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A Longitudinal Analysis of the Impact of Daily Hassles, Life Stressors, and Chronic Medical Conditions on Salivary Alpha Amylase in Young Adults

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Biography

The author was born in Birmingham, United Kingdom, December 18, 1991. She graduated from Lakota East High School in Liberty Township, Ohio. She received her Bachelor of Arts degree from Miami University in 2014. She received his Master of Arts with distinction in Clinical-Child Psychology in 2019 from DePaul University in Chicago, Illinois, and is currently pursuing a Ph.D. in Clinical-Child Psychology at DePaul University. She is completing her American Psychological Association accredited pre-doctoral residency in pediatric psychology at Nationwide Children's Hospital.

Abstract

Young adulthood is a critical point of transition accompanied by a number of different stressors. Exposure to a stressor activates two systems – the hypothalamic pituitary axis (HPA) and sympathetic adrenal medullary (SAM) system. Research has primarily examined HPA axis and its corresponding stress hormone, salivary cortisol with little attention on sympathetic nervous system (SNS) markers. However, emerging research has proposed salivary alpha amylase (sAA) as a potential surrogate for SNS activity. The existing neuroendocrine research on sAA has largely focused on acute stressors and it is important to understand how sAA behaves in response to different levels of stressors – both proximal and distal stressors. This study sets out to examine the impact of daily hassles, life stressors, and chronic medical conditions on sAA in young adults. Exploratory analyses were conducted to understand how life stressors and chronic medical conditions influence diurnal patterns of sAA. Longitudinal data were collected from 83 young adults (63.9% female) between the ages of 18-24 at a large Midwestern University. Results found a significant relationship between the number of life stressors and average sAA output where average sAA output increased as the number of life stressors increased. Diurnal patterns of sAA demonstrated significant differences in high life stressors groups compared to the life stressors group 30 minutes after waking and in the evening. Daily hassles and CMCs did not significantly influence sAA output. Results suggest distal stressors impact SAM sensitivity. Future longitudinal research is warranted to further substantiate sAA as a measure of SNS activity and better understand how different types of stressors impact SNS activity.

A Longitudinal Analysis of the Impact of Daily Hassles, Life Stressors, and Chronic Medical Conditions on Salivary Alpha Amylase in Young Adults

Introduction

Stress is a complex phenomenon that is variable in intensity and impact and occurs in the context of many other factors. Exposure to stressors during a vulnerable period of development, young adulthood, could also worsen the effects of these stressors. Young adulthood is a critical developmental period during which individuals experience new stressors, such as financial independence and independent living, which individuals might not be equipped with coping skills to handle these stressors leaving them vulnerable to negative consequences of stress (Coiro, Bettis, & Compas, 2017). A history of significant life stress, experiencing a high number of daily hassles, or living with a chronic medical condition could potentially worsen the psychological and physiological effects of stress on the body. Examining how biomarkers of stress, such as salivary alpha amylase (sAA), function against the backdrop of these different stressors can help to explain how the body responds to stressors. Therefore, the current study aims to explore the impact of daily hassles, life stressors, and chronic medical conditions on sAA in young adults.

The terms “stress” and “stressors” are defined inconsistently in the literature. However, experts in the field have conceptualized and defined these constructs in a way that accounts for both the practical and theoretical significance. The stimulus model, according to Grant and colleagues (2003), defines “stressors” as the actual environmental experiences that negatively impact the individual whereas “stress” is an all-encompassing term that refers to the environmental events and accounts for the consequences of exposure to the stressor (Grant et al.,

2003). For the purpose of the study, we will be looking at stress from the stimulus model by examining stress as the objective count of stressful experiences.

Stress disrupts the body's homeostatic setpoint and leads to psychological and physical consequences (Goldstein & McEwen, 2002). The hypothalamic pituitary axis (HPA) and the sympathetic nervous system (SNS), specifically the sympathetic-adrenal-medullary system (SAM) are activated following exposure to stress. Neuroendocrine research examining the stress response primarily focuses on HPA axis activity and its corresponding stress hormone, cortisol. However, the SAM, in addition to the HPA axis, plays a critical role in the stress response. Cortisol is released slowly and often used to measure long-acting responses to stress (e.g., chronic stress) (Skosnik, Chatterton Jr, Swisher, & Park, 2000), whereas sAA is immediately released following exposure to a stressor (Kirschbaum, Pirke, & Hellhammer, 1993). While there are several ways to measure SNS activity such as measuring norepinephrine level in cerebrospinal fluid or epinephrine in urine samples, sAA offers a much less invasive approach to measure SNS activation as it can be examined via saliva (Yoon & Weierich, 2016). Emerging research provides support for the use of this salivary enzyme as a surrogate of the SNS (e.g., (Kirschbaum et al., 1993; Nater et al., 2005; Yoon & Weierich, 2016)).

