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Salivary alpha-amylase reactivity during discussion of the death of a spouse in recently bereaved elders

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SALIVARY ALPHA-AMYLASE REACTIVITY DURING DISCUSSION OF THE DEATH OF A SPOUSE IN RECENTLY BEREAVED ELDERS

by

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I would like to dedicate this document to Dr. Eric Bui, who took the time and effort to guide me during the study.

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ABSTRACT

In this study, bereavement-related biological and physiological stress reactivity during the discussion of a loss are evaluated by collecting saliva samples in patients aged 55 or more who have lost a spouse within the last 12 months. The concentration of salivary α-Amylase, a marker for stress, is measured. Grief symptoms are also examined in these individuals.

Background

Grief is a complex psychobiological phenomenon triggered by the loss of a loved one, and that comprises emotions (e.g., yearning, longing, intense feeling of sorrow/emotional pain), thoughts (e.g., thoughts or images about the deceased, difficulty accepting the death, guilt), and behaviors (e.g., avoidance of reminders, spending time with deceased belonging). Because bereavement is a major stressor, it has been suggested that the grief reaction is associated with increased sympathetic activity. Salivary α-Amylase levels have been proposed to be a reliable non invasive biomarker of sympathetic activity. The present study aims to examine salivary α-Amylase reactivity to discussion of the death of a spouse in older adults, and the relationship between this reactivity and grief symptom severity.

Methods

Participants were N=8 individuals who have lost a spouse within the last year (Mean(SD)age=72.8(7.5); 60% women); Mean(SD) time since death=10.6(2.5) months). Participants completed self-reported measures including the 19-item Inventory of Complicated Grief (ICG; total score ranges 0-76; Prigerson et al. 1995). The Mean(SD) ICG score was 30.5(11.5). Saliva samples were collected using cotton swabs and Salivettes at four time points during the screen: right before discussion of the loss (T1), right after discussion of the loss (T2), before screening for psychiatric disorders (T3), and at the end of the screening (T4). Salivary α-Amylase samples were frozen and assayed using a kinetic enzyme assay kit specifically designed and validated for the kinetic measurement of salivary α-Amylase activity. A repeated measure ANOVA, with α-Amylase as the dependent variable, and time as the within subject repeated factor were conducted to examine differences in α-Amylase activity across time points. Further, we examined the association between rise in α-Amylase activity (change between T1 and T2) and ICG scores.

<u>Results</u>

The ANOVA revealed a marginally significant main effect of time ($F(3,20)=2.74$, $p=0.07$), with α-Amylase activity being more elevated right after discussion of the loss (T2; Mean (SD)=194.9(79.4) µg/dL) compared to the other time points (T1: 140.00(77) µg/dL; T3: 140.5(56) µg/dL; T4: 143.0(70.3) µg/dL). Further, the rise in α-Amylase activity between T1 and T2, was marginally significantly associated with greater grief symptoms ($r=0.69$, $p=0.08$).

<u>Conclusion</u>

Our preliminary findings suggest that discussion of the loss of a spouse may increase salivary α-Amylase activity, reflecting a potential increase in sympathetic activity. Targeting loss-related sympathetic reactivity might be an interesting avenue to decrease grief related distress and impairment. Limitations for this study include a small sample size and the absence of a real control condition. Future research examining sympathetic activity in grief and its relationship with persistent complex bereavement disorder pathophysiology is warranted.

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LIST OF ABBREVIATIONS

ACh .... Acetylcholine

ACTH .... Adrenocorticotropic hormone

ANOVA .... Analysis of Variance

CG .... Complicated Grief

CGI-S .... Clinical Global Impression-Severity

Cort .... Cortisol

CRH .... Corticotropin-Releasing Hormone

DSM-5 .... Diagnostic and Statistical Manual of Mental Disorders

Epi .... Epinephrine

HPA .... hypothalamic-pituitary-adrenal

ICG .... Inventory of Complicated Grief

IRB .... Institutional Review Board

Ne .... Norepinephrine

NO .... Nitric Oxide

PCBD .... Persistent Complex Bereavement Disorder

PGD .... Prolonged Grief Disorder

PNS .... Parasympathetic Nervous System

PTSD .... Post Traumatic Stress Disorder

SCI-CG .... Structured Clinical Interview for Complicated Grief

SCID .... Structured Clinical Interview

SMART .... Stress Management and Resiliency Training

SNS .... Sympathetic Nervous System

3RP... Relaxation Response Resiliency Program

INTRODUCTION

Types of Grief and Symptoms

Death is an inevitable natural phenomenon. Different cultures throughout time have unique perspectives and ideations about death. However, a universal challenge is coping with the death of a loved one such as a family member, relative, or close friend, as this can be one of the most painful events to experience. Often times, grief can have a significant negative effect on the daily life and routines of individuals. Grief is a complex psychobiological phenomenon (LeBlanc, 2016) triggered by the loss of a loved one, comprising of complex emotions (e.g., yearning, longing, intense feeling of sorrow/emotional pain), thoughts (e.g., thoughts or images about the deceased, difficulty accepting the death, guilt), and behaviors (e.g., avoidance of reminders, spending time with deceased belonging). For most individuals, the intensity of the grief will subside in the following weeks or months and they will be able to return to their daily routines and normal functions. However, some individuals may experience a more severe case of grief called Persistent Complex Bereavement Disorder (PCBD) also known as Complicated Grief (CG) or Prolonged Grief Disorder (PGD). This disorder is listed in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as a subtype of "Other Specified Trauma-and Stressor-Related Disorders" with explicit criteria listed under "Conditions for Further Study." Complicated Grief is a form of prolonged (more than six months) (Robinaugh, 2014) grief reaction that increases suicidality (Latham and Prigerson 2004) and morbidity (Prigerson et al. 1997) among survivors adding a major health burden to society and to the millions of individuals who suffer from it. Recent research conducted on Complicated Grief has reported that loss-related emotional pain and loss-related yearning were the hallmark features of the grief reaction (Bui et al, 2015). About 7% of bereaved individuals will experience Complicated Grief with marked distress and functional impairment for years after the death (Kersting et al, 2011). Symptoms have shown to be associated with lower quality of life, significant sleep disruption, negative health consequences, and suicidal ideation (Marques et al, 2013).

Stress

Stress is the process through which environmental demands tax or exceed the adaptive capacity of an organism, resulting in distress (Park et al. 2013). Stress may manifest as psychological and/or biological changes that place individuals at risk for disease such as heart diseases and diabetes (McEwen, 1998). Common life stressors include work, education, marriage, and finance. When considering the stress that individuals feel when a spouse passes, the premise dates back to evolutionary times. Humans have learned that bonding with others and being surrounded by others allowed for a higher chance for survival as individuals helped one another in different crucial tasks including gathering food and protecting one another from dangers (Brown et al. 2009). It has been wired in our biology that losing a spouse threatens our safety and causes us to fear for our own survival (Bowlby, 1969). Because we feel that we are in danger, our bodies react accordingly to protect ourselves or to keep us safe which triggers our stress response (Shear & Shair, 2005) as shown in Figure 1. However, loss is not something that we can protect ourselves from or something that we can escape. Even though our brains have triggered us to take action, we cannot undo a loss. This leads to an immense amount of stress on the body. Because bereavement is a major stressor, it has been suggested that the grief reaction is associated with increased sympathetic activity (O'Connor et al, 2007).

