by group, revealed that there was no significant interaction ($F_{(1,46)} = 2.48$, $p = .12$), however a significant group effect was shown ($F_{(1,46)} = 3.99$, $p = .05$) (Figure 4.10).

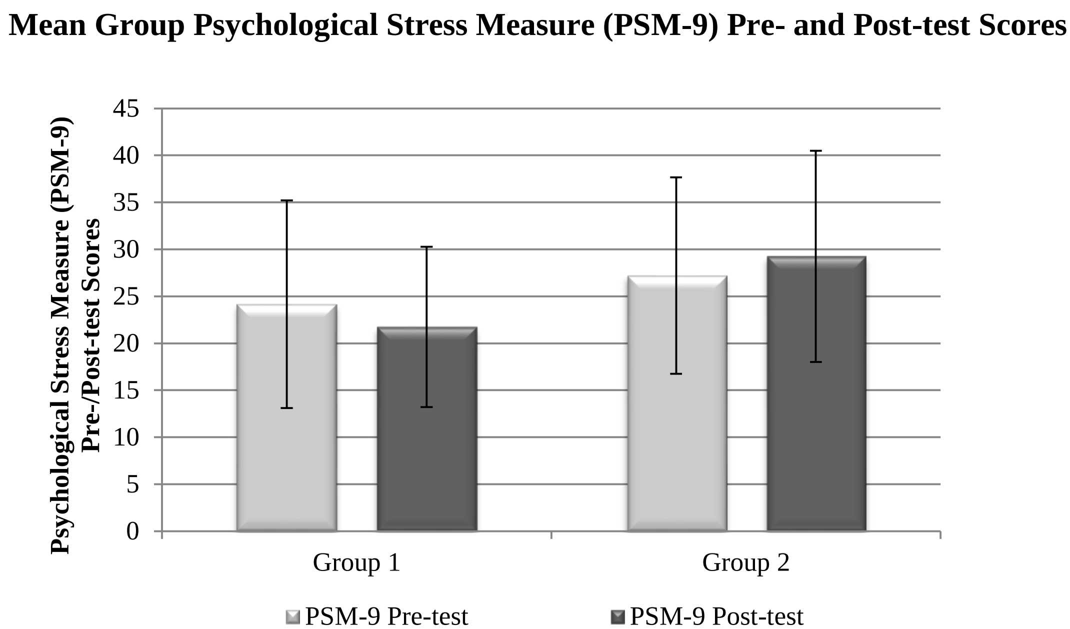

Figure 4.10 Mean Psychological Stress Measure (PSM-9) pre- and post-test scores were calculated by group (Group 1, N = 24; Group 2, N = 24). A 2x2 mixed model ANOVA revealed that there was a significant group effect (F(1,46) = 3.99, p = .05). This finding shows that the groups differed overall for PSM-9 scores (Group 2 had significantly higher pre- and post-test scores as compared to Group 1).

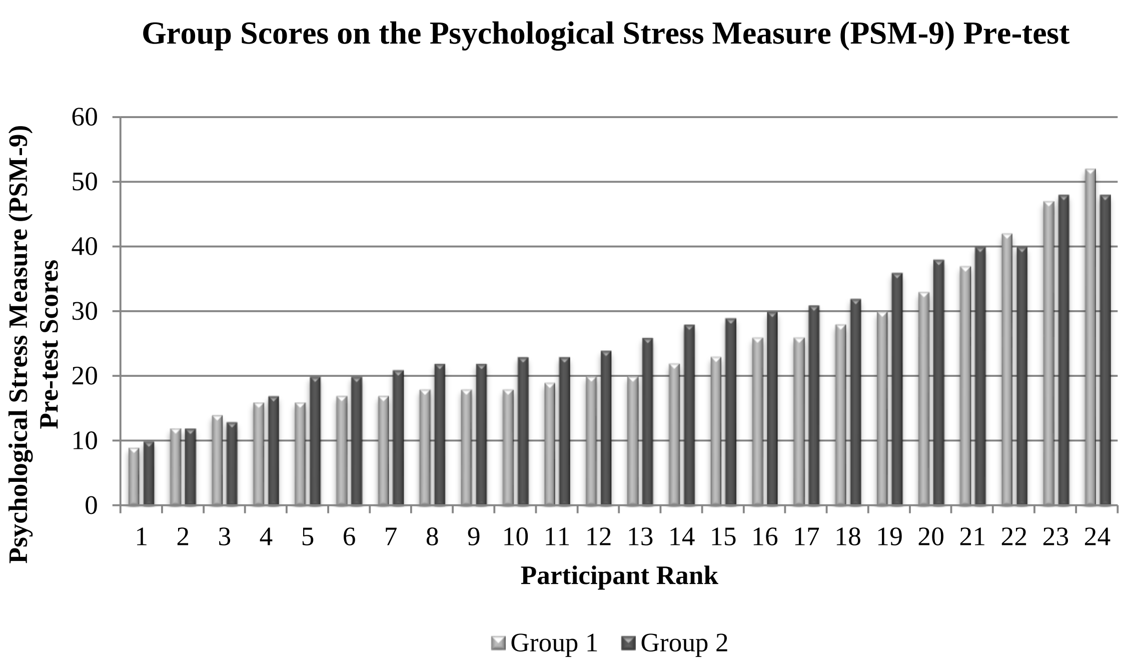

Figure 4.11 The Psychological Stress Measure (PSM-9) pre-test was administered to participants (Group 1, N = 24; Group 2, N = 24) prior to the TSST stress induction procedure. The measure was administered in order to assess the extent to which the participants' mood/state differed in response to the study.

Group Scores on the Psychological Stress Measure (PSM-9) Post-test

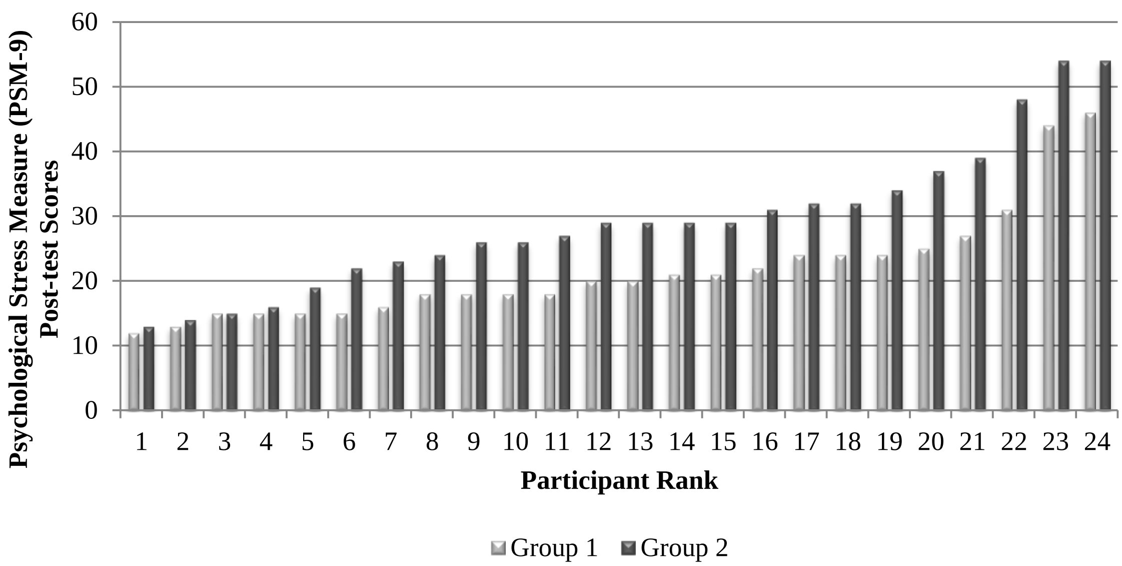

Figure 4.12 The Psychological Stress Measure (PSM-9) post-test was administered to participants (Group 1, N = 24; Group 2, N = 24) after completion of the TSST stress induction procedure.

4.3.5 Heart Rate (HR)

Heart rate (HR) was monitored continuously throughout the TSST procedure (for approximately 30 minutes). Average HR was calculated for three time periods, including during resting baseline, during the speech task, and during the arithmetic task. Heart rate was monitored as a second biomarker for determining whether groups differed with regard to physiological response to stress. Heart rate measures during the speech and arithmetic tasks were further examined for each participant. It was determined that HR levels peaked during the first minute of both the speech and arithmetic tasks, therefore average HR during the first minute was calculated and used in the analysis. The difference between average HR (measured throughout the entire stress induction tasks) and first minute HR during both the speech and arithmetic tasks are shown in Table 4.5.

Descriptive analysis showed that HR during the first minute of the speech task was highest in both groups; HR levels during the first minute of the arithmetic task were similar to those seen during the baseline/resting period in both groups.

Table 4.5 Mean difference in heart rate (HR) between overall heart rate and first minute heart rate during stress induction tasks in Group 1 (N=24) and Group 2 (N=24).

Mean Heart Rate Difference During Speech Task		Mean Heart Rate Difference During Arithmetic Task	
Group 1	Group 2	Group 1	Group 2
6.32 (3.61)	17.72 (14.23)	3.28 (2.38)	2.86 (2.29)

4.3.6 Salivary alpha-amylase (sAA)

Salivary alpha-amylase (sAA) was collected at three stages of the study, including immediately before the TSST procedure ("baseline"); immediately after completion of the speech task; and immediately after the arithmetic task. (The formula for AA enzymatic activity (U/mL) is given in Methods, page 33, paragraph 6.)

Salivary alpha-amylase enzymatic activity was measured at each stage four times, at 0 seconds, 60 seconds, 120 seconds, and 180 seconds. Means and standard deviations for sAA enzymatic activity at these subsequent time points are shown in Table 4.6. sAA activity was estimated by subtracting the level at each subsequent time point from the level obtained at the "zero" time point. For baseline measures, an average of the four samples was used. The time at which sAA level peaked however (at 60, 120 or 180 sec) was expected to differ across subjects. Thus, for the purposes of this study, the largest difference of sAA level, indicating the highest sAA activity, was used to indicate peak sAA for each subject.

Descriptive analysis showed that peak sAA levels were highest following the speech task; sAA levels during the arithmetic task were higher as compared to baseline levels for all participants. Group 1 participants produced peak sAA levels during the speech task, whereas

Group 2 participants produced similar peak alpha-amylase levels during the speech and arithmetic task. Furthermore, examining the peak speech alpha-amylase levels suggested that Group 1 exceeded Group 2, this difference however was not significant ($F_{(1,47)}$ = 1.31, p = .30) because of the large standard deviation in both groups.

Table 4.6 Means and standard deviations of salivary alpha-amylase (sAA) enzymatic activity measured at 0, 60, 120, and 180 seconds in Group 1 (N=24) and Group 2 (N=24).