Previous psychoneuroendocrinology research on sAA has largely focused on examining sAA response immediately following exposure to a stressor (e.g., (Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009; Kirschbaum et al., 1993; Takai et al., 2004)). For example, lab-based studies and studies replicated in real-world settings have shown immediate elevations in sAA following exposure to a stressful event (e.g., (Bosch, de Geus, Veerman, Hoogstraten, & Amerongen, 2003; Granger et al., 2006; Het et al., 2009; Kang, 2010; Kirschbaum et al., 1993). However, there is also recent evidence to show the long-term impact of stressors on sAA

reactivity (Feldman, Vengrober, Eidelman-Rothman, & Zagoory-Sharon, 2013; Kinney et al., 2021; Morris & Rao, 2013). Therefore, it is critical to understand how sAA behaves in response to different levels of stressors, such as daily hassles, life stressors, and chronic medical conditions and whether sympathetic activation differs when examining these proximal and distal stressors.

A typical diurnal pattern of sAA shows a decrease in sAA 30 minutes after waking followed by an increase in sAA levels throughout the day (Nater et al., 2006). Preliminary findings show that significant life stressors may alter the daily response pattern (Feldman et al., 2013; Kinney et al., 2021). However, the literature on sAA and distal stressors is sparse and has showed some variability in response depending on severity of the stressors and other contextual factors. There is conflicting evidence on alterations in diurnal patterns in individuals with a history of trauma. One study showed more elevated afternoon levels of sAA (Feldman et al., 2013) whereas another study revealed higher waking sAA and a slower diurnal increase (Kinney et al., 2021). The differences in SNS response underscore the importance of considering both proximal and distal influences on sAA.

Hassles and sAA

Lazarus (1986) defined daily hassles as, “Experiences and conditions of daily living that have been appraised as salient and harmful or threatening to the endorser's well-being.” Hassles provide insight into the daily variability in stress that might not be captured by other related variables that focus on single events (e.g., life events and potentially traumatic events). Understanding the day-to-day fluctuation in stress is important in elucidating the stress response (DeLongis, Coyne, Dakof, Folkman, & Lazarus, 1982) and can help to better understand the sensitivity of this stress system. Though there are no studies to our knowledge assessing the

impact daily hassles have on the neuroendocrine response, it is likely that there would be an observable increase in sAA in response to these disruptions in daily living due to past research showing stressful events associated with elevations in sAA.

Life Stressors and sAA

Life stressors are a heterogenous category of potentially traumatic events and include events such as experiencing a serious accident, financial difficulties, sudden loss of a loved one and abuse (Dohrenwend, 2006). Little is known about the neuroendocrine response related to life stressors specifically as it related to SNS activity. However, cortisol research shows evidence to support a less pronounced response and recovery profile or an attenuated stress response (Bosch, van den Keijbus, Ligtenberg, van Nieuw Amerongen, & Brand, 1995; Goldstein & McEwen, 2002; Gordis, Granger, Susman, & Trickett, 2008). It is possible that the severe trauma could desensitize the SNS response thus resulting in a less pronounced profile (Morris & Rao, 2013). Given the many contextual variables, it is likely that there are other factors that might cause this response to behave differently. For instance, the number of life stressors could impact the stress response.

Chronic Medical Conditions and Stress

Individuals with a chronic medical condition (CMC) often experience stress related to management and maintenance of their condition. Living with a life-long illness requires a great deal of ongoing care many of which involve daily demands. The complex relationship between stress and disease is best conceptualized as a bidirectional relationship where stress is a consequence of a CMC and stress can negatively impact health. Because having a CMC requires constant management, it is possible that there is a continuous activation of the SNS stress response which could lead to more wear and tear on the body, commonly referred to as allostatic

load, where the body learns to adapt to the negative psychological and physiological effects (McEwen & Wingfield, 2003). Though the research on sAA and CMCs is limited, increasing evidence demonstrates a relationship between certain CMCs and either over or underactivity of the SNS (Fu, 2012). During young adulthood, individuals manage their CMC with increasing independence which may lead to heightened stress as they learn to navigate CMC management.

Rationale

There are a number of factors that likely affect the overall stress response and diurnal sAA pattern including severity of trauma, psychosocial factors, and the number, types, and frequency of stressors. Given the paucity of research on sAA as a biomarker of stress, it is difficult to predict how these different factors, might affect sAA output. The overall aims of this study help to fill an important gap by examining the effects of different levels of stressors on sAA output. We hypothesized that greater daily hassles, greater number of life stressors, and the presence of a CMC would be related to higher sAA levels. Exploratory aims examined how different types of stressors (life stressors and CMC) impact the diurnal response profile of sAA. We hypothesized that participants with greater life stressors will demonstrate differences in diurnal patters compared to individuals with fewer life stressors. Similarly, we hypothesized that the presence of a CMC will demonstrate differences in diurnal patterns compared to those without a CMC.