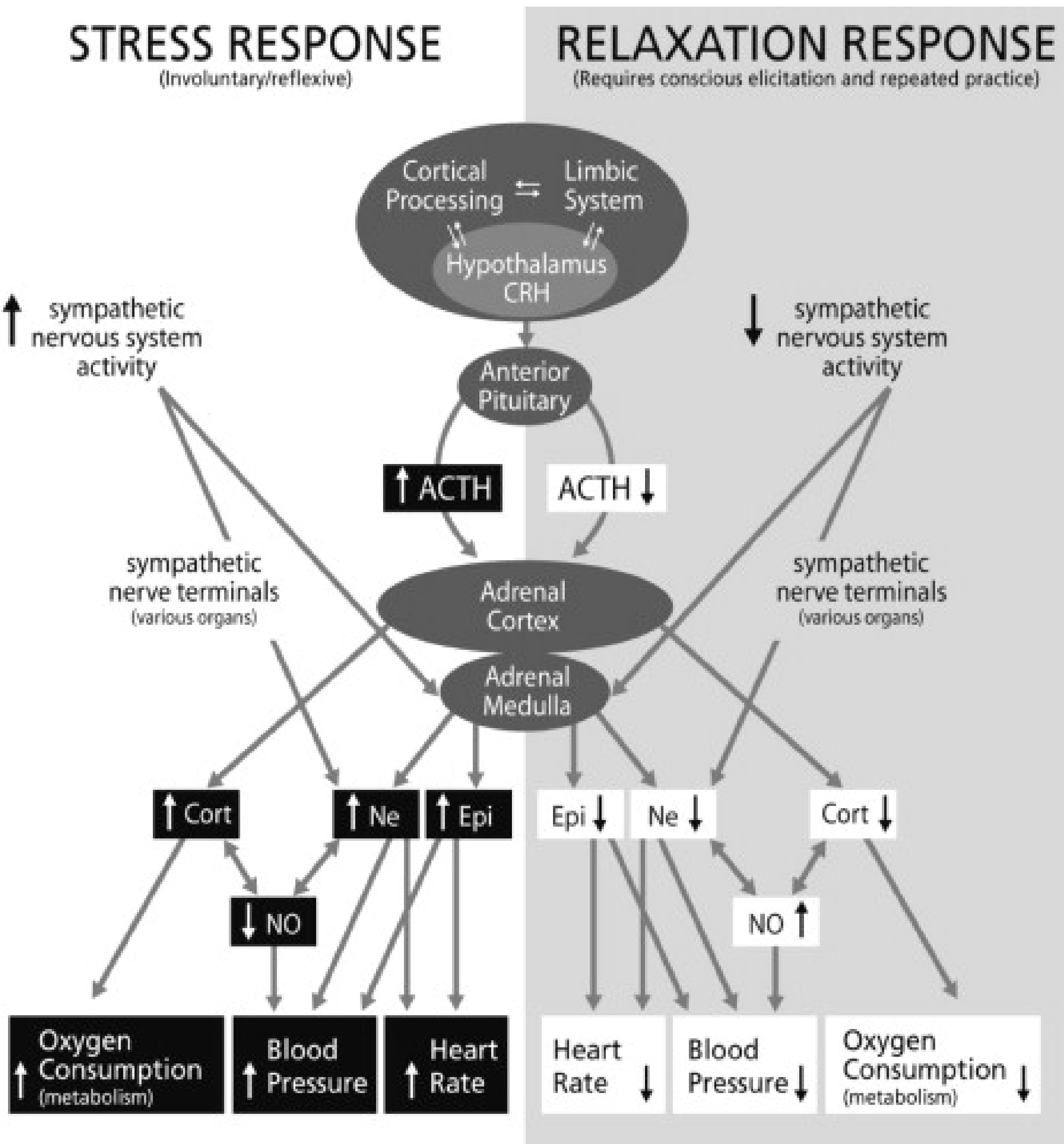

FIGURE 1.
Stress Response and Relaxation Response.
HPA Axis that demonstrates how a stress response or relaxation response occurs in the body. The hypothalamus, a part of the limbic system that supports functions including emotion and behavior, links the nervous system to the endocrine system through the pituitary gland. Once the hypothalamus is stimulated, it synthesizes and secretes releasing hormones which stimulate or inhibit the secretion of pituitary hormones by the pituitary gland. The increase of pituitary hormones or decrease of pituitary hormones has a direct effect on the hormones secreted by the adrenal gland which consist of the adrenal cortex and adrenal medulla. The increase or decrease in hormones produced by the adrenal gland has a direct effect on the function of different body organs such as oxygen consumption, blood pressure, and heart rate, that are associated with a stress response or relaxation response.
A change in sympathetic nervous system activity can also have a direct effect on the adrenal medulla and the norepinephrine that the adrenal medulla produces.
Dusek and Benson (2009) Minnesota Medicine.

Nervous System

The physiological effects due to stress are controlled by the nervous system. The nervous system coordinates the voluntary and involuntary actions and transmits signals to and from different parts of the body. Two components of the nervous system are the parasympathetic nervous system (PNS) and sympathetic nervous systems (SNS) which work together to maintain homeostasis (Pocock, 2006). The parasympathetic system conserves energy as it slows the heart rate, increases intestinal and gland activity, and relaxes sphincter muscles in the gastrointestinal tract. It is known as the rest-and-digest response (McCorry, 2007). The sympathetic nervous system is complementary to the parasympathetic nervous system and stimulates activities associated with the fight-or-flight response (Cannon, 1932). The effects of the sympathetic nervous system and parasympathetic nervous system on different organs are shown in Figure 2.

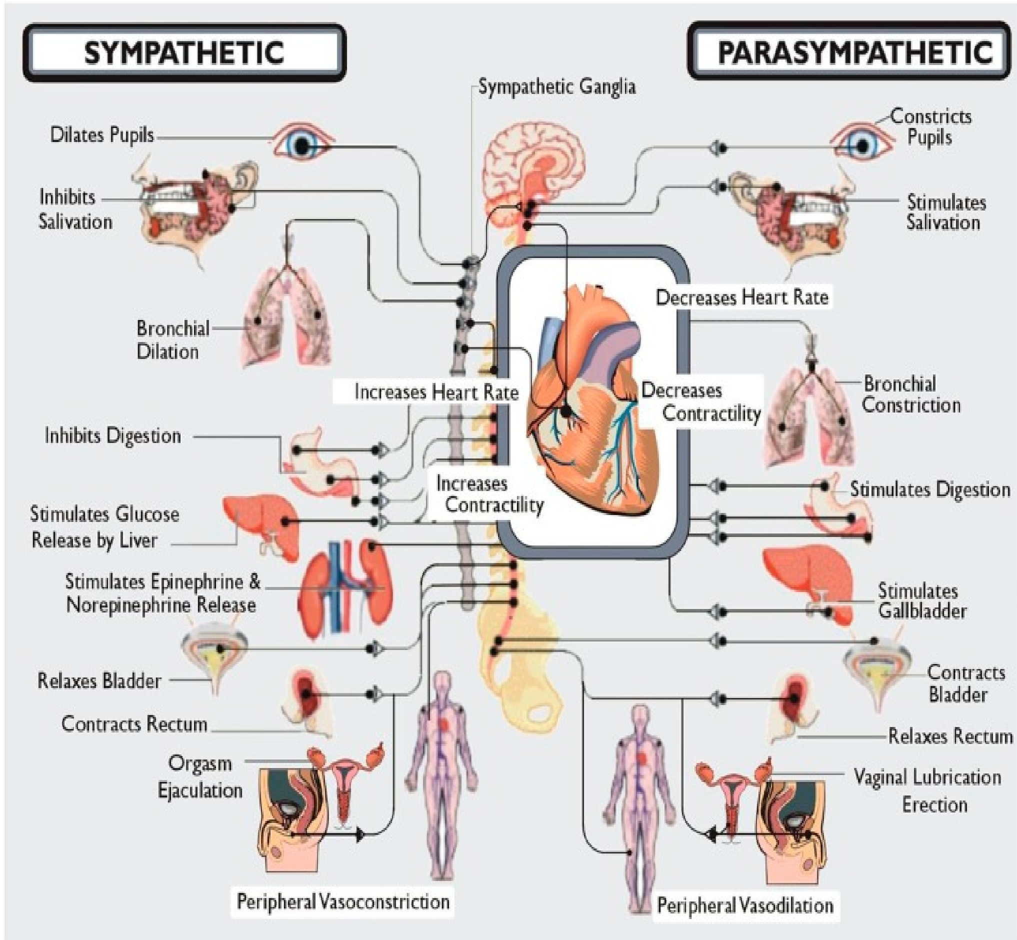

Figure 2.
Diagrammatic illustration of the role of the two arms of the autonomic nervous system.
The effects of the sympathetic nervous system and parasympathetic nervous system are shown. In general, the two branches of the nervous system have opposite effects on organ functions.
Vinik (2012) Frontiers in Endocrinology.