	Group 1		**Group 2**	
Study Condition	Time Point of Absorbance Measure	Mean (SD)	Time Point of Absorbance Measure	Mean (SD)
Baseline	0	.09 (.02)	0	.09 (.03)
	60	.14 (.05)	60	.13 (.05)
	120	.18 (.08)	120	.17 (.08)
	180	.23 (.12)	180	.20 (.11)
Speech	0	.11 (.05)	0	.10 (.04)
	60	.18 (.10)	60	.15 (.09)
	120	.26 (.16)	120	.21 (.15)
	180	.34 (.23)	180	.27 (.21)
Arithmetic	0	.10 (.03)	0	.11 (.04)
	60	.16 (.08)	60	.16 (.09)
	120	.22 (.12)	120	.22 (.14)
	160	.28 (.17)	160	.28 (.19)

4.4 Primary Analyses

A 3 x 2 mixed model ANOVA with time as the within subject factor and group (high vs. low stress) as the between subject factor was used to compare biomarkers outcomes (HR and alpha-amylase).

4.4.1 Heart Rate (HR)

There was a significant main effect of stress induction ("time") on HR ($F_{(2,45)}$ = 68.99, p = .00) suggesting that HR changed significantly across the three study stages (baseline, speech task, arithmetic task) (Figure 4.14). Examination of post-hoc analyses (Fisher's PLSD) showed

that the significant difference occurred between baseline and the speech task, and between the arithmetic task and the speech task, with no difference between baseline and the arithmetic task (Figure 4.15).

There was no effect of group, suggesting that group membership did not predict overall HR mean levels ($F_{(1,46)}$ = .00, p = .95). Also, there was no significant interaction between group and HR time points ($F_{(2,45)}$ = 0.61, p = .55). Thus, for this biomarker, contrary to our hypothesis, women in the high-risk stress group did not experience different amounts of HR change as compared with the low-risk stress group.

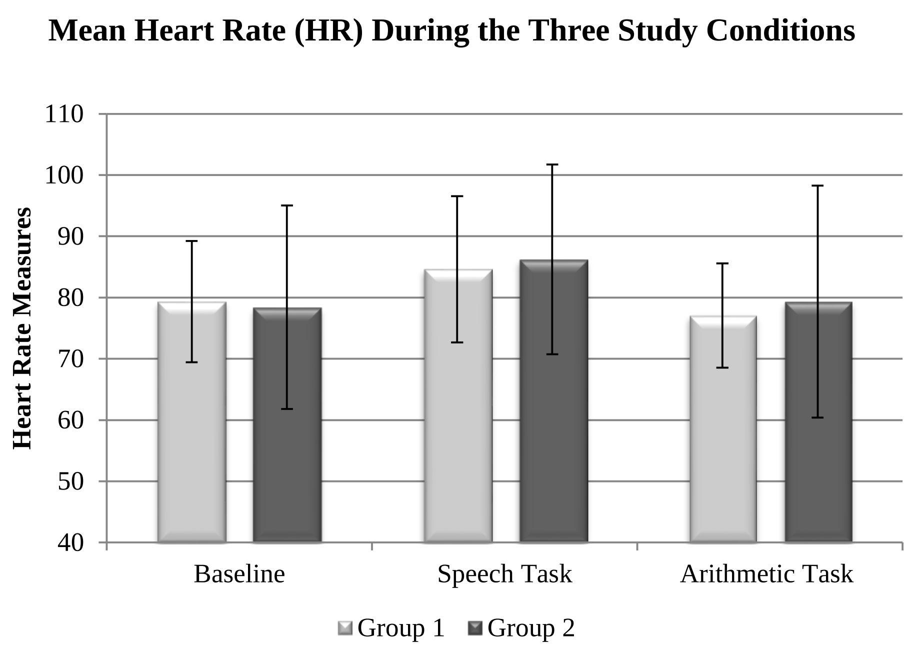

Figure 4.13 Mean heart rates (HR) during the three study conditions (baseline, speech task, arithmetic task) were calculated by group (Group 1, N = 24; Group 2, N = 24).

Mean First Minute Heart Rate (HR) During the Three Study Conditions

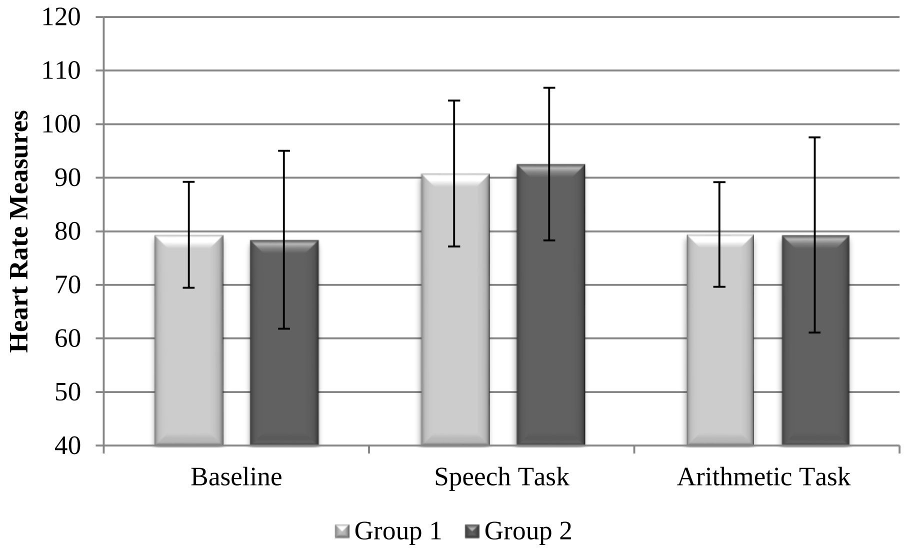

Figure 4.14 Mean first minute heart rates (HR) during the three study conditions (baseline, speech task, arithmetic task) were calculated by group (Group 1, N = 24; Group 2, N = 24). HR measures were examined during the stress induction tasks and it was determined that HR levels peaked during the first minute of both the speech and arithmetic tasks, therefore average HR during the first minute was calculated and used in the analysis. A mixed model ANOVA showed that there was a significant main effect of stress induction ("time") on HR ($F(2,45) = 68.99$, $p = .00$) suggesting that HR changed significantly across the three study stages (baseline, speech task, arithmetic task).

Mean First Minute Heart Rate (HR) Change from Baseline to Speech Task

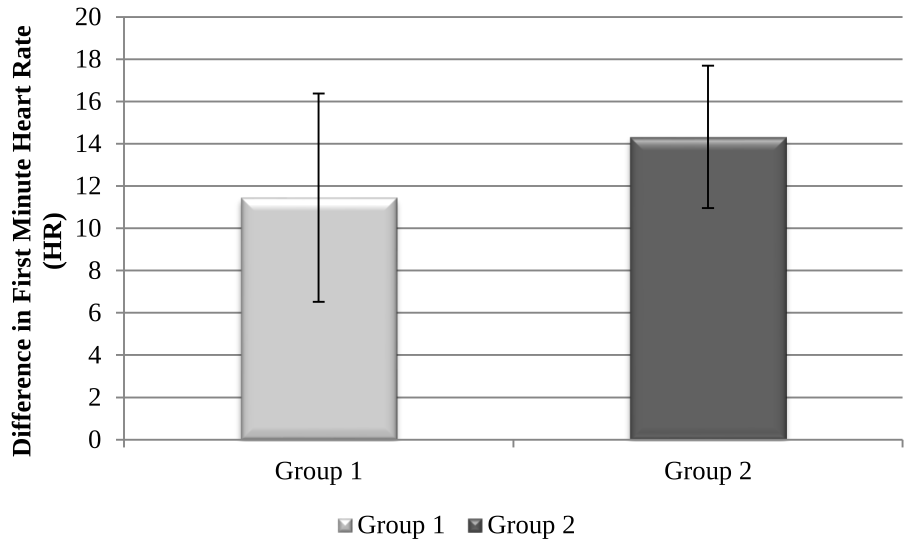

Figure 4.15 A mixed model ANOVA showed that there was a significant main effect of stress induction ("time") on HR suggesting that HR changed significantly across the three study stages (baseline, speech task, arithmetic task). Fisher's PLSD post-hoc analysis showed that the significant difference occurred between baseline and the speech task. Mean peak HR activity from baseline to the speech task was calculated for Group 1 (N=24) and Group 2 (N=24).

4.4.2 Salivary alpha-amylase (sAA)

There was a significant main effect of stress induction (time) on sAA ($F_{(2,45)} = 15.09$, $p = .00$). Overall sAA changed significantly across the three time points (Figure 4.16). Examination of post-hoc analyses (Fisher's PLSD) showed that the significant difference occurred between baseline and the speech task, and between the arithmetic task and the speech task, with no difference between baseline and the arithmetic task (Figure 4.17).

There was no effect of group on sAA ($F_{(1,46)} = 1.42$, $p = .24$) suggesting that high- vs. low-stress women did not differ with regard to sAA activity. Moreover, there was no significant interaction between the groups and sAA activity levels ($F_{(2,45)} = .71$, $p = .49$).

Mean Peak Salivary Alpha-amylase (sAA) Activity at Baseline and After the Stress Inductions Tasks

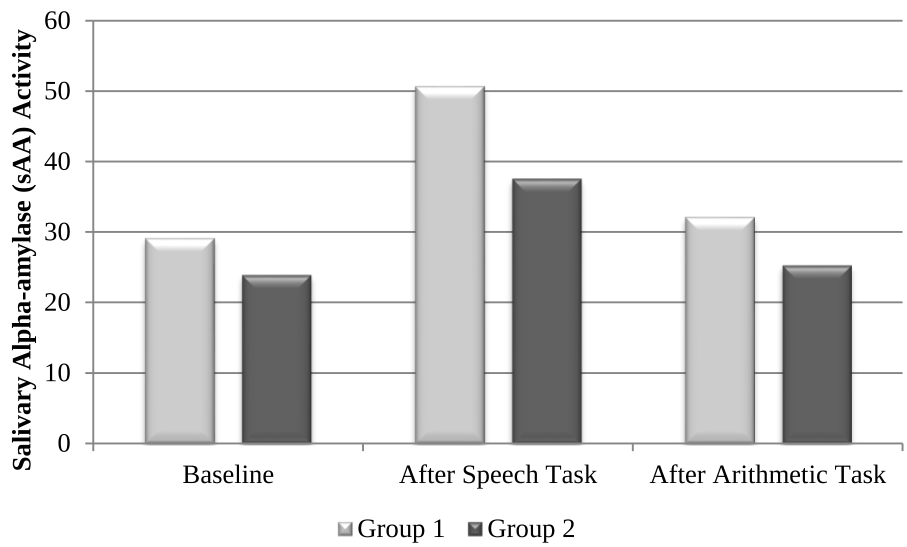

Figure 4.16 Mean peak salivary alpha-amylase (sAA) activity at baseline and after the two stress induction conditions (speech task and arithmetic task) were calculated by group (Group 1, N = 24; Group 2, N = 24). Salivary alpha-amylase enzymatic activity was measured at each stage four times, at 0 seconds, 60 seconds, 120 seconds, and 180 seconds. sAA enzymatic activity (U/mL) was calculated using the following formula: ΔAbs./min x TV x DF)/(MMA x SV x LP. sAA activity was estimated by subtracting the level at each subsequent time point from the level obtained at the "zero" time point. For baseline measures, an average of the four samples was used. The time at which sAA level peaked however (at 60, 120 or 180 sec) was expected to differ across subjects therefore the largest difference of sAA level, indicating the highest sAA activity, was used to indicate peak sAA for each subject. A mixed model ANOVA showed that there was a significant main effect of stress induction ("time") on sAA ($F(2,45) = 15.09$, $p = .00$) suggesting that sAA changed significantly across the three study stages (baseline, speech task, arithmetic task).

