ALPHA-AMYLASE AND CORTISOL IN WOMEN WITH ENDOMETRIAL CANCER: COMPARING DIURNAL PROFILES IN RELATION TO STRESS

By

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To my mother and father

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Abstract of Thesis Presented to the Graduate School
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Alpha-amylase and cortisol are stress hormones produced via independent stress systems. Following stress, alpha-amylase is released by the sympathetic nervous system (SNS), while cortisol is released by the hypothalamic-pituitary-adrenal (HPA) axis. Although the psychoneuroimmunologic (PNI) mechanisms involved in HPA stress responses have been studied in medical populations, less is known about mechanisms of limbic HPA-independent alpha-amylase, particularly in cancer. This study (1) compared salivary alpha-amylase and cortisol levels, and (2) examined stress/anxiety and alpha-amylase relations among women scheduled for surgery for endometrial cancer.

Participants included 15 women drawn from a larger study of PNI relations in endometrial cancer. Stress was measured with the Perceived Stress Scale and stress ratings (0 [*no stress*] to 10 [*highest stress possible*]) obtained at the time of saliva collection. Anxiety was measured using the Millon Behavioral Medicine Diagnostic and the Structured Interview Guide for the Hamilton Anxiety/Depression Scales. Saliva was collected at 8 AM, 12 PM, 5 PM, and 9 PM the day prior to surgery and assayed using ELISA.

A moderate effect size correlation (r = -.34) emerged between greater 8 AM cortisol and lower 8 AM alpha-amylase. A moderate effect size correlation (r = .39) also emerged between greater changes in cortisol and greater changes in alpha-amylase per unit time. Furthermore, a large and statistically significant effect size correlation (r = .58) was revealed between greater 8 AM stress levels and greater 12 PM alpha-amylase. Statistically significant correlations emerged between greater anxiety and less change in alpha-amylase per unit time (r = -.72, p<.05) and less total diurnal output (r = -.71, p<.05), a possible pattern of SNS activity blunting. Although based on a small sample, these findings suggest stress/anxiety may be associated with SNS-dependent alpha-amylase in women with cancer. Future research should replicate and expand upon these findings in a larger sample.

CHAPTER 1
INTRODUCTION

Epidemiology of Endometrial Cancer

Endometrial cancer is a type of uterine cancer affecting the lining of the uterus, called the endometrium. It is the most common gynecologic cancer and the fourth most common type of cancer affecting women in the United States. In 2008, the estimated number of new endometrial cancer cases was 40,100, with an estimated number of deaths from endometrial cancer reaching 7,470. The American Cancer Society predicts that in 2009, the number of new cases will increase to 42,160, with an expected 7,780 deaths. In 2006, approximately 572,603 women in the United States had a history of endometrial cancer (National Cancer Institute, 2009). Mortality related to endometrial cancer is highest in African-Americans, with 7.2 per 100,000 deaths compared to 3.9 per 100,000 deaths in Caucasians. The five-year survival rate for women diagnosed with endometrial cancer is 88%; however, the five-year survival rate for women who are diagnosed early in the disease process increases to approximately 95% (American Cancer Society, 2008). Endometrioid adenocarcinoma, the most common type of endometrial cancer, has well-established risk factors, including obesity, early onset of menses and/or late menopause, diabetes, and never having given birth. However, little research has focused on how biobehavioral factors (i.e., psychological, behavioral, and physiological factors, in concert) may influence the progression of endometrial cancer.

Biobehavioral Factors Involved in Tumor Biology

Several studies have provided evidence for the influence of stress and other psychological factors on cancer progression (Antoni, Lutgendorf, Cole, Dhabhar, Sephton, McDonald, Stefanek, & Sood, 2006). Central nervous system activated

responses to stress are initiated by both environmental and psychosocial processes. These stress responses follow two pathways: the autonomic nervous system (ANS; fight-or-flight) and the hypothalamic-pituitary adrenal axis (HPA; defeat/withdrawal). These parallel stress systems both release hormones (i.e., glucocorticoids and catecholamines) in response to stress that can subsequently impact immune functioning due to dysregulation of the feedback system.

Mechanisms for Regulation of the Endocrine System

The endocrine system is regulated by the HPA, which releases hormones in response to stress. Through the initiation of a feedback loop, the hypothalamus is stimulated by the neurotransmitter gamma-aminobenzoic acid (GABA). As a result, the hypothalamus secretes corticotrophin releasing hormone (CRH), which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). Finally, glucocorticoids including cortisol are released by the stimulation of the adrenal cortex by ACTH. Homeostasis is maintained because circulating cortisol levels determine whether further stimulation of the hypothalamus and release of CRH and ACTH is required (Kaye & Lightman, 2005). For example, low cortisol levels will prevent the suppression of CRH and ACTH release, while higher levels of circulating cortisol will reduce the amount of CRH and ACTH that is produced. However, under conditions of chronic stress and sustained circulation of glucocorticoids, this negative feedback system may cease to function, resulting in continued HPA activation and secretion of glucocorticoids (Antoni et al., 2006).

Impact of Stress on Endocrine Functioning, Immunity, and Cancer

Endocrine factors can have a significant impact on immune function, particularly in response to the presence of stress. Stress modulates, or alters, immunity through

increases in HPA and SNS activity, particularly affecting suppression of anti-tumor immunity (Sephton & Spiegel, 2003). Although HPA and SNS activation in response to acute stress is adaptive, HPA and SNS activation in response to chronic stress is maladaptive, such that it decreases cell-mediated immunity through suppressed synthesis of proinflammatory cytokines (Antoni, Lutgendorf, Cole, Dhabhar, Sephton, McDonald, Stefanek & Sood, 2006; Thaker, Lutgendorf, & Sood, 2007). A decrease in cell-mediated immunity makes it harder for individuals to fight off infection. There is some evidence that cortisol may enhance this higher vulnerability to infection by SNS induced immunity implicated in cancer cell growth (Nakane, Szentendrei, Stern, Virmani, Seely, & Kunos, 1990), but less is known regarding the impact of alpha-amylase on immunity.