Types of Neurons in the Sympathetic Nervous System

There are two neurons in the sympathetic nervous system: the pre-ganglionic and post-ganglionic. The shorter pre-ganglionic neuron travels to the ganglion that synapses with the post-ganglionic neuron. Pre-ganglionic neurons release the neurotransmitter acetylcholine that activates nicotinic acetylcholine receptors on post-ganglionic neurons. Once activated, a majority of post-ganglionic neurons release the catecholamine norepinephrine which activates adrenergic receptors on peripheral target tissues as shown in Figure 3. This activation leads to the physiological effects associated with the sympathetic nervous system (Drake et al. 2015).

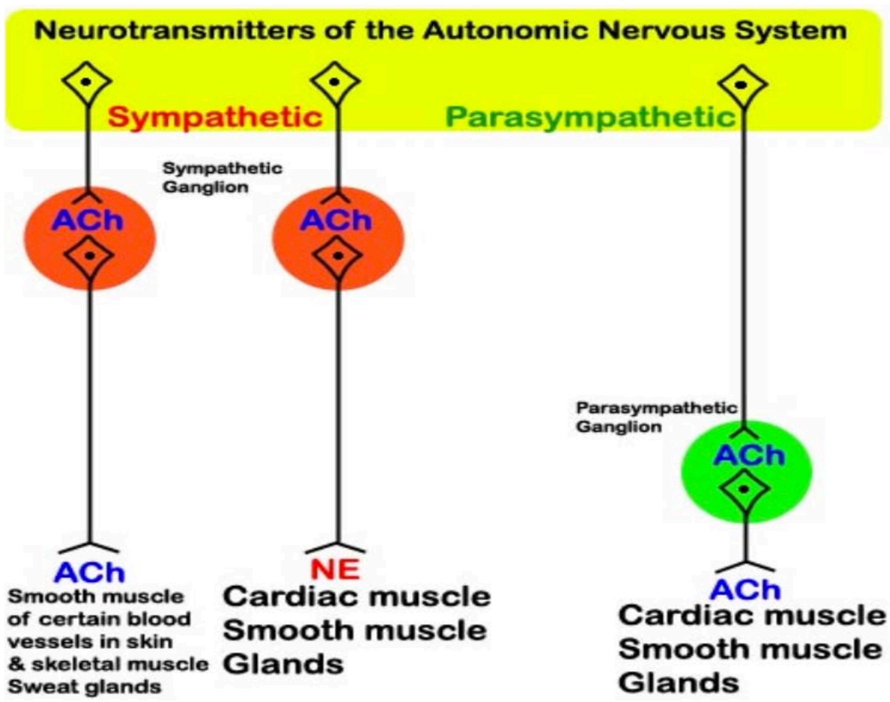

Figure 3.
Pre-ganglionic and Post-ganglionic neurons.
The pre-ganglionic neuron originates in the brainstem or the spinal cord and is shorter in the sympathetic nervous system than the parasympathetic system. Both pre-ganglionic neurons release acetylcholine which activates the ganglion cells, or post-ganglionic neurons which lies outside of the central nervous system. The post-ganglionic neuron release acetylcholine which effects cardiac muscle, smooth muscle, and glands. Post-ganglionic neurons in the sympathetic nervous system can release either acetylcholine or norepinephrine which effect the effector organs.
(2017) Michigan State University College.

Salivary α-Amylase

One of the stimulatory effects of the sympathetic nervous system is the release of the salivary enzyme, α-Amylase (Nater et al. 2009) shown in Figure 4. Salivary α-Amylase, also known as 1,4-α-D-Glucan glucanohydrolase, is a protein enzyme that hydrolyzes alpha bonds of large, alpha-linked polysaccharides such as starch and glycogen into the monosaccharide glucose, and disaccharide maltose (Schenkels et al. 1995).

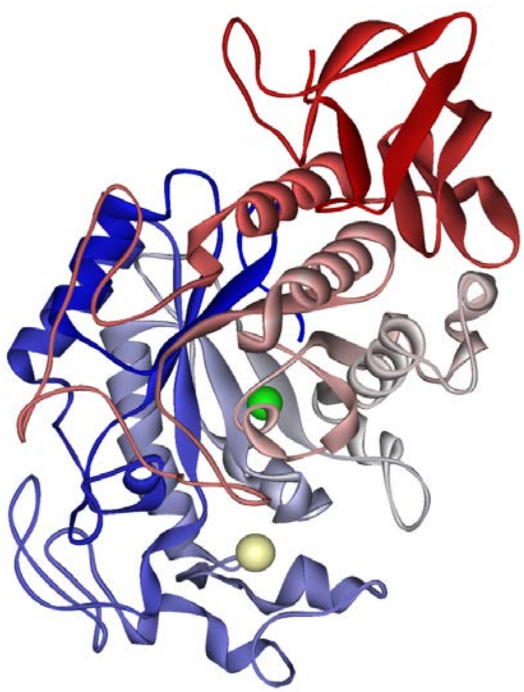

Figure 4.
Salivary α-Amylase molecule
A 3-dimensional picture of the enzyme molecule that hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen into glucose and maltose. It is the major form of amylase found in humans and other mammals. *(2017) Salimetrics.*

Glucose and Maltose both have free aldehyde groups and have the ability to form strong hydrogen bonds with sweet taste receptors which activate the receptors and allow us to taste sweetness (Temussi, 2007) shown in Figure 5.

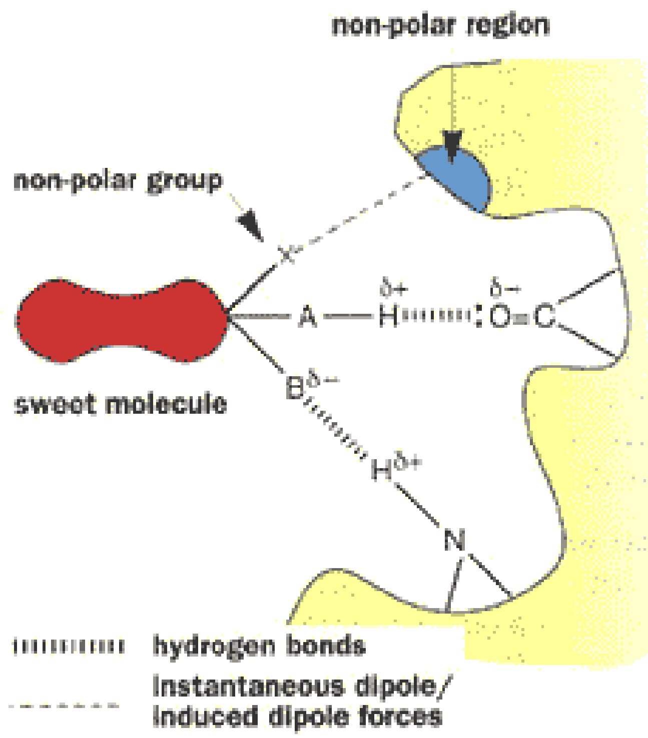

Figure 5.
Sugar Molecule Binding To Receptor.
The sugar molecule forms different bonds with the sweet receptor including hydrogen bonds, dipole bonds and non-polar bonds. These receptors are found in the circumvallate papillae and the foliate papillae near the back of the tongue and the roof of the mouth. Once the sugar molecule binds to the receptor and the receptor is activated, the cell synapses with the chorda tympani and glossopharyngeal nerves to send signals to the brain that a sweet substance is being consumed.
(2017) Unilever Education.