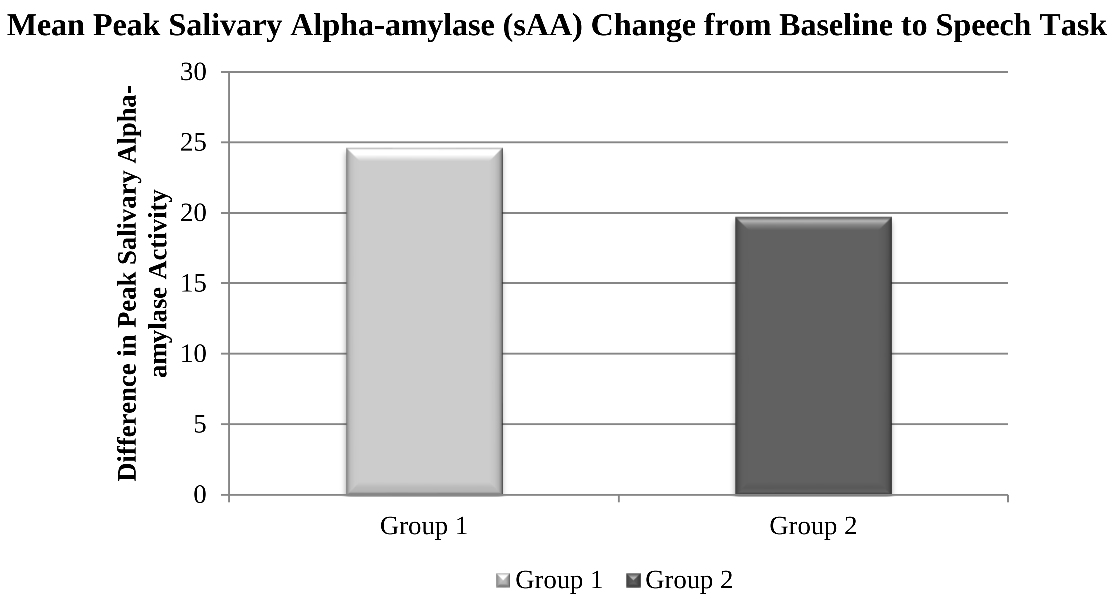

Figure 4.17 A mixed model ANOVA showed that there was a significant main effect of stress induction ("time") on sAA suggesting that sAA changed significantly across the three study stages (baseline, speech task, arithmetic task). Fisher's PLSD post-hoc analysis showed that the significant difference occurred between baseline and the speech task. Mean peak sAA activity from baseline to the speech task was calculated for Group 1 (N=24) and Group 2 (N=24).

4.5 Secondary Analyses

Primary analyses showed that groups did not differ with regard to the biomarker outcome variables HR or sAA. Since the goal of this study was to determine whether and how perception of stress and stress coping was associated with quantifiable physiological biomarkers of stress, secondary analyses were conducted to explore whether components of the ACTS scale might better predict the biomarkers of interest (HR and sAA).

The primary analyses showed that groups did not differ with regard to the outcome measures analyzed and for the secondary analyses all subjects were treated as one group. We considered the possibility that, while seemingly logical, the criteria used for group membership might have combined too many aspects of the stress experience to be uniquely predictive of biological responsivity. Thus for secondary analyses, we conducted multiple regression

analyses, predicting biomarker outcome (HR and sAA) from components of the ACTs scores, including Total ACTS Stress Score and Total ACTS Coping Score, as well as the two ACTS domains for which subjects reported the broadest score range (Unexpected Medical Events and Social Anxiety Events).

Results showed that Total Stress, Total Cope, Unexpected Medical Events Domain, or Social Anxiety Events Domain did not significantly predict peak speech sAA. Table 4.7 summarizes the results from the multiple regressions.

Table 4.7 Summary results from the multiple regression analyses predicting sAA from ACTS scale component scores (N = 48).

Predictor Variable	B	SE B	ß	t	p	R^2
Unexpected Medical Events Domain Score	-4.43	8.33	-.08	-.53	.60	.01
Anxiety Events Domain Score	11.53	9.45	.18	1.22	.23	.03
Total Stress	-.26	.45	-.09	-.58	.56	.01
Total Cope	-.16	.41	-.06	-.40	.69	.00

Similar findings were seen when examining HR. Total Stress, Total Cope, Unexpected Medical Events Domain, or Social Anxiety Events Domain did not significantly predict first minute speech HR. Table 4.8 summarizes the results from the multiple regressions.

Table 4.8 Summary results from the multiple regression analyses predicting heart rate from ACTS scale component scores (N = 48).

Predictor Variable	B	SE B	ß	t	p	R^2
Unexpected Medical Events Domain Score	-2.54	2.87	-.13	-.89	.38	.02
Anxiety Events Domain Score	-.45	3.33	-.02	-.13	.89	.00
Total Stress	.17	.15	.16	1.09	.28	.03
Total Cope	.16	.14	.16	1.11	.27	.03

Chapter 5: Discussion

If a person experiences chronic stress, this has been shown to have detrimental effects on the person's physical and psychological well-being, and it can increase the individual's susceptibility to a number of different chronic diseases. Medical professionals who are aware of the damaging effects of stress might attempt to determine whether their patients are at heightened risk of stress-related illness, and would do so by simply asking their patients to "self-report," that is, they depend on patients' subjective perceptions of experienced stress to determine risk. Few studies however have examined the extent to which patient self-report of stress predicts the physiological changes ("biomarkers") that are the known indicators of physical vulnerability and potential damage leading to chronic disease.

The goal of this study was to determine whether young women's cognitive perceptions of their stress response predicted physiological response to stressful situations. Using the bi-dimensional ACTS scale, two groups of young women were identified, including those who tended to appraise potentially stressful situations as threatening and perceived themselves as having inadequate coping abilities (High Stress-Low Coping); and those who tended to appraise potentially stressful situations as challenging rather than threatening and perceived themselves to have adequate coping abilities (Low Stress-High Coping). Physiological responsivity to stress was quantified by measuring levels of salivary alpha-amylase (sAA) and heart rate (HR) before, during, and after administering the TSST stress induction paradigm. Baseline demographic, health and recent stressful experiences data, and blood pressure readings were also collected. It was hypothesized that as compared to low stress-high coping women, high stress-low coping women have increased levels of sAA and increased HR following the stress induction.

There were four main findings in this study. A 3 x 2 mixed model ANOVA with time as the within subjects factor and group as the between subjects factor was used for the analyses. First, findings showed that the TSST procedure induced significant changes in HR and sAA levels regardless of group association. Second, no significant differences in HR or sAA levels were seen between the two groups of women; that is, self-reported stress appraisal did not predict participants' physiological responses to stress. Third, divergent findings for changes in HR and sAA suggested that these two biomarkers respond differently to stress, or perhaps measure different aspects of physiological stress. Significant changes in sAA levels (within all participants) were seen only between baseline and the speech stress induction task; differences in HR levels were seen between baseline and the speech task, and between the speech task and the arithmetic task, such that during the arithmetic task, HR level returned to the baseline level. Together the results suggested that increased stress reactivity for all participants occurred following the speech task and not following the arithmetic task. Fourth, a comparison of pre- and post-test mood state (PSM-9) scores showed a significant main effect for group. As compared to the high-stress/low-coping individuals, low stress-high coping individuals had significantly higher scores before and after the TSST procedure suggesting that the high-stress/low-coping individuals may be vulnerable to emotional blunting. These findings will be considered in detail below.

5.1 The TSST procedure Induced changes in sAA

5.1.1 History of sAA as a stress measure and its response to ANS activation

Research studies conducted in the 1970s began to identify salivary alpha-amylase (sAA) as a reliable biomarker of psychological stress (Gilman et al., 1979a). One of the first studies

conducted by Gilman et al. showed that sAA concentration levels increased when subjects were exposed to hyperbaric pressure for 8 days. The researchers suggested that increases in sAA concentrations were not only due to the hyperbaric exposure but also due to the psychological strain caused by the procedure. After Gilman's studies other researchers continued to examine the reliability and validity of salivary parameters in response to stress (Morse et al., 1983a, 1983b; Borgeat et al, 1984). At the time, findings from these studies were unclear and inconsistent; initial findings showed that sAA changes were not a result of stress-related conditions as previously suggested. However, as research designs became more sophisticated, more rigorous conditions were employed (e.g., the use of control groups) and findings verified that, as Gilman had stated, sAA levels increased with stress exposure (Nater and Rohleder, 2009).

Prior to any of these studies ever being conducted Speirs et al. (1974) examined whether the autonomic nervous system (ANS) had any effect on increased levels of sAA in response to stress. His findings led him to believe that there was an association between stress-induced levels of sAA and activation of the sympathetic nervous system (SAM). One of his first studies showing evidence of this involved a stress-induction that immersed subjects in waist high cold water. His findings showed that sAA concentrations in the parotid gland increased and this led other researchers to further investigate the effects of sAA in response to stress tests. Speirs's findings are considered to be one of the first to show that the ANS plays a powerful role in the secretion of sAA.

Recent findings continue to show evidence that sAA is not only a highly sensitive stress-induced biomarker but that its elevated levels in response to stress are due to the activation of the HPA axis and the sympathetic system (SAM). Chatterton and colleagues (1996) have shown

that the activation of the SAM by a stressor causes alpha-amylase to be secreted by the salivary glands. This is of great importance because both sympathetic and parasympathetic nerves are branched throughout the salivary glands indicating that sAA is an index of SAM activity (Nater, et al., 2005; Takai et al., 2004). Thus increased levels of sAA can be expected during psychological stress when autonomic levels are activated.

5.1.2 Various stress induction techniques activate the ANS generating increased sAA levels

Researchers have further explored the effects of stress exposure on the ANS and have consistently shown that the sAA measurement is a powerful and highly sensitive tool in stress research (Nater et al., 2005; Gilman et al., 1979a, 1979b; Nexo et al., 1988; Steerenberg et al., 1997; Chatterton et al., 1996; Walsh et al., 1999). The use of psychological stressors such as public speaking, examinations, and violent or suspenseful films have been used to activate the ANS and increase sAA levels (Takai, N., et al., 2004). For example, Chatterton and colleagues (1997) saw an increase in sAA levels when exposing participants to stressful tasks/procedures such as a parachute jump; similar results were seen when using a video game to induce stress (Skosnik et al., 2000). Bosch and colleagues (1996) also showed a two-fold increase in sAA levels when using a written examination as a psychological stressor.