Biobehavioral Mechanisms of Alpha-Amylase Production

Salivary alpha-amylase is an enzyme secreted into the oral mucosa by the salivary glands and released by activation of the sympathetic nervous system (SNS) in response to stress (Granger, Kivlighan, El-Sheikh, Gordis & Stroud, 2007). Although it can be measured in multiple bodily fluid compartments, it is often measured in saliva as it is the least invasive collection method. Primarily, alpha-amylase functions biologically to aid in digestion. However, it plays a secondary role in the prevention and clearance of bacteria from the mouth, which highlights its role in facilitating immunocompetence through the promotion of wound healing via inflammatory processes.

Among healthy, non-stressed individuals, alpha-amylase can be found in relatively high concentrations in the salivary glands, where it peaks quickly and declines rapidly upon awakening. Diurnally, alpha-amylase increases throughout the day following the initial rapid decline. Much like cortisol, acute physiological and

psychological stress can cause levels of alpha-amylase to rise. Several studies have shown that alpha-amylase baseline concentrations and stress-related changes are highly correlated with patterns of plasma norepinephrine release, suggesting adrenergic regulation of the stress system (Granger, Kivlighan, Blair, El-Sheikh, Mize, Lisonbee, Buckhalt, Stroud, Handwerger, & Schwartz, 2006; Granger et al., 2007; Nater & Rohleder, 2009).

Biobehavioral Mechanisms of Cortisol Production

Cortisol is a stress hormone, specifically a glucocorticoid. It is produced by the adrenal cortex and it is released by activation of the HPA axis. Under normal conditions, cortisol follows a typical diurnal pattern that is characterized by increased levels in the morning and decreased levels in the evening. During acute stress, cortisol levels rise and stay high throughout the course of the day, which is illustrated by a flattened slope. However, under conditions of chronic stress, the HPA system may become fatigued, resulting in a blunting of the diurnal cortisol rhythm.

Comparing Alpha-Amylase and Cortisol Stress Profiles

Some studies have shown that cortisol has a similar diurnal profile to alpha-amylase in healthy individuals (Granger et al., 2007); however, other studies have presented conflicting evidence, suggesting that alpha-amylase has a large initial decline upon awakening followed by steadily increasing levels across the day. While mechanisms involved in the HPA stress-response associated with chronic illness have been studied extensively (Kumar, Kumar, Waldrop, Antoni, Schneiderman & Eisdorfer, 2002; Reiche, Nunes, & Morimoto, 2004; Spiegel, Giese-Davis, Taylor & Kraemer, 2006), the study of limbic HPA-independent alpha-amylase profiles in medical populations has not received much attention. The majority of studies indicate that

individuals with chronic stress show increased cortisol and alpha-amylase levels (Granger et al., 2007; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007; Van Veen, Van Vliet, DeRijk, Van Pelt, Mertens, & Zitman, 2008). Obtaining knowledge regarding stress related changes in physiological processes both within and outside the HPA axis is critical to a comprehensive view of the stress-response system. This information will be particularly useful in medically ill populations (i.e., cancer) who experience chronic stress secondary to diagnosis and treatment.

Psychosocial and Endocrine Relationships

There is a robust body of literature linking psychosocial factors to cortisol in cancer populations. Previous research has demonstrated connections between cortisol and several psychosocial factors in cancer patients, including depression (Jehn, Kuehnhardt, Bartholomae, Pfeiffer, Krebs, Regierer, Schmid, Possinger, & Flath, 2006; Reiche, Morimoto, & Nunes, 2005; Spiegel & Giese-Davis, 2003), anxiety (Miller, Ancoli-Israel, Bower, Capuron & Irwin, 2008; Vedhara et al., 2003), PTSD (Cohen, Moor, Devine, Baum, & Amato, 2001), social support (Turner-Cobb, Sephton, Koopman, Blake-Mortimer, & Spiegel, 2000), sleep difficulties and fatigue (Bower, Ganz, Dickerson, Petersen, Aziz, & Fahey, 2005; Bower, Ganz, & Aziz, 2005; Sephton, Sapolsky, Kraemer, & Spiegel, 2000), and benefit finding (Cruess, Antoni, McGregor, Kilbourn, Boyers, Alferi, Carver, & Kumar, 2000). For instance, in one study of cancer patients, depression was associated with higher cortisol concentrations at 8 AM and 8 PM and dysfunctional cortisol variations (Jehn et al., 2006). Another study of stress, depression, and anxiety in breast cancer patients revealed no correlations between psychological distress and levels of cortisol taken at specific time points across the day, but associations were found between psychological distress (particularly stress and

anxiety) and non-linear rate of decline in cortisol (Vedhara, Miles, Bennett, Plummer, Tallon, Brooks, Gale, Munnoch, Schreiber-Kounin, Fowler, Lightman, Sammon, Rayter, & Farndon, 2003). Despite this wealth of literature, little is known about relationships between cortisol and psychosocial factors in endometrial cancer, the 4th most common cancer among women.

There is also a significant gap in the literature about how psychological factors may be associated with alpha-amylase in both healthy and disease populations. Only one published study, to our knowledge, has assessed the relationship between alpha-amylase and psychological factors (vanVeen et al., 2008). In this study, salivary alpha-amylase and cortisol were measured in a sample of adults diagnosed with social anxiety disorder (also known as social phobia) and were compared to a sample of healthy adults in a non-stressed condition and following a low dose of dexamethasone, a synthetic glucocorticoid hormone that suppresses HPA functioning. Results indicated that diurnal alpha-amylase profiles for individuals with social anxiety disorder were significantly different from cortisol profiles for both conditions, such that diurnal alpha-amylase and alpha-amylase levels post-dexamethasone were found to be higher than cortisol levels. No significant differences were found between individuals with social anxiety disorder and healthy controls. Additionally, there is little known regarding potential relationships between psychosocial factors and alpha-amylase, particularly in chronic, life-limiting illnesses, including cancer.