Salivary α-Amylase has a daily secretion pattern of a sharp decrease in the first hour after awakening followed by an increase throughout the day. Momentary stress are relatively independent to diurnal salivary α-Amylase but chronic stress and mood is significantly associated (Nater et al., 2007). Alpha-Amylase has been shown to be released by acinar cells in the salivary gland when activated by the autonomic sympathetic nervous system (Nater et al, 2006). It is not controlled by the activity of the limbic hypothalamic-pituitary-adrenal (HPA) axis such as the glucocorticoid, cortisol (Nater et al. 2009). The HPA axis, a major neuroendocrine system that controls reactions to stress is another method to evaluate a stress response (Bellavance & Rivest, 2014). However, α-Amylase has a quicker response to a stress stimuli and α-Amylase is a non-invasive marker (Thoma et al, 2012) as it is present in saliva whereas cortisol is present in blood. Alpha-Amylase concentrations are predictive of plasma catecholamine levels, particularly norepinephrine, under stressful conditions (Chatterton Jr et al, 1996). Alpha-Amylase is an important and accurate non-invasive marker for both physical and psychological stress (Thoma et al, 2012).

Summary

Data suggest that bereavement is a stressor that will elicit a stress response in individuals. The stress response may be triggered by reminders of the death and can be measured by salivary α-Amylase. However, the mechanism behind being reminded of the death generating a stress response remains unclear. Studies have suggested that grief-related panic symptoms may be prevalent among grieving individuals (Bui et al, 2014) indicating that thoughts about the death may trigger the activation of the sympathetic system. Being able to confirm this may lead to a better understanding of the grieving process in recently bereaved elders and the possibility of finding more effective treatments for grieving individuals.

AIMS/OBJECTIVES

The purpose of this study is to examine the sympathetic reactivity during reminder of the recently bereaved subject's loss by examining the quantity of salivary α-Amylase produced by subjects during the discussion of the loss of a spouse. The magnitude of reactivity associated with grief symptom severity is also assessed and the association between salivary α-Amylase reactivity to discussion of loss and grief symptom severity is examined.

METHODS

Participants

Bereaved adults were recruited through advertisement and referral. Inclusion criteria included male or female outpatients 55 years of age or greater, widowed (spouse or partner) in the past year; bereavement-related distress defined by CGI-S scale greater than three regardless of presence of psychiatric diagnosis; being psychotropic medication free for more than two weeks or on a stable dose for more than six weeks prior to baseline; and willing to comply with study protocol and ability to participate in the intervention. Exclusion criteria included inability to understand study procedures or to provide informed consent; serious medical illness or instability likely requiring hospitalization within 6 months; current active suicidal ideation; lifetime history of psychotic disorder; alcohol or substance abuse or dependence within the past six months; practice of yoga, meditation, guided imagery or other mind-body techniques eliciting the Relaxation Response (Figure 1), once per week for 45 minutes or more within past three months; current medical condition that would preclude safe and effective practice of the intervention. Inclusion and Exclusion Criteria are shown in Table 1.

Table 1. Inclusion / Exclusion Criteria (Widowed Participants)

Inclusion Criteria	***Rationale***
Male or female outpatients aged ≥55, widowed (spouse or partner) in the past year	Population studied
Clinically significant bereavement-related distress defined by CGI-S scale ≥ 3 regardless of presence of psychiatric diagnosis*	Population studied
Psychotropic medication free (≥2 wks) or stable dose (≥6 wks) prior to baseline	Confound

Willingness to comply with study protocol and ability to participate in the intervention	Ethical concern, Feasibility
Exclusion Criteria	***Rationale***
Inability to understand study procedures or to provide informed consent	Ethical concern
Serious medical illness or instability likely requiring hospitalization within 6 months	Feasibility, safety
Current active suicidal ideation (assessed with item 12 of the QIDS)	Safety
Lifetime history of schizophrenia, bipolar disorder, or other psychotic disorder	Confound
Alcohol/substance abuse or dependence within the past 6 months	Confound
Practice of yoga, meditation, guided imagery or other mind-body techniques eliciting the RR, once per week for 45 min or more within past 3 months	Confound
Current medical condition that would preclude safe and effective practice of the intervention (physical injuries and musculoskeletal problems)	Ethical concern, safety
**Note: Using a multidimensional approach, we will not require any specific diagnosis, similar to an ongoing study (R01MH101486). Further, somatic complaints will be factored into the CGI-S.*	

Data collection

Psychopathological measures (shown in appendix) were evaluated by the Structured Clinical Interview (SCID) and the Structured Clinical Interview for Complicated Grief (SCI-CG) (Bui et al, 2015). The SCID is used to make major DSM-5 Axis I disorder diagnoses. A SCID summary is included in the appendix. The SCI-CG is a 31-item clinician-administered instrument to assess specifically Complicated Grief symptoms. Score ranges from 31-84 with higher scores indicating higher symptom severity. Participants completed self-reported measures including the 19-item Inventory of Complicated Grief (ICG) (Prigerson et al, 1995) which measures pathological grief concerning the subject's bereavement-related thoughts and behaviors. Scores range from 0 to 76 in which higher scores indicated higher grief symptom severity.

Saliva was collected with salivettes at four different time points; before the discussion of the loss, right after the discussion of the loss, before screening of psychiatric disorders, and at the end of the screen. This allowed for the comparison of α-Amylase concentrations during discussion of the loss to neutral situations. Saliva samples were sent to Salimetrics to have salivary α-Amylase quantitatively measured.

Procedures

Patients were contacted and invited through an IRB-approved recontact list that included bereaved participants from prior studies conducted by the Center for Anxiety and Traumatic Stress Disorders and Complicated Grief Program. Additional participants were self-referred, recruited online, or through IRB-approved advertisements on public transportation, or locations throughout the surrounding area. Patients were reminded the day before the visit not to have alcohol or nicotine for 12 hours before the visit, food or exercise for two hours before the visit, and anything to drink an hour before the visit. Once patients arrive, they first provide signed consent and participate in the screening in which the psychiatrist determines if they qualify for a larger IRB-approved study aiming to evaluate the effect of Stress Management and Resiliency Training: A Relaxation Response Resiliency Program on grief. If eligible for the study, personal scripts are created from each individual's experience. Saliva samples were collected using cotton swabs and Salivettes. Patients were instructed to keep the swab in place for 2 minutes under the tongue. Saliva Sample 1 was collected right before discussion of the loss (T1).

Saliva Sample 2 was collected right after discussion of the loss (T2). Saliva Sample 3 was collected before screening for psychiatric disorders (T3). Saliva Sample 4 was collected at the end of the screening (T4) (Figure 6). The average time between when Sample 1 and Sample 2 were taken was 28 minutes with a standard deviation of 5.3. The average time between Sample 2 and Sample 3 was 14 minutes with a standard deviation of 1.2. The average time between Sample 3 and Sample 4 was 28 minutes with a standard deviation of 6.3.

Patients complete the ICG and Quick Inventory of Depressive Symptomatology Questionnaires after meeting with the physician. Saliva samples were centrifuged (3000rpm for 15 min) with supernatant pipetted into tubes and frozen at -70°C until processing. Salivary α-Amylase was assayed using a commercially available kinetic reaction assay (Salimetrics, State College, PA).

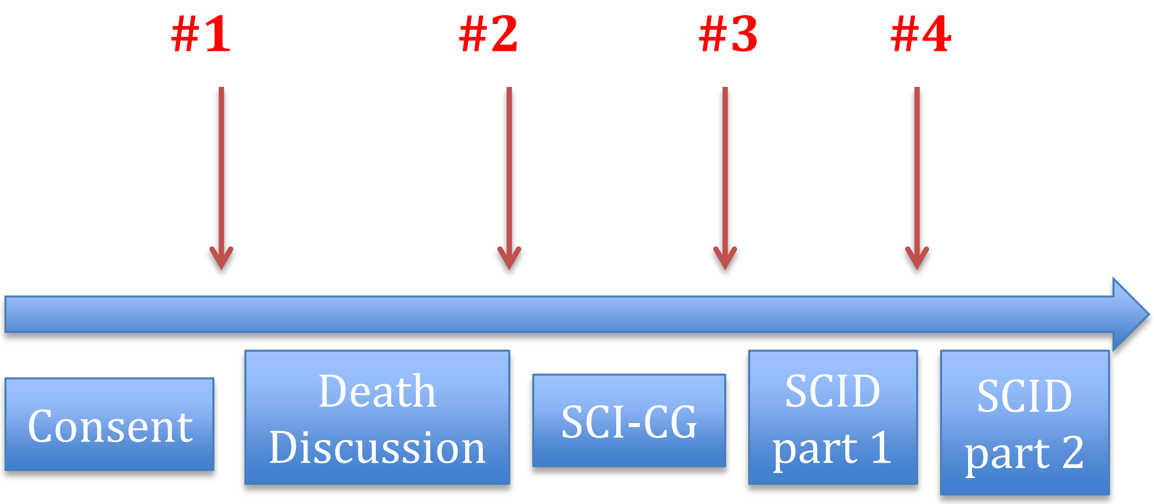

Figure 6. Saliva Sample Collection timeline

Saliva Sample 1 was collected after the consent form was filled out right before discussion of the death. Saliva Sample 2 was collected after discussion of the death. Saliva Sample 3 was collected after the Structured Clinical Interview for Complicated Grief was complete. Saliva Sample 4 was collected after the Structured Clinical Interview in which subjects were screened for psychiatric disorders.