5.1.3 Differences in sAA levels in response to the TSST procedure

As study designs have evolved, researchers have relied on feasible and reliable stress-induction methods that can be used in a variety of settings and with a variety of individuals. One such stress induction procedure is the Trier Social Stress Test (TSST). The TSST is composed of two tasks, a speech task and an arithmetic task, that have been shown to effectively generate

stress responses in individuals. The findings in this study are consistent with past studies using the TSST procedure. For example, a study conducted by Rohleder and colleagues (2004) examined the associations between stress responses and the TSST. Findings from 12 healthy subjects (7 women aged 41.56 ± 2.53 years and 5 men aged 39.25 ± 9.23 years) showed that exposure to the TSST induced significant increases of sAA. A second study of 114 men and 71 women, aged 25.0 ± 3.8 years, examined the stress responsiveness within the sympathetic adrenomedullary (SAM) system following the TSST and found rapid responses in sAA reactivity, and peak levels immediately after the stress induction (Maruyama et al., 2012). Thoma and colleagues (2012) also found significant increases in sAA activity in 40 women and 26 men, aged 24.30 ± 4.24 years, and additionally found an association between stress responses and plasma norepinephrine.

5.1.4 sAA responded very well to the TSST

A general linear mixed model was used to investigate the impact of a stress-induction procedure (TSST) on participants' sAA biomarker levels across three time periods (baseline, speech task, arithmetic task). As with previous findings, the current study showed that the TSST procedure was effective in inducing changes in sAA levels in all participants (regardless of ACTS scores). High stress-low coping (Group 1) and low stress-high coping (Group 2) individuals experienced increased sAA levels in response to the TSST procedure. Thus, the current findings add to the literature on the sensitivity of sAA to psychological stress. The fact that sAA changed in the expected direction in all groups is validation that the TSST induction was effective in increasing stress in research participants.

5.2 The TSST speech task and not the arithmetic task was associated with increased sAA

Whereas the speech task was effective in inducing a stress response, the arithmetic task did not induce a significant increase in sAA levels in either group. Examination of post-hoc analyses showed that that there was no significant difference between the baseline and the arithmetic task or between the speech task and the arithmetic task. Therefore, the speech task was the only task effective in inducing sAA levels that differed from baseline in all participants and suggests that the speech task was experienced as more stressful than the arithmetic task.

As a reminder, the TSST is composed of five phases – resting, anticipatory speech preparation, speech performance, arithmetic performance, and recovery. The TSST begins with a 5-15 minute resting period prior to the stress-induction where participants are left alone and encouraged to relax. Participants are then instructed to prepare a 5-minute public speech and are given 2 minutes to mentally prepare and organize their thoughts. The speech task is followed by a 5-minute verbal arithmetic task where participants are asked to perform basic subtraction. After the 5-minutes elapse the participants are given 5 additional minutes to recover and are debriefed about the goals and objectives of the study.

For the current study the more typically used sampling protocol was changed in order to allow testing of biomarker changes following each distinct phase of the TSST procedure. We did not want to simplify the TSST and consider it as one stress-induction but instead we wanted to assess the effectiveness of the tasks separately in inducing a stress response.

To the best of our knowledge no other study has attempted this and thus no other study has suggested that the arithmetic task could be less effective than the speech task at inducing stress. A re-reading of the literature confirmed that researchers examining stress-induced biomarkers in response to the TSST procedure did not separately measure sAA responsivity after

the individual tasks comprising the TSST, but instead, measured sAA only at baseline and after administration of the second stress induction task. In other words, most previous studies did not attempt to measure and compare sAA levels after each part of the TSST as a biomarker of psychologically induced stress.

For example, a study conducted by Rohleder et al. (2004) collected blood and sAA samples immediately before and after the complete TSST procedure and at 10 and 20 minutes after the arithmetic (last) task, but not between the two stress-inducing tasks. Similarly, in a study conducted by Strahler et al. sAA and cortisol samples were collected immediately before, immediately after, 10 and 20 minutes after completion of both TSST stress inductions (2010). Previous studies have shown that the TSST is an effective tool for increasing sAA levels. The current study is unique in measuring sAA after the speech task and arithmetic task, and suggested that a reduced form of the TSST could be effective in producing stress associated with increased levels of sAA. Collecting saliva samples after each of the two tasks revealed novel findings about the effectiveness of each task in inducing a stress response.

5.2.1 Experiential differences observed in subjects while completing the tasks

Although not quantifiable, very different behavioral reactions were observed during two phases of the TSST procedure. Participants' demeanors and attitudes differed drastically while completing the speech and arithmetic task and these might have led one to conclude that the arithmetic task, but not the speech task, induced biological stress. In the speech task participants appeared to be confident and excited and did not seem to hesitate when beginning the task. Participants seemed comfortable and willing to share their personal experiences and future endeavors when showcasing their skills and knowledge during the mock job interview.

In the arithmetic task however, the participants seemed to immediately react negatively as soon as they were instructed to subtract by increments of 13 beginning with 1,022. Their facial expressions appeared to convey feelings of frustration, confusion and anxiousness. Participants also seemed to experience feelings of embarrassment when failing to subtract correctly and when instructed to start from the top. Many participants at one time or another apologized for being “bad” at math and made excuses for the length of time they required to perform the subtraction. Some repeatedly asked if they could use their phone or a pen and paper to perform the calculations. Based only on the behavioral reaction to the tasks, it appeared that the arithmetic task was more effective in inducing stress responses and feelings of anxiety/panic.

5.2.2 Variations in recovery time between tasks may explain differences in the stress induction effect on sAA

A methodologic detail might be the most obvious explanation for differences in the stress induction effect on sAA. Variations in recovery time differed broadly among participants and may account for the lack of group differences in stress reactivity during the arithmetic task. Whereas participants all began the speech task at minute 7 of the study, start time for the arithmetic task varied by participant. Arithmetic start time was dependent on the amount of time needed by participants to produce a second sAA sample (post-speech task sAA measure). Time allotted to collect the post-speech task sAA sample was considered a recovery phase because it allowed participants to momentarily rest and regain composure before introduction to the arithmetic task. Participants varied in the ability to produce a sample due to dry mouth or cotton mouth; this was brought to the attention of the researcher by several participants after the study. It appeared that because participants were instructed to speak for 5 minutes and unable to pause

for more than 10 seconds at a time they were unable to quickly produce saliva after the speech task. Approximately half of participants required 3-5 minutes to produce the post-speech sAA sample although others required up to 12 minutes. Therefore participants who had difficulty producing the sAA sample received additional resting time between the tasks.

5.3 Group differences in self-perception of stress and coping did not predict changes in sAA or heart rate following the TSST stress induction

With regard to the central question of the study, the findings showed that self-report perception of primary and secondary appraisal did not predict biomarker levels in response to the TSST procedure. General linear mixed models showed that there was no significant interaction between group and HR time points, nor was there a significant interaction between group and sAA activity levels. Thus, although the two groups had extremely different perceptions of stress and coping abilities, their biological stress reactivity did not differ.

Further exploratory regression analyses using the different ACTS domain subscale scores confirmed that no other components of the ACTS predicted HR or sAA activity following the stress-inducing tasks. The findings suggested that subjects' perceptions of stress and perceptions of their ability to cope were unrelated to the physiological aspects of experienced stress.

The lack of difference between groups with regard to stress responsivity during a stress induction was in fact consistent with the work of other stress researchers who examined associations between self-reported stress and biomarker levels. It should be noted that these past studies used stress report instruments that were far simpler than the ACTS.

For example, a study conducted by van Eck and colleagues (1996) examining the effects of perceived stress on salivary cortisol levels showed no significant associations (41 "high stress"

and 46 "low stress" men). In this study, a repeated measures analysis of variance showed no significant effect of the Perceived Stress Scale (PSS) on cortisol after the stress induction, nor was there a statistically significant association with cortisol and a life event measure (LTE-Q).

Other studies found no significant associations between self-reported stress and hormonal biomarkers (cortisol and CHR) in pregnant women. Harville and colleagues (2009) showed that women (N = 1587) with demographic characteristics associated with poor pregnancy outcomes who reported higher levels of stress did not consistently have higher levels of stress hormones.

We attempted again to examine self-reported stress and biomarker levels because we had the availability of the new ACTS scale which provided a relatively sophisticated approach for grouping subjects according to perceived stress and coping. Unlike past self-report scales of stress, the ACTS separately rates perception of threat associated with everyday stressors, and perception of ability to cope with everyday stressors. The ACTS scale has been shown to be a valid and reliable stress appraisal instrument (Tomaka et al., 2012). Although participant responses on the ACTS did not predict levels of stress-induced biomarkers, the findings in this study do not discredit the ACTS as a useful tool for quantifying individuals' perception of stress.

Perceived stress must first be defined before attempting to explain the observed divergence between the perception of stress and biological stress reactivity. Perceived stress is an individual's perception or thoughts about the amount of stress endured at a given time (Phillips, 2013). For the purposes of this study, perceived stress was defined only according to an individual's perceptions about stress and their ability to cope with the stress. Self-reported measures such as the ACTS are subjective and only measure what the individual believes to be experiencing. Thus, the researcher relies on the introspective ability of the participant to quantify their level of stress and coping appraisal.

Contrary to what was hypothesized, the findings suggested that participants' perception of threat and ability to cope was removed from participants' biological reactivity to induced stress. For future studies, it will be important to consider what variables might moderate the relationship between biological reactivity and a person's perception of threat and ability to cope. In other words, the findings in this study suggested that there was a lack of connection between women's perceptions of stress threats and perceptions of ability to cope, and their biological reactivity to stress. One possibility for this disconnection is that the two systems (cognitive perception and biological reactivity to stress) are completely distinct systems and should not be expected to relate. To some extent, we know this is not true however. Psychological stress, as shown in the stress induction, induces the physiological reaction. Understanding other factors that may over-ride perceptions of their biological reactivity could be important for identifying subgroups of women whose self-perceptions are associated with biological reactivity, or conversely, for determining how women might be taught to over-ride influences from these other factors that result in women's cognitive appraisals becoming disconnected from their biological reactivity.

Two logical categories of variables that might be examined in future studies could be personality characteristics and societal gender expectations. In fact, previous studies have suggested that personality characteristics predict one's perception of coping ability, while societal gender expectations have been shown to predict women's perceptions of threat.

5.3.1 Personality characteristics and one's perception of ability to cope

Several studies using the five-factor model have been conducted and associations between the five determinants of behavior and stress and coping have been reported (Digman, 1990; Goldberg, 1981; McCrae and Costa, 2003). These findings suggested that personality

characteristics/factors influence an individual's perception of stress and coping. For example, neuroticism has been shown to predict the tendency to appraise stressful events as threatening and possessing low coping abilities (Bolger and Zuckerman, 1995; Grant and Langan-Fox, 2007; Gunthert et al., 1999; Penley and Tomaka, 2002; Suls and Martin, 2005). Conscientiousness has been shown to be associated with perceptions of low stress (Lee-Baggley et al., 2005; Vollrath, 2001). Agreeableness has been associated with low interpersonal conflict (conflict between two individuals) and therefore low social stress (Asendorpf, 1998). Lastly, extraversion, conscientiousness, and openness have all been associated with perceiving stress situations as challenging and possessing high coping ability (Penley and Tomaka, 2002; Vollrath, 2001). When combining dimensions of personalities, high neuroticism and low conscientiousness has been shown to predict heightened levels of stress perception and perceptions of threat, whereas low neuroticism and high extraversion or high conscientiousness predicts both low stress and low perceptions of threat (Grant and Langan-Fox, 2006; Vollrath and Torgersen, 2000).