Current Study

The current study intends to address these gaps in the literature by exploring the following specific aims:

Specific Aim 1: To examine the relationship between waking levels/diurnal slopes of cortisol and alpha-amylase in women with endometrial cancer.

Hypothesis 1a: At least a moderate negative effect size correlation will emerge between levels of alpha-amylase at 8 AM and levels of cortisol at 8 AM.

Hypothesis 1b: Alpha-amylase diurnal slope will reflect an overall increase in values across the day, while cortisol diurnal slope will reflect an overall decrease in values across the day (i.e., alpha-amylase will have a more positive diurnal slope than cortisol).

Specific Aim 2: To examine the association between perceived stress/anxiety and alpha-amylase/cortisol slope in women with endometrial cancer.

Hypothesis 2a: At least a moderate effect size correlation will emerge between greater perceived stress and (i) less decrease in cortisol per unit time, and (ii) greater increase in alpha-amylase per unit time (i.e., patterns suggestive of a blunted cortisol response and an exaggerated alpha-amylase response, respectively).

Hypothesis 2b: At least a moderate effect size correlation will emerge between greater anxiety and (i) less decrease in cortisol per unit time, and (ii) greater increase in alpha-amylase per unit time (i.e., patterns suggestive of a blunted cortisol response and an exaggerated alpha-amylase response, respectively).

Exploratory Aim 3: To explore relationships between stress/anxiety and additional markers of alpha-amylase/cortisol levels (values at 8 AM, 12 PM, 5 PM, and 9 PM; and total diurnal output) in women with endometrial cancer.

Hypothesis 3: Given the exploratory nature of this aim, no specific hypotheses are offered.

CHAPTER 2
METHODS

Design

The current study utilized a nonexperimental, cross-sectional design. Briefly, participants provided psychosocial data immediately prior to surgery for suspected endometrial cancer. Furthermore, they collected saliva samples four times a day for three consecutive days prior to surgery (see “Procedures” below for additional detail).

Participants

Participants for this study were 15 women drawn from a larger, parent study jointly funded by the American Cancer Society and the National Cancer Institute (PI: Deidre Pereira, Ph.D. R03). Inclusion criteria for the parent study were: (a) women with suspected primary endometrial cancer who were 18 or older, (b) undergoing a total abdominal hysterectomy with bilateral salpingo oophorectomy (TAH-BSO), and (c) fluent in spoken English. Exclusion criteria were: (a) a diagnosis of recurrent endometrial cancer, (b) metastasis from another site, (c) undergoing pre-operative chemotherapy or radiotherapy, and (d) a current psychotic disorder or suicidal intent. Women were selected for this sub-study if they had complete physiological (i.e., assayed cortisol and alpha-amylase samples) and psychosocial data.

Procedures

Participants for this study were recruited from the Gynecologic-Oncology Clinic at the University of Florida. Women who were potentially eligible for participation were identified during their pre-operative consultation visits with a team of physicians, residents, and nurses. If the patient expressed interest in study participation to the medical staff, she then met with a trained member of the research team to discuss study

procedures and address subsequent questions and concerns. Following confirmation that the patient was interested in participating, the patient read and signed an IRB-approved Informed Consent Form. Upon providing consent and enrolling in the study, the participant underwent a brief psychiatric screening assessment. If the screening measure indicated a lack of psychiatric disturbance, the participant was then scheduled for a psychosocial interview during their pre-operative visit to the Gynecologic Oncology Clinic. Participants were given questionnaires to complete and were instructed to collect saliva samples for the three days prior to their return appointment. At this appointment, participants underwent a psychosocial interview in one of the clinic rooms and returned their completed materials. Upon completion of the psychosocial interview and collection of materials, participants received $20 compensation to reimburse parking and transportation expenses.

Psychosocial Assessment

Anxiety

Two instruments were used to assess anxiety. The Structured Interview Guide for the Hamilton Anxiety/Depression Scales (SIGH-AD) (Williams, 1988) was used to assess symptoms of anxiety over the past week. This measure is a semi-structured interview based on the Hamilton Anxiety Scale (Hamilton, 1959) and the Hamilton Depression Scale (Hamilton, 1960) and has been used in the past with medical populations (Brown, Rundell, McManis, Kendall, Zachary, & Temoshok, 1992). In order to reduce patient burden and to exclude items that may also be associated with endometrial cancer symptomatology, the present study used an abbreviated version that consisted of 15 items assessing depression and 9 items assessing anxiety. Scores for the anxiety subscale ranged from 0 (no anxious symptoms) to 29 (severe anxious

symptoms). This abbreviated version of the SIGH-AD demonstrated good concurrent validity. SIGH-AD depression scores (with and without organic ratings) were significantly correlated with Beck Depression Inventory – second edition (BDI-II; Beck, Steer, & Brown, 1996), $r = .477$, $p < .05$ and $r = .623$, $p < .001$ respectively. Additionally, SIGH-AD anxiety scores were significantly correlated with Affects Balance Scale (ABS; Derogatis, 1975) scores, $r = .624$, $p < .001$.

The Millon Behavioral Medicine Diagnostic (MBMD) Anxiety-Tension subscale was used to measure symptoms of anxiety that may interfere with medical care. The MBMD is a 165-item self-report inventory that measures several psychological facets that can affect treatment progress and outcomes in patients with medical illnesses (Millon, Antoni, Millon, Minor & Grossman, 2001). The measure includes 29 subscales in addition to Response Pattern scales (i.e., disclosure, desirability, and debasement), a Validity indicator, and Negative Health Habits indicators (i.e., alcohol, drug, eating, caffeine, inactivity, and smoking). The Anxiety-Tension subscale is considered a psychiatric indicator and elevations suggest that additional assessment of anxious symptomatology should be evaluated to determine an appropriate DSM-IV diagnosis. The MBMD was normed on a medical population and demonstrated adequate reliability (internal reliability = .79; internal consistency = .83) in medical populations (Cruess, Minor, Antoni, & Millon, 2007).