α-Amylase Assay

A kinetic enzyme assay kit specifically designed and validated for the kinetic measurement of salivary α-Amylase activity was used. A chromogenic substrate, 2-choro-p-nitrophenol linked with maltotriose is added to α-Amylase and the reaction produces 2-chloro-p-nitropheniol which can be spectrophotometrically measured at 405 nm.

Data analyses

A repeated measure ANOVA, with α-Amylase as the dependent variable, and time as the within subject repeated factor was conducted to examine differences in α-

Amylase across time points to determine if α-Amylase levels increase during discussion of a loss.

The association between the variables, rise in α-Amylase activity (change between T1 and T2) which allowed α-Amylase levels to be compared during discussion of the loss to baseline level, and ICG scores which reflect grief symptom severity was evaluated. This determined if there was a significant correlation between grief symptom severity and change in α-Amylase levels during discussion of the loss.

Further, the Area Under the Curve of α-Amylase with respect to increase was calculated from the equation shown in Figure 7.

$$AUC_I = \left(\sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2} \right) - \left(m_1 \cdot \sum_{i=1}^{n-1} t_i \right)$$

Figure 7. Area Under the Curve with respect to increase equation
Formula used to calculate the Area Under the Curve with respect to increase. m_i denotes the single measurements, t_i denotes the time distance between the measurements, and n denotes the total amount of measurements.
(2003) Pruessner et al.

The Area Under the Curve can be used to estimate changes of secretion over a specific time period (Pruessner, 2003). The association between Area Under the Curve and ICG scores was analyzed as an alternate method to evaluate the correlation between grief symptom severity and change in α-Amylase levels during discussion of the loss.

All analyses were conducted using Stata version 12, and the level of statistical significance was set to 0.05 (two-tailed).

RESULTS

The basic descriptive statistics of the sample is shown in Table 1. The mean age was 72.8 with a standard deviation of 7.5 years. The age range was from 63 to 86. 60% of the subjects were female in the study. The mean ICG Score was 30.5 with a standard deviation of 11.5. ICG scores ranged from 12 to 45. The average time since death was 10.6 months with a standard deviation of 2.5. The range for average time since death was 7.1 months to 12.7 months. The causes of the deaths included cancer, heart diseases, and Amyotrophic lateral sclerosis.

Table 2. Demographics of n=8 Individuals with Complicated Grief

n=8	Mean (SD) or % (n)
Age, years, Mean (SD)	72.8 (7.5)
Gender, Female, % (n)	60% (5)
ICG Score, Mean (SD)	30.5 (11.5)
Time Since Death, Months, Mean (SD)	10.6 (2.5)

Salivary α-Amylase Reactivity During Discussion of Loss

Examination of Salivary α-Amylase levels at different time points showed a change in enzyme quantity. Alpha-Amylase activity was more elevated right after discussion of the loss (T2; Mean (SD)=194.9(79.4) µg/dL) compared to the other time points (T1: 140.00(77) µg/dL; T3: 140.5(56) µg/dL; T4: 143.0(70.3) µg/dL). This elevation in enzyme production by the subjects suggest that they experience stress responses while discussing the loss of their loved ones. The ANOVA revealed a marginally significant main effect of time ($F(3,20)=2.74$, $p=0.07$). Alpha-Amylase quantities decreases back to around baseline level (T1) at T3 after subjects complete the Structured Clinical Interview for Complicated Grief and remains stable while subjects were being screened for psychiatric disorders (T4). This decrease in α-Amylase level suggest that there was no longer a stress response once the subjects started discussing matter not pertaining to the loss. The results of α-Amylase levels at different time points are shown in Figure 8.

The range of α-Amylase levels at T1 is from 12 µg/dL to 217.1 µg/dL. The range at T2 is from 50.8 µg/dL to 312.9 µg/dL. The range at T3 is from 31.5 µg/dL to 214.2 µg/dL. The range at T4 is from 48.9 µg/dL to 270.8 µg/dL. These ranges show that the quantity of α-Amylase produced by each individual varies greatly.

The average time between when Sample 1 and Sample 2 were taken was 28 minutes with a standard deviation of 5.3. The average time between Sample 2 and Sample 3 was 14 minutes with a standard deviation of 1.2. The average time between

Sample 3 and Sample 4 was 28 minutes with a standard deviation of 6.3. Different subjects had his or her saliva samples collected at different times during the day.

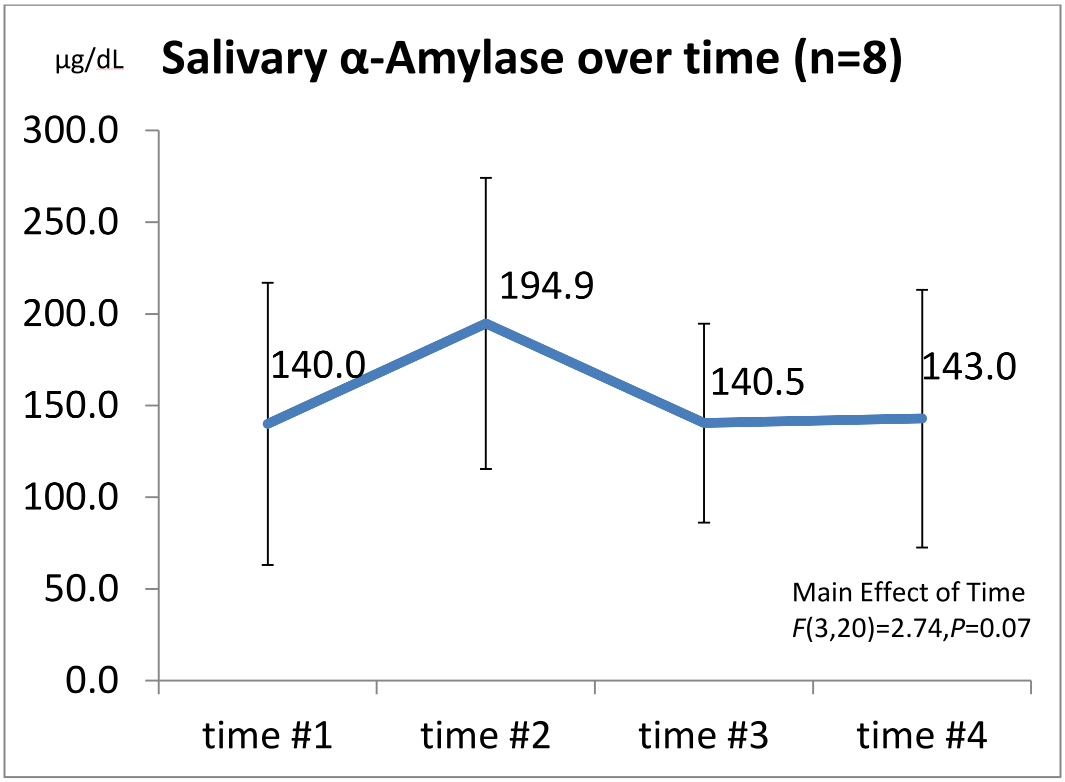

Figure 8. Quantity of salivary α-Amylase at the 4 different time points graph
The average enzyme concentration at each time point is shown measured in µg/dL at each timepoint. Time #1 is right before discussing the loss. Time #2 is right after discussing the loss. Time #3 is before the screen for psychiatric disorders. Time #4 is at the end of the screen. Also displayed are standard deviation bars that show both 1 standard deviation above and below the averages.