Other studies, not using the five-factor model, have also reported interesting findings when examining associations between personality factors and stress/coping appraisals. For example, researchers have found an association between Type A behaviors and patterns of coping (Pittner and Houston, 1980; Vickers et al., 1981; Vingerhoets and Flohr, 1984), between internal control and problem-oriented coping (Anderson, 1977; Parkes, 1984) and between trait anxiety and maladaptive coping (Parasuraman and Cleek, 1984). Other more extensive studies on personality characteristics and coping showed that self-esteem was weakly related to coping whereas self-denial (tendency to avoid thinking of one's own negative aspects) and nondisclosure (tendency to avoid sharing one's own problems with others) were associated with coping. Furthermore, findings have shown that individuals scoring high on extraversion and

neuroticism have poor adaptability in response to stress and therefore have inadequate coping abilities (Denney and Frisch, 1981; Duckitt and Broll, 1982).

In the current study, the lack of group differences in the physiological reactivity to stress might have been attributable to broad individual differences in both groups with regard to some or all of the above considered personality characteristics. Moreover, these studies suggest many possibilities for investigating which personality characteristics might create individual differences in the extent to which stress perception predicts biological reactivity to stress.

5.3.2 Societal gender expectations predict women's perception of threat

Findings from previous studies have also suggested that societal gender expectations may predict women's perception of threat. For example, researchers have shown that compared to men, women appear to be more likely to overestimate the probability of threat and anticipate poor coping ability due to encouraged gender conforming behaviors (Thorpe and Salkovskis, 1995). From an evolutionary perspective it has been hypothesized that women tend to perceive situations as more threatening than men because they have been encouraged and taught to preserve their safety and the safety of their children (Wood and Eagly, 2002). It has also been suggested that because women have been taught to be the caretakers of the family, their attention to threat is heightened and ambiguous situations are more frequently judged as threatening.

Furthermore, it is hypothesized that social patterns of reinforcement for girls and boys may play a critical role in understanding gender differences in response to anxiety. For example, Bem (1981) theorized that because boys and girls are taught to socialize and respond to anxiety/stress differently, their coping ability and responsiveness to threat differs as well. He suggested that fearful behavior in boys is less tolerated because the expression of anxiety is not

accepted in the male gender role whereas girls are encouraged by caregivers (e.g., teacher, peers) to conform to behaviors that are contrary to those of boys. Moreover, research has shown that parents consider behaviors of withdrawal and inhibition to be more acceptable in girls as they get older, but not for boys (Stevenson-Hinde and Shouldice, 1993).

Additionally, studies have provided evidence showing that gender role is significantly associated with symptoms of fear and anxiety (Chambless and Mason, 1986; Ollendick et al., 2002). These studies have shown a significant association between scores on the Fear Survey Schedule (Wolpe and Lang, 1977) and the Fear Survey Schedule for Children-II (Gullone and King, 1993), and the Bem Sex Role Inventory, which measures the degree to which individuals possess personality characteristics consistent with social conceptions of masculine and feminine gender roles. It was found that among children, greater fear reporting was associated with higher levels of femininity (Muris et al., 2005) and lower levels of masculinity (Ginsburg and Silverman, 2000). Studies examining the relationship between fear and femininity saw similar findings among adults as well (Dillon et al., 1985; Tucker and Bond, 1997); others found that both high femininity and low masculinity were related to elevated fear (Carey et al., 1988).

Bem (1981) has suggested that boys may learn purposeful coping behavior if the masculine role was less accepting of the expression of fear and avoidance. Young girls on the other hand may have more opportunity to exert control and feel more capable to cope with potentially threatening situations if they were not encouraged by caregivers to be timid and fearful of stressful events. Thus, women may learn to respond to threat differently rather than default to the reinforced traditional gender behavior. As findings have shown, not only do women tend to perceive more situations as threatening due to the evolutionary perspective that has emphasized the importance to protect and nurture offspring but also because of the gender

socialization processes that have encouraged women to express feelings of worry, sensitivity and avoidance in the face of stressful situations (McLean and Anderson, 2009).

Similar to the studies on personality characteristics, these studies suggest additional factors that might be explored in planned studies that examine which gender-related variables might predict individual differences in women's perceptions of stress and how these individual differences perhaps diminish the association between stress perception and biological reactivity to stress.

5.4 Mood/emotional state worsened in low stress-high coping participants

Comparing PSM-9 mood scores before and after the TSST stress induction also revealed very interesting findings. A 2 x 2 mixed model analysis of variance using pre- and post-test PSM-9 scores (within subject) and the two groups of women (between subjects) showed a significant group effect indicating that groups differed in PSM-9 pre- and post-test scores. Interestingly, low stress-high coping participants (Group 2) had significantly higher pre- and post-test scores as compared to high stress-low coping participants (Group 1). This finding may suggest that Group 1 participants may be vulnerable to emotional blunting as demonstrated by the lower PSM scores.

5.4.1 Dissociation and stress responses

Lower emotion scores in the high stress-low coping participants may have indicated that these individuals experienced emotional blunting. Dissociation from stress has been found in individuals who have had repeated or severe forms of trauma that have caused to some degree a division of personality; in this case, personality is defined as a system that determines an

individual's characteristic behavior and thought (van der Hart et al., 2005). Dissociation therefore occurs when sensory perceptions or affective states that were generated from fragments or portions of a stressful experience influence an individual's personality and system of ideas. The ability to dissociate from stressful events is associated with a pattern of posttraumatic stress response, which can be described as a dissociative part of the personality mediated by action systems of daily life and defense. In other words, when a traumatized individual is detached from a stressful event and subjected to systems of daily life, they can appear to be unphased and continue with their routine (van der Hart et al. 2005). However this is accomplished because a part of their personality has allowed them to remove themselves mentally from any trauma-related cue.

It is possible that high stress-low coping individuals (Group 1) had lower scores on the PSM-9 because their perceptions of stress have led to psychological dissociation from their emotional states. It may be that those individuals mentally disengaged themselves during the tasks as a form of defense, therefore inhibiting their ability to emotionally react and respond. Whether this emotional blunting is actually part of a psychological mechanism that modifies a person's perception of biological stress reactivity could be examined in future studies.

Despite these individuals' status as psychologically typical (individuals were screened for current psychological disorders and did not report current diagnoses), the finding suggested that Group 1 differed clinically from Group 2 and responded in a way that has not been seen in other studies of typical individuals. As stated above, this finding suggested that high stress-low coping individuals may be more vulnerable to emotional blunting.

5.5 Limitations

There were several limitations in this study. No methods were used to independently verify self-reported health questionnaire data. The researcher relied on participants to self-report existing health conditions and indicate the use of medications that may obstruct the validity of the findings. Although the health assessment questionnaire was one measure used to determine exclusion in the study, the researcher did not verify that the information provided by participants was accurate. Similarly, there was no method in place to verify that participants were not diagnosed with health conditions or to indicate that medications were not used 48 hours prior to the study. Additionally, the researcher could not verify for certain that participants were fasting prior to the study. Diagnoses of health conditions, medications, and the consumption of food and beverages (other than water) could have potentially altered biomarker readings of sAA and HR. Also, in the current study participants may have divulged details of the procedures to potential qualifying participants creating bias or expectations that altered responses. Because the women were recruited mostly from the same courses, it is possible that participants who completed the study shared information with others that were also scheduled to participate. This could have allowed scheduled participants to anticipate the protocol involved, therefore potentially reducing their stress responses or reactivity when subjected to the TSST procedure.

Conclusions

The TSST procedure was effective in inducing a stress response regardless of group membership, thus adding to the literature on the sensitivity of sAA to psychological stress. By using a different sampling protocol from those of previous studies, the current study also showed that the only the speech task and not the arithmetic task was effective in inducing significant changes in sAA and HR levels. To the best of our knowledge, this study is the first study to report a difference in effectiveness of TSST tasks in inducing stress reactivity. Contrary to our hypothesis that as compared to low-stress/high-coping women, high stress-low coping women have increased levels of sAA and HR following the stress induction, no significant differences in change from baseline to stress induction were seen between the two groups of women. The findings suggested that other variables may moderate the relationship between biological reactivity and a person's perception of threat and ability to cope. In secondary analyses, comparisons of mood state (PSM-9) scores showed a significant group effect, in which, paradoxically, high-stress/low-coping women reported less emotional response than low-stress/high-coping women. Replication of these results are necessary, nonetheless, this finding may suggest that high stress-low coping individuals may be vulnerable to emotional blunting.

This study was undertaken because the ACTS provided a unique quantification of two dimensions of stress, perceived threat and perceived ability to cope. Although the newly developed ACTS was useful in separately assessing threat and challenge aspects of stressful life events, it did not predict levels of stress-induced biomarkers. This finding indicates that health care workers should not attempt to use the ACTS when attempting to determine whether individuals are at risk of stress-related disease.

References

Almeida, D., Kessler, R.C. (1998). Everyday stressors and gender differences in daily distress. *Journal of Personality and Social Psychology*, 75, 670-680.

Anderson, C.R. (1977). Locus of control, coping behaviors, and performance in a stress setting: A longitudinal study. *Journal of Applied Psychology*, 62, 446-451.

Arnold, M. B. (1960). *Emotion and Personality* (2 vols.). New York: Colombia University Press.

Asendorpf, J.B. (1998). Personality effects on social relationships. *J. Personal. Soc. Psychol*., 74, 1531-44.

Bard, P. (1934). On emotional expression after decortication with some remarks on certain theoretical views: Part I. *Psychological Review*, 41(4), 309-329.

Barnett, R.C., Biener, L., Baruch, G.K. (1987). *Gender & stress*. New York: The Free Press.

Bassett, J. R., Marshall, P. M., Spilane, R. (1987). The physiological measurement of acute stress (public speaking) in bank employees. *Psychophysiology*, 5, 265-273.

Bem, S.L. (1981). Gender schema theory: A cognitive account of sex typing. *Psychological Review*, 88(4), 354-364.

Birkett, M.A. (2011). The Trier Social Stress Test protocol for inducing psychological stress. *J Vis Exp*., 19(56), 3238.

Blascovich J., Katkin ES, editors. (1993) Cardiovascular reactivity to psychological stress and disease. Washington, DC: *American Psychological Association*.

Blascovich, J., Mendes, W.B. (2000). Challenge and threat appraisals: The role of affective cues. In J. Forgas (Ed.), *Feeling and thinking: The role of affect in social cognition* (pp. 59-

82). Paris: Cambridge University Press.

Bolger, N., Zuckerman, A. (1995). A framework for studying personality in the stress process. *J. Personal. Soc. Psychol*., 69, 890-902.