Perceived Stress

Perceived stress was assessed in two ways. First, participants completed the Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983), a 14-item self-report scale used to measure an individual's appraisal (perceptions) of situations as stressful during the week prior to surgical evaluation. Participants rated the frequency

of these 14 feelings, cognitions, and situations on a 5-point scale (0 = never; 4 = very often). Examples of items included: “In the last week, how often have you felt nervous and stressed?” and “In the last week, how often have you felt that you were unable to control the important things in your life?” Several items were reverse scored so that higher ratings corresponded to greater perceived stress. Ratings were then summed to yield a total perceived stress score, with higher scores indicating greater perceptions of stress.

Second, participants provided stress ratings at the time of each saliva collection across the three days prior to surgery. Specifically, participants rated their stress levels on a scale of 0 (no stress) to 10 (highest stress possible).

Physiological Assessment

Saliva Collection and Storage

Saliva samples were collected at 8 AM, 12 PM, 5 PM, and 9 PM for 3 consecutive days prior to participants’ pre-operative visit in the Gynecologic Oncology Clinic. Participants were instructed to collect saliva samples using a Salivette (Starstedt, Inc., Newton, N.C.), which is a cylindrical plastic tube containing a cotton roll. Participants were instructed to place the cotton roll in their mouth and allow it to saturate with saliva for approximately 1-2 minutes. Additionally, participants were asked not to smoke, eat, drink liquids, or brush their teeth for the 30 minutes prior to collection. Participants were also instructed to write the exact time of collection and their stress ratings (0 – 10) on the plastic tube. Finally, participants were instructed to refrigerate the samples until they returned to the Gynecologic Oncology Clinic for their pre-operative visit. Once the samples were returned to the researchers, they were stored in a freezer maintained at -80 degrees Celsius. Batches of samples were then shipped to

Salimetrics, Inc. (State College, PA), where they were assayed. Only day 3 saliva collection will be used in the current study.

Quantitation of Salivary Cortisol

Salivary cortisol concentrations were obtained using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Salimetrics, Inc., State College, PA). This method of kinetic measurement is commonly used in laboratory settings and involves combining an unknown amount of antigen with an antibody that is linked to an enzyme so that they may bind. Finally, a substance is added to aid in the enzyme's conversion resulting in the formation of various complexes that can be seen via the magnitude of fluorescence that they emit. This magnitude is read by a standard plate reader, which detects the optical density and determines cortisol levels based on the intensity of the color following binding with the substrate (tetramethylbenzidine).

Quantitation of Salivary Alpha-Amylase

Salivary alpha-amylase concentrations were also obtained using an ELISA kit (Salimetrics, Inc., State College, PA), which is based on the same principles of measurement present in cortisol. The main differences lie in the type of substrate used (2-chloro-p-nitrophenol) in the enzymatic reaction with alpha-amylase and the amount of nanometers (405) required to measure optical density.

Operationalization of Cortisol and Alpha-Amylase

Cortisol and alpha-amylase levels were examined in several ways. First, morning (i.e., awakening) values were assessed by examining cortisol/alpha-amylase concentrations at 8 AM on Day 3 of collection. Morning values reflect the body's ability to respond physiologically to the stress of transitioning from sleep to wakefulness. Determination of "abnormal" 8 AM values is challenging. In psychoneuroendocrinology

research, individual participants' 8 AM values of cortisol and alpha-amylase are generally compared to those of other participants in the overall sample. 8 AM values that are either greatly elevated or much lower than other participants' 8 AM values may be viewed as abnormal, suggesting either an exaggerated or blunted response to waking.

Second, cortisol/alpha-amylase slopes on Day 3 of collection were generated by regressing cortisol/alpha-amylase concentrations at 8 AM, 12 PM, 5 PM, and 9 PM on time of collection. Slopes were represented by the unstandardized beta weights generated by these regression analyses and equaled the average change in cortisol/alpha-amylase per unit time. As per standard convention in the psychoneuroendocrinology literature, a steeper, negative cortisol slope (i.e., greater decrease in cortisol per unit time) indicated a more normal rhythm, whereas a flattened (i.e., smaller decrease per unit time) or positive slope (i.e., increase in cortisol per unit time) indicated a more abnormal rhythm (Sephton et al., 2000). In the alpha-amylase literature, an overall steep, positive slope would be consistent with an exaggerated, abnormal stress response (Nater et al., 2007)

For the exploratory aim, cortisol/alpha-amylase area under the curve with respect to ground (AUCg) was calculated (Vedhara, Tuinstra, Miles, Sanderman, & Ranchor, 2006) using a previously published trapezoidal formula (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). AUCg represented the total diurnal output of cortisol/alpha-amylase and is thought to represent basal physiological stress reactivity.

Statistical Analyses

Descriptive statistics were calculated on all variables of interest. The distributions of cortisol and alpha-amylase values were examined for normality and

transformed, as needed, in order to allow for the use of parametric statistics. Due to the small sample size, the relationship between biobehavioral control variables and cortisol/alpha-amylase were not examined.

Analyses of Specific Aims

Specific Aim 1: To examine the relationship between waking levels/diurnal slopes of cortisol and alpha-amylase in women with endometrial cancer.

Bivariate correlation analyses were performed to estimate the effect size of the relationship between Day 3 8 AM values of cortisol and alpha-amylase and slopes of cortisol and alpha-amylase. The slopes of cortisol and alpha-amylase were then compared using the methods of Meng, Rosenthal, & Rubin (1992). This method compares two correlated correlation coefficients. In this instance, the slope for cortisol and slope for alpha-amylase shared time as a predictor.

Specific Aim 2: To examine the association between perceived stress/anxiety and alpha-amylase/cortisol slope in women with endometrial cancer.

Bivariate correlation analyses were performed to estimate the effect sizes of the relationships between perceived stress and cortisol/alpha-amylase slopes on Day 3. Parallel analyses were also performed between anxiety and cortisol/alpha-amylase slopes.

Analysis of Exploratory Aims

Exploratory Aim 3: To explore relationships between stress/anxiety and additional markers of alpha-amylase/cortisol levels (values at 8 AM, 12 PM, 5 PM, and 9 PM; and total diurnal output) in women with endometrial cancer.