Correlation Between Change of Salivary α-Amylase During Discussion of Loss and Grief Symptom Severity

Next, the two variables, change in α-Amylase between T1 and T2 and grief symptom severity measured by the Inventory of Complicated (ICG) score were examined to determine if subjects experiencing greater grief symptoms had greater increases in α-Amylase levels while discussing their loss. The ICG's reliability in assessing grief symptoms in individuals is evident by the high internal consistency and test-retest reliabilities. Individuals with ICG scores greater than 25 were significantly more impaired in social, general, mental, and physical health functioning and pain than those with scores below 25 (Prigerson et al, 1995). The average ICG score was 30.5 with a standard deviation of 11.5 which means that most subjects were experiencing more severe grief symptoms. The lowest ICG score was 12 and the highest ICG score was 45. The smallest change in α-Amylase between T1 and T2 was -34.1 μg/dL and the greatest change in α-Amylase between T1 and T2 was 115.1 μg/dL. Obtaining a negative value for the change in α-Amylase between T1 and T2 was interesting as this suggest that this particular subject did not have a stress response while discussing the loss. The rise in α-Amylase activity between T1 and T2 was marginally significantly associated with greater grief symptoms (r=0.69, p=0.08).

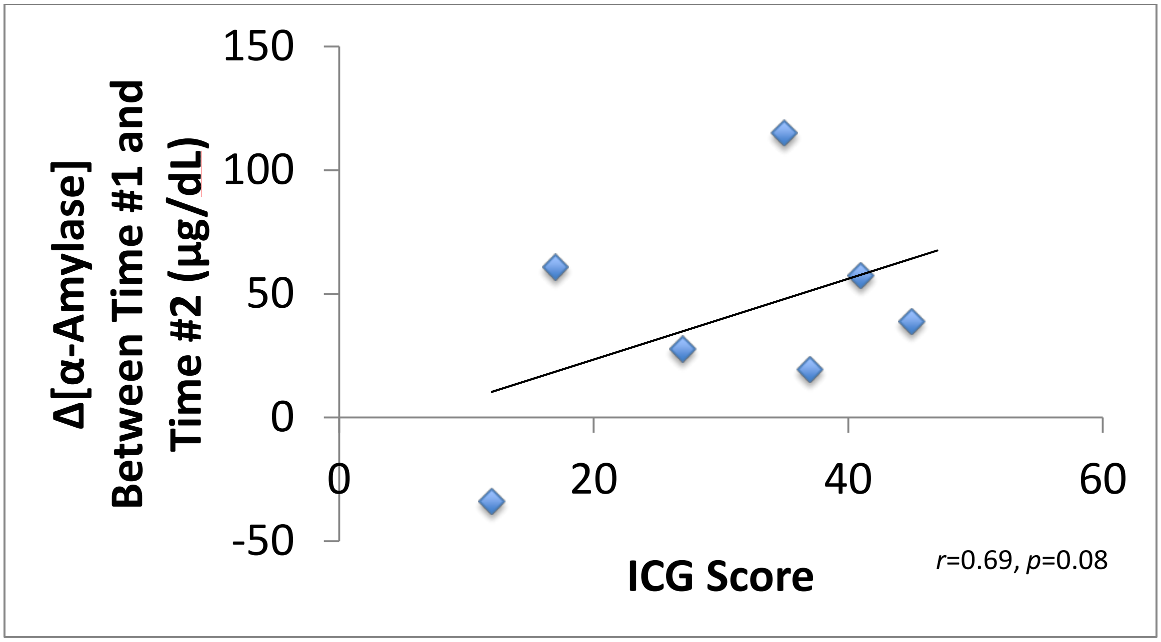

Figure 9. Change in α-Amylase between T1 and T2 and ICG Score graph
The change in α-Amylase between T1 and T2 shown measured in µg/dL. ICG scores showing grief symptom severity are recorded with the lowest possible score being 0 and the highest possible score being 76.

Alternative Correlation Between Change of Salivary α-Amylase During Discussion of Loss and Grief Symptom Severity

The correlation between the Area Under Curve and ICG score was examined as an alternative method to investigate the correlation between Change of Salivary α-Amylase during discussion of the loss and grief symptom severity. The Area Under Curve variable was used because it is related to the sensitivity of the system, pronouncing changes over time (Pruessner, 2003). The Area Under Curve reflects both the intensity and the duration of the stress response. The Area Under Curve with respect to the increase is calculated with reference to the first value instead of the distance from zero for all measurements (Pruessner et al, 2003). The average Area Under Curve value was 60.3 with a standard deviation of 60.2. The lowest Area Under Curve value was -59.95 and the highest Area Under Curve value was 168. Obtaining a negative value for the Area Under Curve was interesting as this suggest that this particular subject did not have a stress response while discussing the loss. The average ICG score was 30.5 with a standard deviation of 11.5 which means that most subjects were experiencing more severe grief symptoms. The lowest ICG score was 12 and the highest ICG score was 45. The Area Under Curve with respect to increase and ICG Score was not significant.

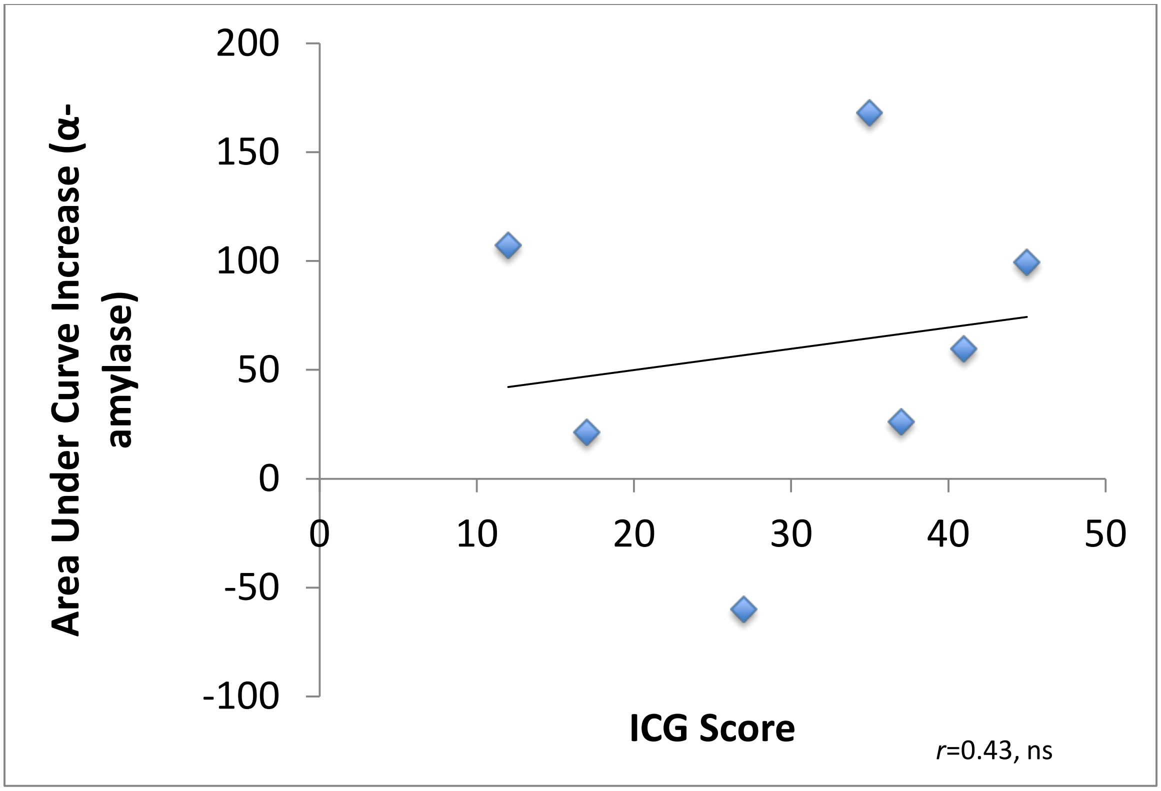

Figure 10. Area Under Curve with respect to increase and ICG Score graph
Area Under Curve with respect to increase shows the intensity and duration of the change in α-Amylase. ICG scores show grief symptom severity with the lowest possible score being 0 and the highest score being 76.