Borgeat, F., Chagon, G., Legault, Y. (1984). Comparison of the salivary changes associated with a relaxing and with a stressful procedure. *Psychophysiology*, 21, 690-698. Bortner, R.W., Rosenman, R.H. (1967). The measurement of pattern A behavior. *J. Chronic Dis.*, 20(7): 525-33.

Bosch, J.A., Brand, H.S., Ligtenberg, T.J. Bermond, B., Hoogstraten, J., Nieuw Amerongen, A.V. (1996). Psychological stress as a determinant of protein levels and salivary-induced aggregation of *Streptococcus gordonii* in human whole saliva. *Psychosom. Med*., 58, 374-82.

Cannon, W. B. (1914). The emergent function of the adrenal medulla in pain and the major emotions. *American Journal of Physiology*, 33, 356-372.

Cannon, W. B. (1932). *The wisdom of the body*. New York: W. W. Norton.

Carey, M.P., Dusek, J.B., Spector, I.P. (1988). Sex roles, gender and fears: A brief report. *Phobia Practice and Research Journal*, 1(1), 114-120.

Chambless, D.L., Mason, J. (1986). Sex, sex-role stereotyping and agoraphobia. *Behaviour Research and Therapy*, 24(2), 231-235.

Chatterton Jr. R.T., Vogelson, K.M., Lu, Y.C., Ellman, A.B., Hudgens, G.A. (1996). Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin. Physiol*., 16, 433-448.

Chatterton, Jr. R.T., Vogelson, K.M., Lu, Y-C. et al. (1997). Hormonal responses to psychological stress in men preparing for skydiving. *J. Clin. Endocrino. Metab*., 82, 2503-09.

Cohen, S., Kessler, R. C., Gordon, L. U. (1995). *Measuring Stress: A Guide for Health and Social Scientists*. New York: Oxford University Press.

Denney, D.R., Frisch, M.B. (1981). Depression and coping in stressful episodes. *Journal of Abnormal Psychology*, 90, 439-447.

Dickerson, S.S., Kemeny, M.F. (2004). Acute stressors and cortisol response: a theoretical integration and synthesis of laboratory research. *Psychol. Bull.*, 130(3):355-391.

Digman, J.M. (1990). Personality structure: emergence of the five-factor model. *Annu. Rev. Psychol.*, 41, 417-40.

Dillon, K.M., Wolf, E., Katz, H. (1985). Sex roles, gender, and fear. *Journal of Psychology*, 119, 355-359.

Duckitt, J., Broll, T. (1982). Personality factors as moderators of the psychological impact of life stress. *South African Journal of Psychology*, 12, 76-80.

Duhachek, A., Kelting K. (2009). Coping repertoire: Integrating a new conceptualization of coping with the transactional theory. *Journal of Consumer Psychology*, 19, 473-485.

Ferguson, E., Matthews, G., Cox, T. (1999). The Appraisal of Life Events (ALE) scale: Reliability and validity. *British Journal of Health Psychology*, 4(2), 97-116.

Gilman, S., Thorton, R., Miller, D., Biersner, R. (1979a). Effects of exercise stress on paratid gland secretion. *Horm. Metab. Res.*, 11(7), 454.

Gilman, S.C., Fischer, G.J., Biersner, R.J., Thorton, R.D., Miller, D.A. (1979b). Human paratid alpha-amylase secretion as a function of chronic hyperbaric exposure. *Undersea Biomed. Res.*, 6(3), 3030-307.

Ginsbur, G.S., Silverman, W.K. (2000). Gender role orientation and fearfulness in children with anxiety disorders. *Journal of Anxiety Disorders*, 14(1), 57-67.

Goldberg, L.R. (1981). Language and individual differences: the search for universals in personality lexicons. *In Review of Personality and Social Psychology*, ed. L Wheeler. 2, 141-65. Beverly Hills, CA: Sage.

Goodyer, I.M., Herbert, J., Tamplin, A., Altham, P.M.E. (2000). Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high risk adolescents. *British Journal of Psychiatry*, 177, 499-504.

Goodyer, I.M., Park, R.J., Herbert, J. (2001). Psychosocial and endocrine features of chronic first-episode major depression in 8-16 year olds. *Biological psychiatry*, 50, 351-357.

Gordis, E.B., Granger, D*.A.*, Susman, *E*.J., Tickett, P.K. (2008). Salivary alpha amylase-cortisol asymmetry in maltreated youth. *Horm. Behav.*, 53(1), 96-103.

Gordis, E.B., Margolin, G., Spies, L., et al. (2010). Interparental aggression and parent-adolescent salivary alpha amylase symmetry. *Physiol. Behav.*, 100(3), 225-33.

Granger, D.A., Kivlighan, K.T., El-Sheikh, M., et al. (2007). Salivary α-amylase in biobehavioral research: Recent developments and applications. *Ann N Y Acad Sci*, 1098, 122-44.

Grant, S., Langan-Fox, J. (2006). Occupational stress, coping and strain: the combined/interactive effect of the Big Five traits. *Personal. Individ. Differ.*, 41, 719-32.

Grant, S., Langan-Fox, J. (2007). Personality and the occupational stressor-strain relationship: the role of the Big Five. *J. Occup. Health Psychol*., 12, 20-33.

Gullone, E., King, N.J. (1993). The fears of youth in the 1990s: Contemporary normative data. *Journal of Genetic Psychology*, 154, 137-153.

Gunthert, K.C., Cohen, L.H., Armeli, S. (1999). Role of neuroticism in daily stress and coping. *J. Personal. Soc. Psychol*., 77, 1087-100.

Harris, T.O., Borsanyi, S., Messari, S., Stanford, K., Cleary, S., Shiers, H.H., et al. (2000). Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. *British Journal of Psychiatry*, 177, 505-510.

Harville, E. W., Savitz, D.A., Dole, N., Herring, A.H., Thorp, J.M. (2009). Stress Questionnaires and Stress Biomarkers during Pregnancy. *J Womens Health (Larchmt)*, 18(9): 1425-1433.

Hellhammer, D. H., Kirschbaum, C., Y Belken, L. (1987). Measurement of salivary cortisol under psychological sitmulation. In J. N. Hingtgen, D. H. Hellhammer, D. Huppmann (Eds.), *Advanced methods in psychobiology*. (pp. 281-289). Toronto: Hogrege.

Hellhammer, J. (2011). The Trier Social Stress Test (TSST) - A valid tool for clinical studies. *European Journal of Pharmacology*, 668(1), e45-e46.

Herrero, S.G., Saldana, M.A.M., Rodriguez, J.G., Ritzel, D.O. (2012). Influence of task demands on occupational stress: Gender differences. *Journal of Safety Research*, 43, 365-374.

Hinkle, L. E., Jr. (1977). The concept of "stress" in the biological and social sciences. In Z. J. Lipowski, D. R. Lipsitt, P. C. Whybrow (Eds.) *Psychosomatic medicine: Current trends and clinical implications*. New York: Oxford University Press.

Jacobs, R., Pawlak, C.R., Mikeska, E., Meyer-Olson, D., Martin, M., Hijnen, C.J., Schedlowski, M., Schmidt, R.E. (2001). Systemic lupus erythematosus and rheumatoid arthritis patients differ from healthy controls in their cytokine pattern after stress exposure. *Rheumatology (Oxford),* 40, 868-875.

Jenkins, C.D., Zyzanski, S.J., Rosenman, R.H. (1971). Progress toward validation of computer-scored test for the type A coronary-prone behavior pattern. *Psychosomatic Medicine*, 33,

193-202.

John, O. P., Srivastava, S. (1999). The Big-Five trait taxonomy: History, measurement, and theoretical perspectives. In L. A. Pervin and O. P. John (Eds.), *Handbook of personality: Theory and research* (Vol. 2, pp. 102–138). New York: Guilford Press.

Kajantie E., Phillips, DI. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology*, 2006;31:151–78.

Keller, P.S., El-Sheikh, M., Vaughn, B., Granger, D.A. (2009). Salivary Alpha-amylase as a longitudinal predictor of children's externalizing symptoms: Respiratory sinus arrhythmia as a moderator of effects. *Psychoneuroendocrinology*, 34(5), 633-43.

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

Kirschbaum, C., Wolf, O.T., May, M., Wippich, W., Hellhammer, D.H. (1996). Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sciences*, 58, 1475-1483.

Kirschbaum, C., Wust, S., Hallhammer, D. (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosom. Med*., 54(6), 648-57.

Kudielka BM, Kirschbaum C. (2005) Sex differences in HPA axis responses to stress: A review. *Biol Psychol*., 69:113–32.

Kudielka, B. M., Hellhammer, D. H., Kirschbaum, C. (2007). Ten years of research with the Trier Social Stress Test—revisited. *Social neuroscience: Integrating biological and psychological explanations of social behavior*, 56-83.

Kirschbaum, C. (2010). Trier social stress test. *Encyclopedia of psychopharmacology: Trier*

social stress test. Berlin Heidelberg: Springer-Verlag.

Kunz-Ebrecht, S.R., Mohamed-Ali, V., Feldman, P.J., Kirschbaum, C., Steptoe, A. (2003). Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. *Brain, Behavior, and Immunity*, 17, 373-383.

Lazarus, R.S. (1966). *Psychological Stress and the Cognitive Process*. New York: McGraw-Hill.

Lazarus, R.S., Launier, R. (1978). Stress-related transactions between person and environment. In L.A. Pervin and M. Lewis, (Eds.), *Perspectives in interactional psychology*. New York: Plenum.

Lazarus, R.S., Folkman, S. (1984). *Stress, appraisal, and coping*. New York: Springer.

Lazarus, R.S., Folkman, S. (1987). Transactional theory and research on emotions and coping. *European Journal of Personality*, Vol. 1, 141-169.

Lazarus, R.S. (1991). *Emotion and Adaptation*. New York: Oxford University Press.

Lazarus, R.S. (1993). From Psychological Stress to the Emotions: A History of Changing Outlooks. *Annu. Rev. Psychol*., 44, 1-21.

Lee-Baggley, D., Prece, M., DeLongsi, A. (2005). Coping with interpersonal stress: role of Big Five traits. *J. Personal*., 73, 1141-80.

Lehnert, H., Beyer, J., Walger, P., et al. (1989). Salivary cortisol in normal men. In Weiner, I. Florin, and D. H. Hellhammer (Eds.), *Frontiers in stress research* (pp. 392-394). Toronto: Huber.

Lemyre, L., Lalande-Markon, MP. (2009). Psychological Stress Measure (PSM-9): integration of an evidence-based approach to assessment, monitoring, and evaluation of stress in physical therapy practice. *Physiother Theory Pract*., 25(5-6): 453-62.

Levin, A., Zagoory-Sharon, O., Feldman, R., Lewis, J. G., Weller, A. (2007). Measuring cortisol in human psychobiological studies. *Physiology and Behavior*, 90:43-53.

Lumsden, D. P. (1981). Is the concept of "stress" of any use, anymore? In D. Randall (Ed.), *Contributions to primary prevention in mental health: Working papers*. Toronto: Toronto National Office of the Canadian Mental Health Association.