For the exploratory analyses, bivariate correlation analyses were also performed to (a) estimate effect sizes of the relationships between stress ratings at the time of

saliva collection and cortisol/alpha-amylase values at each time of collection, and (b) perceived stress scores/anxiety and cortisol/alpha-amylase AUCg on Day 3.

CHAPTER 3
RESULTS

Preliminary Analyses

Normality Assumptions

Alpha-amylase day 3 individual time point values were determined to be non-normally distributed. This included values for 8 AM (Skewness = 2.417, SE = .550; Kurtosis = 7.122, SE = 1.063), 5 PM (Skewness = 2.566, SE = .550; Kurtosis = 8.182, SE = 1.063), and 9 PM (Skewness = 2.012, SE = .550; Kurtosis = 3.877, SE = 1.063). Additionally, the alpha-amylase day 3 slope was non-normally distributed (Skewness = 1.829, SE = .580; Kurtosis = 4.147, SE = 1.121). As a result, the Blom transformation (Blom, 1958) was used in order to normalize the data so that parametric statistics could be used.

Cortisol day 3 time point values were also determined to be non-normally distributed. This included values for 12 PM (Skewness = 2.147, SE = .616; Kurtosis = 6.147, SE = 1.191), 5 PM (Skewness = .847, SE = .616; Kurtosis = 1.508, SE = 1.191), and 9 PM (Skewness = 2.931, SE = .616; Kurtosis = 9.464, SE = 1.191). The Blom transformation was employed to normalize these data, as well.

Finally, curve fit estimation was utilized to fit non-transformed cortisol and alpha-amylase values to a regression line in order to obtain slopes. The resulting cortisol slope was determined to be normal; however, the alpha-amylase slope was determined to be non-normally distributed and, subsequently, the Blom transformation was used to normalize these data.

Descriptive Results

A total of 130 women met the eligibility requirements for participation and were subsequently enrolled in the parent study. A subset of 15 women had full psychosocial data and day 3 physiological data and were thus selected for participation in the present study. Participants in the sub-study ranged in age from 44-70 years old (*M*=58.67 years, *SD*=8.60 years) and the majority of women reported that they were Caucasian (86.7%) and non-Hispanic (80.0%).

The majority of participants in the sub-study had Stage I disease (52.9%); however 17.6% had Stage II disease and 11.8% had Stage III disease. Additionally, 17.6% were classified as having benign endometrial disease following surgery. Of those with cancer, the primary diagnosis was endometrial adenocarcinoma, endometrioid type (58.8%).

The number of days between study enrollment and surgery ranged from 6-18 (*M*=11.60, *SD*=3.89) and the number of days between the pre-operative visit and surgery ranged from 1-8 days (*M*=1.93, *SD*=1.98).

Comparison to the Parent Study Sample

T-tests and chi-square analyses indicated that there were no statistically significant differences between the group with incomplete psychosocial and physiological data (*n* = 115) when compared to the group with complete psychosocial data and Day 3 physiological data (*n* = 15) across age or disease stage. However, significant differences were present between the two groups across histology ($X^2(12, N = 130) = 23.894$, $p = .021$) and race ($X^2(2, N = 130) = 7.459$, $p = .024$). With regard to histology, the majority of participants in the sample with incomplete data reported having endometrial adenocarcinoma, endometrioid type, while the sample with complete data included women with less common histologic types of endometrial

cancer (Table 3-1). The significant difference in race between the two groups as detected by the chi square analysis is likely the result of an inaccurate inference due to the small sample size, which is illustrated in Table 3-2.

Analyses of Specific Aims

Specific Aim 1: Comparison of Alpha-Amylase and Cortisol 8 AM Values/Slopes

Table 3-3 shows the slope generated by regressing alpha-amylase and cortisol levels on time of collection. For alpha-amylase, there was a mean increase of 3.60 Units/mL per hour, while for cortisol, there was a mean decrease in cortisol of 0.01 ug/dL per hour. Figure 1 depicts the slopes for alpha-amylase and cortisol following transformation of the unstandardized betas into standardized betas for direct comparison. As shown in Figure 1, alpha-amylase tended to have a more positive slope than cortisol. Furthermore, alpha-amylase tended to have a more variable course throughout the day with general increases in levels, while cortisol steadily decreased throughout the day.

Next, relationships between cortisol and alpha-amylase 8 AM values and slopes were examined using bivariate correlations (Table 3-4). A moderate negative effect size correlation (r = -.335, p=.222) emerged between cortisol and alpha-amylase values at 8 AM, indicating that low alpha-amylase values at 8 AM were associated with high cortisol values at 8 AM. A moderate effect size correlation (r = .389, p = .152) was also found between cortisol and alpha-amylase slopes. However, there was no statistically significant difference between alpha-amylase and cortisol slopes (z = .86, p = .390).

Specific Aim 2: Stress, Anxiety, and Alpha-Amylase/Cortisol Slopes

Contrary to hypotheses, perceived stress scores were not associated with alpha-amylase slope. However, a large effect size correlation was found between alpha-

amylase slope and MBMD anxiety-tension (r = -.722, p<.05), suggesting that women with more MBMD anxiety-tension had less of an increase in alpha-amylase per unit time (Table 3-5; Figure 3-2).

There was a moderate effect size correlation between greater SIGH-AD anxiety and greater decrease in cortisol per unit time (r = -.507), as well as between greater MBMD anxiety-tension and greater decrease in cortisol per unit time (r = -.619) (Table 3-5).