DISCUSSION

Preliminary findings suggest that discussion of the loss of a spouse may increase salivary α-Amylase activity, reflecting a potential increase in sympathetic activity. While subjects answered the SCID screening questions and the SCI-CG cover sheet questions, both of which required subjects to discuss the memory and moment of the death, salivary α-Amylase concentrations on average increased. This demonstrates the physiological manifestations of a psychological state and shows that there was a stress response and an increase in sympathetic activity during the discussion of the death. While subjects answered SCI-CG and SCID questions, enzyme levels gradually decreased back to baseline level as expected. This means that the subjects' sympathetic activity decreased. The increase in concentration of α-Amylase was also greater in individuals that had greater grief symptoms, as reflected by their ICG Score. This suggests that the more grief one is experiencing, the more stressed they become, which results in higher sympathetic activity. However, this could not be confirmed by the results obtained when examining the correlation between Area Under Curve with respect to increase and ICG score as those results were non significant.

Studies have also demonstrated that the sympathetic nervous system is chronically activated in individuals with Posttraumatic Stress Disorder (PTSD). In one study, saliva samples were collected from sexually abused adolescent girls and compared with the saliva samples from healthy subjects. Results showed total PTSD symptoms and severity were associated with higher overall morning levels of salivary α-Amylase concentrations (Keeshin et al. 2015). In another study, salivary α-Amylase diurnal profiles between

Bosnian War refugees with PTSD and a healthy control group were compared. The profile of salivary α-Amylase was the opposite between the group of subjects with PTSD and the healthy controls as there was an increase level of enzyme in PTSD patients and a decrease for the healthy controls (Thoma et al. 2012). This may suggest that these two psychopathologies, PTSD and grief share the same common stress response. The underlying cause for the stress response can be further investigated.

Everyone will experience the loss of a loved one. This was an intriguing study because, surprisingly, this was the first time that stress reactivity was examined during the discussion of the loss of a spouse. It would be interesting to determine whether thinking about the loss will also lead to the same reactivity as discussing the loss. Normally grief does not need clinical intervention as most bereaved individuals are resilient and do not need mental health treatment. However, knowledge about improved treatment options for those in who experience a more severe and chronic form of grief is crucial to help reduce the pain and agony that these grieving individuals suffer from such as dysfunctional thoughts, maladaptive behaviors and emotion dysregulation. The stress caused by bereavement can also lead to or worsen other physical or mental disorders such as depression, panic disorder, post-traumatic stress disorder, greater suicidality and sleep disorders.

This paper describes the rationale and methods of evaluating stress and grief symptoms in recently bereaved elders and there may be some evidence that grief is correlated with stress reactivity. The findings of this study are expected to be relevant for future studies targeting loss-related sympathetic reactivity, which might be an interesting avenue to decrease grief related distress and impairment including impairment in work and social function, sleep disturbance, disruption in daily activities, suicidal thinking and behavior, substance abuse, and impairment in relationship functioning. Currently, there are programs that aim to reduce stress through psychotherapy such as the Stress Management and Resiliency Training(SMART)-Relaxation Response Resiliency Program(3RP) designed at Massachusetts General Hospital Benson-Henry Institute for Mind Body Medicine. The Relaxation Response Resiliency Program (3RP) is a comprehensive, multimodal treatment designed to promote adaptation to stress and enhance resiliency (Park et al. 2013). Research on Relaxation Response interventions have suggested that it is effective in reducing hypertension, insomnia, anxiety, pain and medication use for a variety of conditions (Mandle et al. 1996). Future research examining sympathetic activity in grief and its relationship with persistent complex bereavement disorder pathophysiology is warranted.

Strengths of this study include (1) a diverse group of qualified subjects, (2) assessment of eligibility not only by self-report questionnaires but also by a clinical interview with a psychiatrist, (3) specifically designed and validated Kinetic Enzyme Assay Kit to measure α-Amylase concentrations. Limitations for this study include (1) a small sample size as there were only 8 subjects, (2) inability to collect select saliva samples, (3) saliva samples obtained at different times during the day for different subjects, (4) absence of a real control condition. The closest condition to a control condition for this study is the duration between Sample 3 and Sample 4 while subjects are discussing information that does not pertain to the loss. The α-Amylase concentration decreases back to around baseline level during this time.

APPENDIX

SCID Summary - Structured Clinical Interview for DSM Summary

Screening ID: ______________________________

SCID Summary

Bipolar Disorder - LIFETIME
- ○ 1 = absent
- ○ 2 = subthreshold
- ○ 3 = present
- ○ not assessed

Bipolar Disorder - CURRENT
- ○ 1 = absent
- ○ 2 = subthreshold
- ○ 3 = present
- ○ not assessed

You indicated the participant has CURRENT but not LIFETIME bipolar disorder.
Please correct any errors above before moving on.

Age of Onset of Bipolar ______________________________

Major Depressive Disorder - LIFETIME
- ○ 1 = absent
- ○ 2 = subthreshold
- ○ 3 = present
- ○ not assessed

Major Depressive Disorder - CURRENT
- ○ 1 = absent
- ○ 2 = subthreshold
- ○ 3 = present
- ○ not assessed

You indicated the participant has CURRENT but not LIFETIME major depressive disorder.
Please correct any errors above before moving on.

Age of Onset of MDD ______________________________

Dysthymia - CURRENT
- ○ 1 = absent
- ○ 2 = subthreshold
- ○ 3 = present
- ○ not assessed

Age of Onset of Dysthymia ______________________________

Psychotic Disorders - LIFETIME
- ○ 1 = absent
- ○ 2 = subthreshold
- ○ 3 = present
- ○ not assessed

Psychotic Disorders - CURRENT
- ○ 1 = absent
- ○ 2 = subthreshold
- ○ 3 = present
- ○ not assessed

You indicated the participant has CURRENT but not LIFETIME psychotic disorder.
Please correct any errors above before moving on.

Age of Onset of Psychotic Disorder ______________________________

Alcohol Abuse - LIFETIME

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

Alcohol Abuse - CURRENT

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

You indicated the participant has CURRENT but not LIFETIME alcohol abuse.
Please correct any errors above before moving on.

Age of Onset of Alcohol Abuse ______________________________

Alcohol Dependence - LIFETIME

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

Alcohol Dependence - CURRENT

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

You indicated the participant has CURRENT but not LIFETIME alcohol dependence.
Please correct any errors above before moving on.

Age of Onset of Alcohol Dependence ______________________________

Substance Abuse - LIFETIME

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

Substance Abuse - CURRENT

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

You indicated the participant has CURRENT but not LIFETIME substance abuse.
Please correct any errors above before moving on.

Age of Onset of Substance abuse ______________________________

Substance Dependence - LIFETIME

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

Substance Dependence - CURRENT

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

You indicated the participant has CURRENT but not LIFETIME substance dependence.
Please correct any errors above before moving on.

Age of Onset of Substance Dependence ______________________________

Panic Disorder - LIFETIME

◯ 1 = absent
◯ 2 = subthreshold
◯ 3 = present
◯ not assessed

Panic Disorder - CURRENT

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

You indicated the participant has CURRENT but not LIFETIME panic disorder.
Please correct any errors above before moving on.

Age of Onset of Panic Disorder ______________________________

Agoraphobia - LIFETIME

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Agoraphobia - CURRENT

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

You indicated the participant has CURRENT but not LIFETIME agoraphobia.
Please correct any errors above before moving on.

Age of Onset of Agoraphobia ______________________________

Social Anxiety Disorder - LIFETIME

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Social Anxiety Disorder - CURRENT

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

You indicated the participant has CURRENT but not LIFETIME social anxiety disorder.
Please correct any errors above before moving on.