Maier, K.J., Waldstein, S.R., Synowski, S.J. (2003). Relation of Cognitive Appraisal to Cardiovascular Reactivity, Affect, and Task Management. *Ann Behav Med*., 26(1):32-41.

Maruyama Y, Kawano A, Okamoto S, Ando T, Ishitobi Y, et al. (2012) Differences in Salivary Alpha-Amylase and Cortisol Responsiveness following Exposure to Electrical Stimulation versus the Trier Social Stress Tests. PLoS ONE 7(7): e39375. doi:10.1371/journal.pone.0039375

Mason, J.W. (1986). A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosomatic Medicine*, 30, 576-607.

McCrae, R.R., Costa, P.T. (2003). Personality in Adulthood: A Five-Factor Theory Perspective. New York: Guilfold 2nd ed.

McDonough, P., Walters, V. (2001). Gender and health: Reassessing patterns and explanations. *Social Science and Medicine*, 52(4), 547-559.

McEwen, B.S., Stellar, E. (1993). Stress and the individual. *Arch Intern Med*., 153:18, 2093-2101.

McEwen, B.S. (1998). Protective and damaging effects of stress mediators. *N Engl J Med*., 338, 171-179.

McEwen, B.S., Wingfield, J.C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, 43:1, 2-15.

McLean, C.P., Anderson, E.R. (2009). Brave men and timid women? A review of the gender differences in fear and anxiety. *Clinical Psychology Review*. 29, 496-505.

Miller, G.E., Chen, E., Zhou, E.S., (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocorticol axis in humans. *Psychol. Bull. 133,* 25-45.

Monks, S.M., Tomaka, J., Palacios, R., Thompson, S.E. (2010). Sexual victimization in female and male college students: examining the roles of alcohol use, alcohol expectancies, and sexual sensation seeking. *Substance Use and Misuse*, 45(13), 2258-80.

Morse, D.R., Schacterle, G.R., Esposito, J.V., Chod, S.D., Furst, M.L., DiPonziano, J., Zaydenberg, M. (1983a). Stress, meditation and saliva: a study of separate salivary gland secretions in endodontic patients. *J. Oral Med*., 38, 150-160.

Morse, D.R., Schacterle, G.R., Zaydenberg, M., Furst, M.L., Pollack, R.L., Moskow, A., Esposito, J.V. (1983b). Salivary volume and amylase activity. I. Relaxation versus relaxed chewing. J. Am. Soc. *Psychosom. Dent. Med*., 30, 85-96.

Muris, P., Meesters, C., Knoops, M. (2005). The relation between gender role orientation and fear and anxiety in nonclinic-referred children. *Journal of Clinical Child and Adolescent Psychology*, 34, 326-332.

Nater, U.M, Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., Ehlert, U. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradighm. *International Journal of Psychophysiology*, 55, 333-342.

Nater, U.M., Rohleder, N. (2009). Salivary Alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology,*34(4), 486-96.

Nexo, E., Hansen, M.R., Konradsen, L. (1988). Human salivary epidermal growth factor,

haptocorrin and amylase before and after prolonged exercise. *Scand. J. Clin. Lab. Invest*., 48(3), 269-273.

Nolen-Hoeksema, S., Larson, J., Grayson, C. (1999). Explaining the gender differences in depressive symptoms. *Journal of Personality and Social Psychology*, 77, 1061-1072.

O'Connell, K., Badrick, E., Kumari, M., Steptoe, A. (2008). Psychological coping styles and cortisol over the day in healthy older adults. *Psychoneuroendocrinology*, 33:601-611.

Ollendick, T.H., King, N.J., Muris, P. (2002). Fears and phobias in children: Phenomenology, epidemiology, and aetiology. *Child and Adolescent Mental Health*, 7, 98-106.

Owen, N., Steptoe, A. (2003). Natural killer cell and proinflammatory cytokine responses to mental stress: associations with heart rate and heart rate variability. *Biological Psychology*, 63, 101-115.

Parkes, K.R. (1984). Locus of control, cognitive appraisal, and coping in stressful episodes. *Journal of Personality and Social Psychology*, 46, 655-668.

Peacock, E.J., Wong, P.T.P. (1990). The stress appraisal measure (SAM): A multidimensional approach to cognitive appraisal. *Stress Medicine*, 6, 227-236.

Phillips, A.C. (2013). Perceived Stress. *Encyclopedia of Behavior Medicine*, 1453-1454.

Pittner, M.S. Houseton, B.K. (1980). Response to stress, cognitive coping strategies, and the Type A behavior pattern. *Journal of Personality and Social Behavior*, 19, 2-21.

Proctor, G.B., Carpenter, G.H. (2007). Regulation of salivary gland function by autonomic nerves. *Auton Neurosci*, 133(1), 3-18.

Rhodewalt, F., Sansone, C., Hill, C.A., Chemers, M.M., Wysocki, J. (1991). Stress and Distress as a Function of Jenkins Activity Survey-Defined Type A Behavior and Control Over the Work Environment. *Basic and Applied Social Psychology*, 12(2), 211-226.

Rohleder, N., Nater, U.M., Wolf, J.M., Ehlert, U., Kirschbaum, C. (2004). Psychosocial Stress-Induced Activation of Salivary Alpha-amylase. *Ann. N.Y. Acad. Sci*., 1032, 258-263.

Rohrmann, S., Henning, J., Netter, P. (1999). Changing psychobiological stress reactions by manipulating cognitive processes. *International Journal of Psychophysiology*, 33, 149-161.

Rowley, A.A., Roesch, S.C., Jurica, B.J., Vaughn, A.A. (2005). Developing and validating a stress appraisal measure for minority adolescents. *Journal of Adolescence*, 28, 547-557.

Schmaus, B., Laubmeier, K.K., Boquiren, V.M., Herzer, M., Zakowski, S. (2008). Gender and stress: Differential psydchophysiological reactivity to stress reexposure in the laboratory. *International Journal of Psychophysiology*, 69, 101-106.

Seery, M. D. (2011). Challenge or threat? Cardiovascular indexes of resilience and vulnerability to potential stress in humans. *Neuroscience and Biobehavioral Reviews*, 35, 1603-1610.

Selye, H. (1956). *The stress of life*. New York: McGraw-Hill.

Selye, H. (1964). From Dream to Discovery. New York: McGraw-Hill.

Selye, H. (1974). *Stress without distress*. Philadelphia: J. B. Lippincott.

Selye, H. (1975a). Confusion and controversy in the stress field. *Journal of Human Stress*, 1(2), 37-44.

Selye, H. (1975b). Stress and distress. *Comprehensive therapy*, 1(8), 9-13.

Skosnik, D.P., Chatterton, Jr. R.T., Swisher, T. et al. (2000). Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress. *Int. J. Psychophysiol.*, 36, 59-68.

Stahl, F., Dorner, G. (1982). Responses of salivary cortisol levels to situations. *Endocrinology*, 80, 158-162.

Skinner, N., Brewer, N. (2002). The Dynamics of Threat and Challenge Appraisals Prior to

Stressful Achievement Events. *Journal of Personality and Social Psychology,* 83:3, 678-692.

Speirs, R.L., Herring, J., Cooper, W.D., Hardy, C.C., Hind, C. R. (1974). The influence of sympathetic activity and isoprenaline on the secretion of amylase from the human parotid gland. *Arch. Oral Biol*., 19, 747-752.

Steerenberg, P.A., van Asperen, I.A., Van Nieuw Amerongen, A., Biewenga, J., Mol, D., Medema, G. (1997). Salivary levels of immunoglobulin A in triathletes. *Eur. J. Oral Sci*., 105, 305-309.

Sterling, P., Eyer, J. Allostasis: A New Paradignm to Explain Arousal Pathology. In Fisher, S. and Reason, eds. Handbook of Life Stress, Cognition and Health. New York, John Wiley and Sons. 1988, 629-649.

Stevenson-Hinde, J., Shoudice, A. (1993). Wariness to strangers: A behavior systems perspective revisited. In K. H. Rubin and J.B. Asendopf (Eds.). *Social withdrawal, inhibition, and shyness in childhood* (pp. 101-116). New York: Lawrence Erlbaum.

Strahler, J., Mueller, A., Rosenloecher, F., Kirschbaum, C., Rohleder, N. (2010). Salivary alpha-amylase stress reactivity across different age groups. *Psychophysiology*, 47, 587-595.

Suls, J., Martin, R. (2005). The daily life of the garden-variety neurotic: reactivity, stressor exposure, mood spillover, and maladaptive coping. *J. Personal*, 73, 1485-509.

Takai, N., Yamaguchi, M., Aragaki, T., Eto, K., Uchihashi, K., Nishikawa, Y. (2004). Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. *Arch. Oral Biol*., 49, 963-968.

Thoma, M.V., Kirschbaum, C., Wolf, J.M., Rohleder, N. (2012). Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biological Psychology*,

91, 342-348.

Thorpe, S.J., Salkovskis, P.M. (1995). Phobic beliefs: Do cognitive factors play a role in specific phobias? *Behavior Research and Therapy*, 33, 805-816.

Thoresen, C.E., Powell, H.L. (1992). Type A behavior pattern: new perspectives on theory, assessment, and intervention. *Journal of Consulting and Clinical Psychology*, 60(4), 595-604.

Tomaka, J., Blascovich, J., Kelsey, R.M., Leitten, C.L. (1993). Subjective, physiological, and behavioral effects of threat and challenge appraisal. *Journal of Personality and Social Psychology*, 18, 616-624.

Tomaka, J., Blascovich, J., Kibler, J., Ernst, J.M. (1997). Cognitive and physiological antecedents of threat and challenge appraisal. *Journal of Personality and Social Psychology*, 73, 63-72.

Tomaka, J., Gomez, C., Reyes, F. (2012, April). *The Appraisal of Stressful Events Scale*. Poster seession preseted at the 33rd Annual Meeting & Scientific Session of the Society of Behavioral Medicine, New Orleans, LA.

Treiber, F.A., Kamarck, T., Schneiderman, N., Sheffield, D., Kapuku, G., Taylor T. (2003). Cardiovascular reactivity and development of preclinical and clinical disease states. *Psychosom. Med*., 65(1), 46-62.

Tsigos, C., Chrousos, G.P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, 53:865-871.

Tucker, M., Bond, N.W. (1997). The roles of gender, sex role, and disgust in fear of animals. *Personality and Individual Differences*, 22(1), 135-138.

Turner, R.J., Wheaton, B., Lloyd, D.A. (1995). The epidemiology of social stress. *American*

Sociological Review, 60, 194-205.

van der Hart, O., Nijenhuis, E.R.S., Steele, K. (2005). Dissociation: An insufficiently recognized major feature of complex PTSD. *Journal of Traumatic Stress*, 18(5).

van Stegeren, A., Rohleder, N., Everaerd, W., Wolf, O.T. (2006). Salivary alpha amylase as marker for adrenergic activity during stress: Effect of beta blockade. Psychoneuroendocrinology, 31(1), 137-41.

Vickers, R.R., Hervig, L.K., Rahe, R.H., Rosenman, R.H. (1981). Type A behavior pattern and coping and defense. *Psychomatic Medicine*, 4, 381-396.