In order to examine these relationships further, the alpha-amylase and cortisol slopes of the participant with the highest MBMD anxiety-tension score and the participant with the lowest MBMD anxiety-tension score were graphed (Figure 3-2). As shown in Figure 3-2, the high anxiety-tension participant had a more blunted alpha-amylase slope than the low anxiety-tension participant,

However, the high-anxiety tension participant had a flatter cortisol slope than the low anxiety-tension participant, overall, in spite of having an unstandardized regression coefficient indicating a higher rate of decline across the course of the day compared to the low anxiety-tension participant. Further examination indicated that this discrepancy may be due by the fact that the high anxiety-tension participant's cortisol nadir occurred at 12 PM, which is abnormally early. This abnormally early nadir resulted in a statistically high rate of change between 8 AM and 12 PM, followed by a statistically flattened slope across the remainder of the day. This abnormal pattern produced a more negative overall (i.e., 8 AM to 9 PM) slope compared to the low anxiety-tension participant, resulting in the appearance of a more normal slope. In contrast, the low anxiety-tension participant's cortisol nadir occurred at 9 PM, which is the normal pattern.

In addition, in contrast to the highest anxiety-tension participant, the lowest anxiety-tension participant experienced a relatively steady rate of decline of cortisol across the course of the day. Although this pattern resulted in a slope that is greater ("more abnormal") than that of the high anxiety-tension participant, the visual representation of the slope indicates that this is in fact a more normal pattern. Thus, these results are consistent with initial hypotheses.

Exploratory Aim 3: Stress, Anxiety, and Other Markers of Alpha-Amylase/Cortisol Levels

MBMD anxiety-tension demonstrated a moderate effect size correlation with lower 8 AM alpha-amylase levels (r = -.406) and a large effect size correlation with lower alpha-amylase AUCg (r = -.712, p < .05). This indicated that greater MBMD anxiety-tension was associated with lower 8 AM alpha-amylase and less total diurnal alpha-amylase output (Table 3-6).

Furthermore, moderate effect size correlations emerged between greater perceived stress and greater MBMD anxiety-tension and greater 8 AM cortisol (r = .437 and r = .451, respectively). A large and significant effect size correlation also emerged between greater SIGH-AD anxiety and greater 8 AM cortisol (r = .605, p < .05). Stress/anxiety variables were not associated with total diurnal cortisol output (Table 3-6).

A moderate and significant effect size correlation was found between stress ratings at 8 AM and alpha-amylase levels at 12 PM (r = .575, p < .05), suggesting that greater stress early in the morning was associated with alpha-amylase levels later in the day. A moderate effect size correlation emerged between greater stress at 8 AM and less cortisol at 5 PM (r = -.324). Finally, cortisol at 9 PM demonstrated moderate effect

size correlations with stress at 8 AM (r = .360), 12 PM (r = .355), and 9 PM (r = .339). This indicated that greater stress throughout the day was associated with increased cortisol levels at 9 PM (Table 3-7).

Table 3-1. Histology distribution

	Parent Study	Sub-study
Endometrial adenocarcinoma, endometrioid type	79	9
Complex endometrial hyperplasia with atypia	5	2
Complex endometrial hyperplasia without atypia	1	0
Clear cell carcinoma of endometrium	4	0
Mixed clear cell & endometrioid adenocarcinoma	1	0
Mixed serous & clear cell adenocarcinoma of endometrium	1	0
Endocervical adenocarcinoma, endometrioid type	0	1
Papillary serous carcinoma	3	1
Ovarian endometrioid carcinoma, endometrial adenocarcinoma, endometrioid type	1	0
Endometrioid adenocarcinoma with focal squamous differentiation	2	0
Endometrial adenocarcinoma with focal clear cell features	1	0
Adenomyosis	0	1
No residual tumor	0	1

Table 3-2. Racial distribution

	Parent Study	Sub-study
Black or African-American	8	1
White	103	13
Mixed	0	1

Table 3-3. Regression of cortisol and alpha-amylase (sAA) levels on time of collection

	N	b	β
sAA	17	3.60	0.240
Cortisol	15	0.01	-0.067

Table 3-4. Correlations between waking levels/diurnal slopes of cortisol and sAA

	Cortisol 8 AM	Cortisol slope
sAA 8 AM	-.335*	-
sAA slope	-	.389*

* moderate effect size (*p* = ns)

Table 3-5. Correlations between cortisol/sAA slopes and perceived stress/anxiety scores

	PSS	SIGH-AD Anxiety	MBMD Anxiety-Tension
sAA slope	-.069	-.145	-.722**
Cortisol slope	-.292	-.507*	-.619*

* moderate effect size (*p* = ns); ** large effect size (*p* < .05)

Table 3-6. Correlations between waking levels/total diurnal output of cortisol and sAA, perceived stress, and anxiety

	PSS	SIGH-AD Anxiety	MBMD Anxiety-Tension
sAA 8 AM	-.233	-.199	-.406*
sAA AUCG	-.240	-.253	-.712**
Cortisol 8 AM	.437*	.605**	.451*
Cortisol AUCG	.079	.084	-.043

* moderate effect size (*p* = ns); ** large effect size (*p* < .05)

Table 3-7. Correlations between stress ratings at the time of collection and corresponding cortisol/sAA levels

	Stress 8 AM	Stress 12 PM	Stress 5 PM	Stress 9 PM
sAA 8 AM	-.137			
sAA 12 PM	.575**	.067		
sAA 5 PM	.059	.219	-.057	
sAA 9 PM	.177	.002	-.126	-.184
Cortisol 8 AM	.192			
Cortisol 12 PM	.071	.176		
Cortisol 5 PM	-.324*	.122	.062	
Cortisol 9 PM	.360*	.355*	.132	.339*

* moderate effect size (*p* = ns); ** large effect size (p < .05)