Age of Onset of SAD ______________________________

Specific Phobia - LIFETIME

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Specific Phobia - CURRENT

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

You indicated the participant has CURRENT but not LIFETIME specific phobia.
Please correct any errors above before moving on.

Age of Onset of Specific Phobia ______________________________

Obsessive Compulsive Disorder - LIFETIME

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Obsessive Compulsive Disorder - CURRENT

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

You indicated the participant has CURRENT but not LIFETIME obsessive compulsive disorder. Please correct any errors above before moving on.

Age of Onset of OCD ______________________

Post Traumatic Stress Disorder - LIFETIME
- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Post Traumatic Stress Disorder - CURRENT
- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

You indicated the participant has CURRENT but not LIFETIME post traumatic stress disorder. Please correct any errors above before moving on.

Age of Onset of PTSD ______________________

Generalized Anxiety Disorder - CURRENT
- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Age of Onset of GAD ______________________

Somatization Disorder - CURRENT
- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Age of Onset of Somatization Disorder ______________________

Pain Disorder - CURRENT
- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Age of Onset of Pain Disorder ______________________

Undifferentiated Somatoform Disorder - CURRENT
- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Age of Onset of Undifferentiated Somatoform Disorder ______________________

Hypochondriasis - CURRENT
- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Age of Onset of Hypochondriasis ______________________

Body Dysmorphic Disorder - CURRENT
- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Age of Onset of BDD ______________________

Anorexia Nervosa - LIFETIME

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Anorexia Nervosa - CURRENT

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

You indicated the participant has CURRENT but not LIFETIME anorexia nervosa.
Please correct any errors above before moving on.

Age of Onset of Anorexia Nervosa ______________________________

Bulimia - LIFETIME

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

Bulimia - CURRENT

- ◯ 1 = absent
- ◯ 2 = subthreshold
- ◯ 3 = present
- ◯ not assessed

You indicated the participant has CURRENT bulimia, but does not have LIFETIME bulimia.
Please correct any errors above before moving on.

Age of Onset of Bulimia ______________________________

Primary Diagnosis

- ◯ None - Healthy Control
- ◯ Bipolar Disorder
- ◯ Major Depressive Disorder
- ◯ Dysthymia
- ◯ Psychotic Disorders
- ◯ Alcohol Abuse
- ◯ Alcohol Dependence
- ◯ Substance Abuse
- ◯ Substance Dependence
- ◯ Panic Disorder
- ◯ Agoraphobia
- ◯ Social Anxiety Disorder
- ◯ Specific Phobia
- ◯ Obsessive Compulsive Disorder
- ◯ Post Traumatic Stress Disorder
- ◯ Generalized Anxiety Disorder
- ◯ Somatization Disorder
- ◯ Pain Disorder
- ◯ Undifferentiated Somatoform Disorder
- ◯ Hypochondriasis
- ◯ Body Dysmorphic Disorder
- ◯ Anorexia Nervosa
- ◯ Bulimia

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EDUCATION

Masters of Medical Science, Boston University School of Medicine. Degree expected Spring 2017.

Bachelor of Science, Biotechnology, University of California, Davis. 2011-2015.

Diploma, Lowell High School, San Francisco, CA. 2007-2011.

RESEARCH EXPERIENCE

Clinical Research Coordinator, Massachusetts General Hospital's Center for Anxiety and Traumatic Stress Disorders, Boston, Jan 2017-present

Working on a new clinical trial study that is testing the safety and efficacy of JNJ-42165279, a FAaH inhibitor, on social anxiety. My duties include submitting amendments to the protocol, protocol summary, and other regulatory forms related to the study to the IRB, communicating with representatives from the sponsor ensuring fluidity of the study, recruiting subjects for the study and assisting in the study. Dr. Paolo Cassano, Department of Psychiatry.

Clinical Research Assistant, Massachusetts General Hospital's Center for Anxiety and Traumatic Stress Disorders, Boston, Aug 2016-Dec 2016

Assisted on the Osher Study, a study piloting a tailored mind-body Program (SMART-3RP) in elders who have lost a spouse within the last year. I am also writing my thesis titled "Salivary Alpha Amylase and Cortisol Reactivity During Discussion of the Death of a Spouse in Recently Bereaved Elders." Dr. Eric Bui, Department of Psychiatry.

Berben Lab Research Assistant, UC Davis, Davis, Mar 2013-Jun 2013

Assisted in synthesis and characterization of new inorganic molecules. Main projects involved investigating multi-electron CO2 reduction chemistry with multimetallic clusters, redox chemistry of main group elements; Al(III), GA(III), and Si(IV), and synthesis of Low-Coordinate Complexes using Bulky Acetylide Ligands. Professor Louise Berben, Department of Chemistry.

EXPERIENCE/EMPLOYMENT HISTORY

Surgeon Shadowing (Oct 2014-Feb 2015)

Shadowed surgeons in the operating room at UC Davis Medical Center and attended Grand Rounds weekly. Procedures observed included kidney transplant, superficialization of a basalic vein, Lead Laser Extraction, and an operation involving a pulmonary stenosis and sub aortic stenosis.

Kaiser Permanente Ambassador (ambassador, Dec 2013-Dec 2014)

Provided assistance to doctors, nurses and other members of the staff, greeted and directed patients and visitors, and ran errands

Child Life UC Davis Medical Center (intern, Jun 2014-Dec 2014)

Provide care and support for children who must remain in the hospital for an extended period of time. Accompany children patients to X-ray, chemotherapy, and other treatment, and organize activities with kids

Physician Shadowing (Jun 2014-Sept 2014)

Shadowed Dr. David Shu, a primary care physician who shares a private practice in San Francisco.

UC Davis Community Nutrition (intern, Mar 2014-Jun 2014)

Provided nutrition education to low-income families at SFBFS food distribution sites, assisted with nutrition, health and cooking education classes, and compiled nutrition and health education resources

Sutter Health Hospital Medical/Surgical Nursing Unit (intern, Dec 2013-Mar 2014)

Assisted doctors and nurses, stocked supplies, delivered meals to patients, transport samples to lab, transport samples to lab, ran errands, visit patients, helped with audits, and cleaned

Sutter Health Hospital Birthing Center (intern, Mar 2014-Jun 2014)

Assisted RNs and Nursing Aides on Post Partum, delivered meal trays and offered Snacks to patients and visitors, assembled patient charts and educational materials, restocked supplies, transport samples to lab, performed office duties, and ran errands

UCD Pediatric Emergency Room (intern, Sept 2013-Mar 2014)

Shadowed doctors and observed examinations, diagnoses, procedures and traumas, Provided assistace that was asked of me from nurses, technicians, doctors, and other hospital employees, observed patients that were being seen by doctors and nurses, interacted with patients and comforted patients, cleaned and restocked supplies, directed hospital visitors and patients at the front desk, transport and retrieved samples from lab, ran errands, and answered the phone

UCD Medical Center Pediatric Clinic (intern, Sept 2012-Dec 2012)
Shadowed doctors and helped greet patients, performed vitals, clean, restocked supplies, ran errands, and assisted nurses

ACADEMIC SERVICE

-The National Society of Leadership and Success member
-California Scholarship Federation (2007-2011)-lifetime member

HONORS/AWARDS

-Recipient, Dean's List Award, Fall 2012, Fall 2013, Winter 2014.
-Recipient, UCSF Essay Contest Award Winner, UCSF, 2009.

INTERESTS

Sports

-UC Davis Intramural Sports Team Captain (Sept 2011-Jun 2015)
started a variety of intramural sports team including basketball, softball, water polo, volleyball, and flag football

Volunteering

(see above under experience/employment)
-Glide Memorial church San Francisco volunteer (Jun 2015-Aug 2015, Dec 2015-Jan 2016)
prepared and served food to the city's poor, homeless, and hungry
-12th Annual UC Davis Pre-Medical and Pre-Health Professions National Conference Staff Member (Sept 2014-Oct 2014)
ran workshops and panels by doctors, medical school students, and residents