Vingerhoets, A.J.J. M., Flohr, H. (1984). Type A behavior and self-reports of coping preferences. *British Journal of Medical Psychology*, 57, 15-21.

Vollrath, M. (2001). Personality and stress. *Scand. J. Psychol*., 42, 335-47.

Vollrath, M., Torgersen, S. (2000). Personality types and coping. Personality and Individual Differences. *Personality and Individual Differences*, 29, 367-378.

Walsh, N.P., Blannin, A.K., Clark, A.M., Cook, L., Robson, P.J., Gleeson, M. (1999). The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. *J. Sports Sci*., 17(2), 129-134.

Wolff, H. G., Wolf, S. G., Jr., Hare, C. C. (Eds.) (1950). *Life stress and bodily disease*. Baltimore: Williams and Wilkins.

Wolff, H. G. (1953). *Stress and disease*. Springfield, IL: Thomas.

Wood, W., Eagly, A.H. (2002). A cross-cultural analysis of the behavior of women and men: Implications for the origins of sex difference. *Psychological Bulletin*, 128, 699-727.

Appendix A: Instrument Attachments

Appraisal of Challenge or Threat Scale (ACTS)

Answer each by filling in the correct O with regard to (a) how **demanding** the event would be to you AND (b) how **able you would be to deal** with that event.

Life Event	**How demanding is this event to you?**	**How able are you to deal with it?**
1. You're asked to talk about yourself at a workshop (PS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
2. You find out that you have a chronic disease (UE)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
3. You receive notice of bank overdraft fee (F)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
4. Said something to friend you later regretted (CS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
5. Your rent check bounces (F)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
6. You find that someone has said something negative about you (CS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
7. You're asked to give a major presentation at work or school (PS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
8. You receive unwanted medical news (UE)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
9. You have to juggle financial pressures (F)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
10. Discover friends are talking behind your back (CS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
11. Your car won't start before going to work (T)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
12. Forcing yourself to meet new people (SA)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
13. You encounter unexpected medical expenses (UE)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
14. You find that a co-worker complained to boss about you (CS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
15. Talking to potential romantic partners (SA)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
16. You're asked to introduce yourself in a public forum (PS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
17. Your credit card Increases the minimum monthly payment (F)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
18. Your car breaks down during rush hour (T)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
19. You arrive at a party where you don't know anyone (SA)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much

Life Event	How <u>demanding</u> is this event to you?	How <u>able</u> are you to deal with it?
20. A close relative has a heart attack (UE)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
21. A supervisor asks you to give a speech (PS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
22. You discover a flat tire before work or class (T)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
23. Friend or family member says something bad about you (CS)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much
24. You accidentally lock your keys in the car (T)	O----O----O----O----O not at all very much	O----O----O----O----O not at all very much

Scoring:

Each event is appraised (rated) for perceived demand and ability to cope. For each event, compute the difference between the demand and coping ability ratings. Then average the resulting difference scores for all the events within each domain: Conflict Situation (CS), Unexpected Medical Events (UE), Public Speaking (PS), Transportation and Automotive (T), Social Anxiety (SA), and Financial Concerns (F). Average all events across all domains for one total appraisal score.

The Holmes-Rahe Life Stress Inventory
Social Readjustment Rating Scale (SRRS)

Check all of the life events that have you have experienced and indicate within what time frame they occurred in.

		48 hours	7 days	Year
	Death of a spouse			
	Divorce			
	Marital separation			
	Jail term			
	Death of a close family member			
	Personal injury or illness			
	Marriage			
	Fired at work			
	Marital reconciliation			
	Retirement			
	Major change in health or behavior of a family member			
	Pregnancy			
	Sexual difficulties			
	Gain of a new family member (i.e., birth, adoption, older adult moving in)			
	Major business adjustment			
	Major change in financial state			
	Death of a close friend			
	Changing to a different line of work			
	Major change in number of arguments with spouse			
	Taking on a mortgage or loan			
	Foreclosure on a mortgage			
	Major change in responsibilities at work (i.e., promotion, demotion)			
	Son or daughter leaving home (i.e., marriage, attending college, joined military)			
	Trouble with in-laws			
	Outstanding personal achievement			
	Spouse beginning or ceasing formal schooling			
	Major change in living condition (i.e., new home, remodeling)			
	Beginning or ceasing formal schooling			
	Revision of personal habits (i.e., dress manners, associations, quitting smoking)			
	Troubles with the boss			
	Major changes in working hours or conditions			
	Changes in residence			
	Changing to a new school			
	Major change in usual type and/or amount of recreation			
	Major change in church activity			
	Major change in social activities			
	Taking on a loan (i.e., car, school)			
	Major change in sleeping habits			
	Major change in number of family get-togethers			
	Major change in eating habits			

		48 hours	7 days	Year
	Vacation			
	Major holidays			
	Minor violations of the law (i.e., traffic tickets, jaywalking, disturbing the peace)			

Scoring:

Values are added and a score is determined.
150 pts or less – relative low amount of life change and a low susceptibility to stress-induced health breakdown.
150-300 pts – implies about a 50% change of a major health breakdown in the next 2 years
300 pts or more – raises the odds to about 80%, according to the Holme-Rahe statistical prediction model

		Mean Value
	Death of a spouse	100
	Divorce	73
	Marital separation	65
	Jail term	63
	Death of a close family member	63
	Personal injury or illness	53
	Marriage	50
	Fired at work	47
	Marital reconciliation	45
	Retirement	45
	Major change in health or behavior of a family member	44
	Pregnancy	40
	Sexual difficulties	39
	Gain of a new family member (i.e., birth, adoption, older adult moving in)	39
	Major business adjustment	39
	Major change in financial state	38
	Death of a close friend	37
	Changing to a different line of work	36
	Major change in number of arguments with spouse	35
	Taking on a mortgage or loan	31
	Foreclosure on a mortgage	30
	Major change in responsibilities at work (i.e., promotion, demotion)	29
	Son or daughter leaving home (i.e., marriage, attending college, joined military)	29
	Trouble with in-laws	29
	Outstanding personal achievement	28
	Spouse beginning or ceasing formal schooling	26
	Major change in living condition (i.e., new home, remodeling)	26
	Beginning or ceasing formal schooling	25
	Revision of personal habits (i.e., dress manners, associations, quitting smoking)	24
	Troubles with the boss	23
	Major changes in working hours or conditions	20
	Changes in residence	20

		Mean Value
	Changing to a new school	20
	Major change in usual type and/or amount of recreation	19
	Major change in church activity	19
	Major change in social activities	18
	Taking on a loan (i.e., car, school)	17
	Major change in sleeping habits	16
	Major change in number of family get-togethers	15
	Major change in eating habits	15
	Vacation	13
	Major holidays	12
	Minor violations of the law (i.e., traffic tickets, jaywalking, disturbing the peace)	11

Psychological Stress Measure (PSM-9)

Check the number that best indicates the degree to which each statement has applied to you currently.

Description of Mood	Not at all	Not really	Very little	A bit	Some-what	Quite a bit	Very much	Extremely
	1	2	3	4	5	6	7	8
I feel calm.								
I feel rushed; I do not seem to have enough time.								
I have physical aches and pains; sore back, headache, stiff neck, stomach ache.								
I feel preoccupied, tormented, or worried.								
I feel confused; my thoughts are muddled; I lack concentration; I cannot focus.								
I feel full of energy.								
I feel great weight on my shoulders.								
I have difficulty controlling my reactions, emotions, moods, or gestures.								
I feel stressed.								

Scoring:

Values are added for each statement. The following statements are reverse coded – "I feel calm" and "I feel full of energy".

Appendix B: Salivary Alpha-amylase Collection Method

Salimetrics Salivary Cortisol and Alpha-amylase Collection Method: Adults and Older Children

Passive Drool

A very cost-effective method often used by our customers is the collection of whole saliva by passive drool into a small vial. *Passive drool is highly recommended because it is approved for use with almost all analytes, unlike absorbent devices, which can sometimes cause interference in immunoassays.* It is important to use high-quality polypropylene vials, since other vials can lead to problems with analyte retention or the introduction of contaminants that can interfere with the immunoassay. The vials used must also seal tightly and be able to withstand temperatures as low as -80^{o}C. We sell 2 ml cryovials that meet these requirements (Salimetrics Item No. 5002.01).

Materials required

Plastic drinking straws; Scissors; Cryovials (polypropylene, 2 mL capacity); Labels 4

Prior to Saliva Collection

1. Have research participants rinse their mouth with water 10 minutes prior to collection. Consult the Research Participant Preparation and Documentation section above for additional advice.

2. Cut plastic drinking straws into 2-inch (5 cm) pieces.
3. Give each research participant one straw piece and one cryovial.

Instructions for Collecting Saliva

1. Instruct research participants to allow saliva to pool in the mouth. Some find it helpful to imagine eating their favorite food.

2. With head tilted forward, research participants should drool down the straw and collect saliva in the cryovial. (It is normal for saliva to foam, so we advise using a vial with twice the capacity of the desired sample volume.)

3. Repeat as often as necessary until sufficient sample is collected. One mL (excluding foam) is adequate for most tests. Collection of samples to be analyzed for more than one analyte may require larger vials.

Note: Secretory IgA and DHEA-S concentrations in saliva are affected by saliva flow rates. We recommend recording the amount of time necessary to collect a given volume of saliva so as to express the analyte measured as a function of time. Contact Salimetrics for details.

4. Keep samples cold after collection (4^{o}C) and freeze (-20^{o} to -80^{o}C) as soon as possible.

Vita

Clarissa Gomez was born and raised in El Paso, Texas. She graduated from Bowie High School in 2001 with four academic scholarships. She began pursuing her bachelor's degree in Chemistry at the University of Texas at El Paso during Fall 2001. Her work examining the continuity and thickness of layered double hydroxide (LDH) layers led her to receive her first publication. In 2005, she received the National Science Foundation GK-12 Fellowship Grant where she implemented inquiry based science in EPISD classrooms. She completed her degree in Chemistry with a minor in Biology in May 2006 and was awarded the NSF GK-12 Fellowship Grant for a second year.

She immediately began pursuing her master's degree in the Department of Chemistry and continued her research in an inorganic lab. Her background in biology and interest in immunology led to a collaborative research study with the Department of Public Health in the College of Health Sciences. Her research involved examining the effectiveness of Cu(II) and Zn(II) nanoparticles to deliver DNA vaccines in-vivo and investigate the ability of these complexes to bind and deliver a gene vaccine against *Leishmania mexicana*. She graduated with her master's degree in Chemistry with a minor in Biology in December 2008.

She again, immediately dove right in and began pursuing her third degree in Interdisciplinary Health Sciences. Although she was venturing into a new area of study, her love for science never faltered and is very much integrated and apparent in her dissertation. In 2015, she completed her research examining whether stress appraisal predicted stress biomarker activity levels and graduated with her Ph.D. from the University of Texas at El Paso.

Permanent Address: 2726 Mobile Ave.
El Paso, TX 79930
csgomez80@gmail.com

This dissertation was typed by Clarissa Gomez.