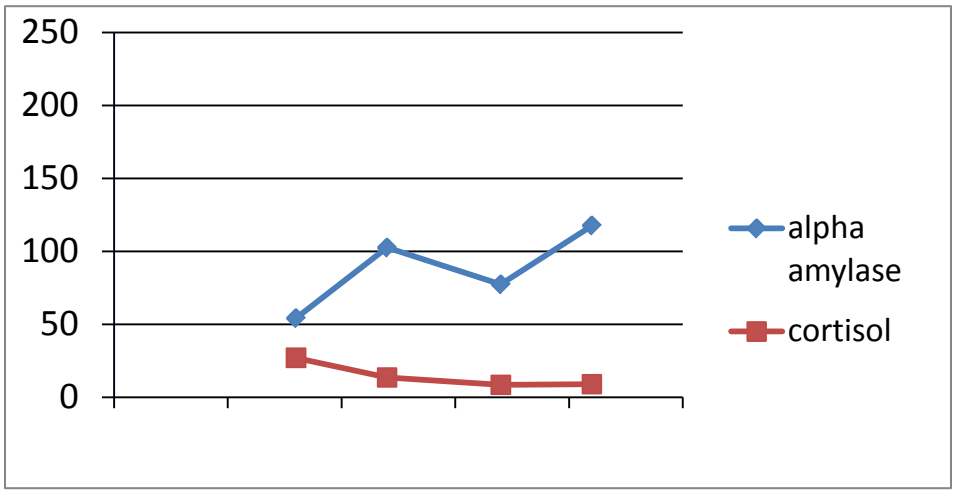
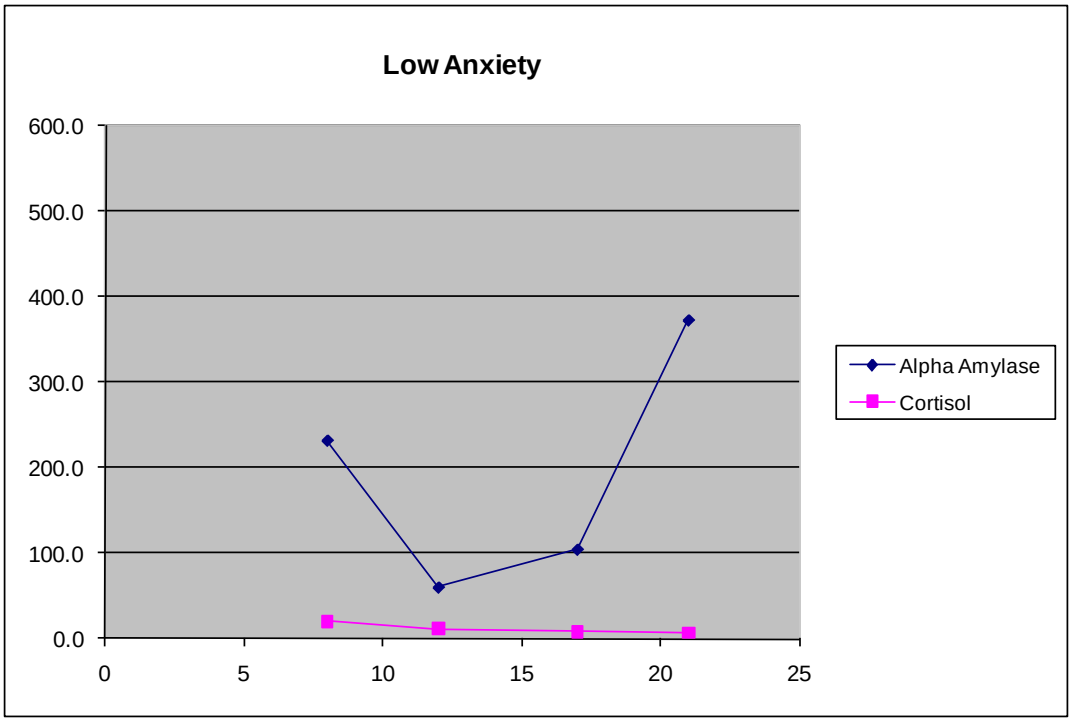


Figure 3-1. Mean alpha-amylase and cortisol slopes (*n* = 15)



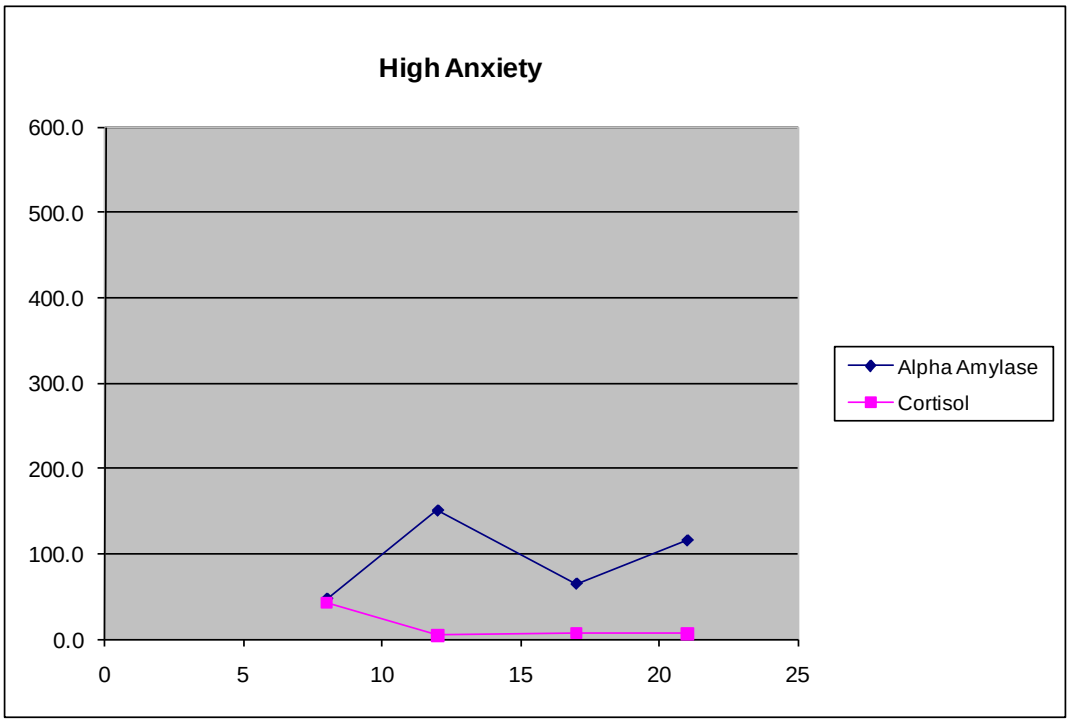


Figure 3-2. sAA and cortisol slopes for high anxiety versus low anxiety participants

CHAPTER 4
DISCUSSION

Previous research on cortisol has demonstrated a well-established diurnal pattern in response to psychological stress that is characterized by a flatter slope signaling abnormality (Nater et al. in 2007; Sephton et al., 2000). However, very little research has investigated the circadian rhythm of alpha-amylase in medical populations. Research has illuminated conflicting evidence for alpha-amylase diurnal responses to stress and sparse associations with psychological factors, suggesting several gaps in the current literature.

In sum, the study's main results indicated that alpha-amylase tended to have a more positive slope than cortisol, although the slopes were not statistically significantly different. Secondly, lower 8 AM levels of alpha-amylase were associated with higher 8 AM levels of cortisol. Finally, a relationship was found between alpha-amylase and cortisol slopes. These findings are consistent with the hypotheses 1a and 1b. Contrary to hypothesis 2a, perceived stress scores were not associated with alpha-amylase slope. Women with more MBMD anxiety-tension had less of an increase in alpha-amylase per unit time (i.e., a blunting effect). Additionally, increased SIGH-AD anxiety and MBMD anxiety-tension were both associated with a greater decrease in cortisol per unit time, which suggests a blunting of the cortisol response as well. While the blunting of alpha-amylase is contrary to hypothesis 2b, the blunting of the cortisol response is consistent with this hypothesis.

These findings suggest that diurnal patterns in this sample are similar to those in published research with healthy individuals. In particular, stress and anxiety may be associated with SNS-dependent alpha-amylase in women with endometrial cancer.

Results that emerged in the unexpected direction may demonstrate blunting of SNS activity under chronic stress, as is often seen with worn out HPA stress systems. Additionally, new discoveries of appropriate methodology for measurement of stress hormones, stress hormone profiles (particularly SNS-dependent markers) and their impact on other physiological and psychological processes continue to emerge in the literature. Results from this study highlight the limitations associated with how the literature currently operationalizes diurnal cortisol/alpha-amylase rhythm. Although the use of regression coefficients to quantify slopes is effective at providing estimations of change across over time, visualizing slopes using growth plots can be an important tool for confirming direction and rate of decline.

Exploratory analyses assessing relationships between stress/anxiety and additional markers of alpha-amylases/cortisol levels revealed interesting relationships; however, results have been interpreted with caution as the literature has not yet attempted to assess alpha-amylase using independent values and stress ratings at various time points. Women with more anxiety-tension showed lower AM alpha-amylase values and less total diurnal alpha-amylase output, suggesting a possible blunting of alpha-amylase throughout the day. With regard to cortisol, higher 8 AM values and a greater decrease in cortisol per unit time were both associated with greater perceived stress and greater anxiety-tension; however, stress and anxiety were not associated with total diurnal cortisol output. These findings may indicate that high levels of stress and anxiety result in a blunted, more abnormal cortisol response.

Study Limitations

Several limitations are present that would suggest caution when making interpretations of these results. Although the majority of published studies assessing

alpha-amylase have utilized small sample sizes, the statistical power associated with the results presented in this paper may be affected by a particularly small sample size. For example, a significant difference between the alpha-amylase slope and the cortisol slope may actually exist; however, there may not have been enough statistical power to detect this relationship. Additionally, there were significant differences in histology and race between those with complete psychosocial and physiological data and those with incomplete data. This appears to be explained by a relatively homogenous sample with regard to histology in the sample with incomplete data. For example, the breakdown of histology in the sample with incomplete data revealed that the majority of patients were classified as having endometrial adenocarcinoma, endometrioid type. In contrast, the sample with complete data demonstrated more heterogeneity across classifications. Another limitation associated with this study is the use of a modified version of the SIGH-AD. This decreases the ability to generalize results from this particular questionnaire to those findings from studies using the full questionnaire. Finally, distinctions between state and trait anxiety were not evaluated in this research, which may confound statistically significant relationships found between endocrine markers and anxiety as a result of participants' stable tendencies to exhibit anxious responses when faced with threatening situations. These limitations serve as important considerations for future research assessing physiological stress profiles and psychological correlates.

Future Directions

Future directions will emphasize the importance of increasing the sample size in order to replicate and expand on these findings. This would also allow for the utilization of linear mixed modeling as a means of determining change over time both between

and within individuals as well as a means of further comparing alpha-amylase and cortisol profiles. Most important is the need to further explore possible immune function correlates in order to gain a better understanding of how these independent stress systems directly affect immunity. A multitude of research has supported various interventions to reduce the effects of psychological stress (Reiche et al., 2005) leading to changes associated with reduced endocrine responses (Cruess et al., 2000) and ultimately improved cell mediated immunity among cancer patients. Expanding on research involving alpha-amylase will hopefully support the need for interventions targeting the sympathetic nervous system stress responses, thereby improving immune function at the cellular level and slowing the progression of cancer.

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BIOGRAPHICAL SKETCH

Stephanie Garey graduated with honors from Towson University in 2007, where she received a Bachelor of Science degree in psychology. During her four years at this institution, she worked as a research assistant on several research studies, including a longitudinal study assessing attachment styles and stable self-esteem evaluations. Stephanie also completed an undergraduate thesis on the associations between perceived social support, level of self-esteem, and depression in college students. During her senior year, Stephanie completed two internships, including a research assistantship at the Uniformed Services University of the Health Sciences (USUHS) and a clinical assistantship on the Neurobehavioral Unit at the Kennedy Krieger Institute at Johns Hopkins. Her academic excellence and her involvement in research, teaching, and clinical work was recognized by the Towson University Department of Psychology through her receipt of a Departmental Achievement Award.

After graduation, Stephanie worked as a full-time research coordinator on a NIH funded study assessing biobehavioral predictors of heart failure conducted jointly at the USUHS and University of Maryland Medical Center. Stephanie began attending graduate school at the University of Florida in the Department of Clinical and Health Psychology in August 2008. She is focusing her research on psycho-oncology, psychoneuroimmunology, and women's health. She is currently pursuing her doctorate in clinical psychology.