Methods

Participants

A subset of a larger study (N=98 out of 265) consented to participate in an optional saliva data collection in addition to participating in the full study including daily diaries. The inclusion criteria for the larger study were: 1) between the ages of 18-24 at the time of consent; 2)

currently enrolled at the university; 3) fluency in English; 4) a mobile phone with unlimited text-message capabilities in order to participate in the daily assessments. Exclusion criteria included: 1) illiterate or inability to provide informed consent; 2) involvement in varsity athletic team.

Eighty-eight participants participated in the saliva data collection, and after removing those with data that could not be analyzed, 83 participants were included in the final analyses. Participants ranged in age from 18-24 (M=19.7, SD=1.6; Table 1). Two-thirds of the sample was female identifying and one-third of the sample was male identifying. None of the participants identified as a gender other than male or female (e.g., transgender, gender non-conforming, gender queer, or other). Notably, the current sample was slightly more diverse than the university population with almost half of the sample representing a diverse racial identity.

Procedure

Participants were recruited through flyers posted around campus at a large Midwestern university and recruitment information was distributed in classes and student organizations. The longitudinal study included an in-person baseline assessment followed by daily tracking over a two-week period. At the conclusion of the two-week period, participants completed an in-person follow-up assessment. At the baseline assessment, participants were given the option to participate in a saliva portion of the study where they provided four saliva samples every day for three consecutive days during the two-week period between the baseline and follow-up assessment. For the purpose of this study, data were examined from baseline, daily tracking, and saliva collection.

Baseline Assessment

Once participants consented, they completed questionnaires and provided their cell phone number in order to receive daily text message reminders to complete daily surveys during the 2-week period. Participants received $15 compensation after completion of the baseline assessment.

Daily Assessment

Participants received daily text message reminders in the evening to complete daily surveys assessing daily hassles. Participants earned $5 compensation each day they completed the survey with an opportunity to earn up to $70 for completing all 14 days of surveys.

Saliva Samples

Participants were provided with a saliva collection kit as well as verbal and written instructions on how to correctly provide saliva (passive drool) samples. Participants were provided a study-developed schedule which indicated what days of the week participants provided their saliva samples. In addition, clear directions with photos were included to explain how to provide saliva samples. Participants were asked to avoid brushing teeth, eating a large meal, smoking and drinking thirty minutes to an hour prior to providing sample.

The saliva kits included an insulated lunch bag with an ice pack, straws, and 12 2mL salivettes. Participants were asked to provide four saliva samples each day for three consecutive days for a total of 12 samples. When participants returned their sample at their follow-up appointment, they were frozen in a freezer at -20°C until the samples were shipped for analyses. Samples were shipped and assayed at the Salimetrics SalivaLab (Carlsbad, CA) using the Salmetrics Salivary Alpha-Amylase Assay Kit (Cat. No. 1-1902), without modifications to the

manufacturer's protocol. Participants earned $15 compensation each day they completed the four samples with an opportunity to earn up to $45.

Measures

Background Information (Baseline)

Demographic information including age, sex, gender, race, and ethnicity were obtained.

Physical Health Information Form (Baseline)

Participants answered a study-developed questionnaire assessing the presence of chronic medical conditions and recurrent physical symptoms (e.g., asthma, diabetes, chronic pain, and chronic headaches).

Life Stressors Checklist Revised (Baseline)

The Life Stressors Checklist Revised (LSC-R) is a self-report measure that assesses 30 life stressors and other potentially traumatic events (e.g., financial difficulties, parental divorce, family member incarcerated, sexual abuse, etc.) (Wolfe, Kimerling, Brown, Chrestman, & Levin, 1996) consistent with DSM-IV Post Traumatic Stress Disorder Criterion A. In addition, the LSC-R evaluates timing, threat or potential death, and relative impact. For the purpose of the study, we examined the number of events endorsed; one point was assigned for each positively endorsed life stressor to derive an overall life stressor score ranging from 0-30.

Hassles (Daily)

The 20-item Brief College Student Hassles Scale (BCSHS) was used in order to assess commonly reported hassles experienced by college students (e.g., academic, financial, and social stress). Participants rated their hassles on a seven-point Likert scale ranging from (1) "No hassle, not persistent at all" to (7) "High occurrence; extremely persistent, high frequency or duration." Initial validation of the BCSHS demonstrated good internal consistency based on total scores

(Cronbach's $\alpha = 0.81$) (Blankstein & Flett, 1992; Blankstein, Flett, & Koledin, 1991). A total mean score is derived with higher scores indicating greater hassles.

Saliva Tracking Log

A study-developed saliva tracking log was filled out by participants on each saliva collection day. Participants were asked to include the time of day they took the four saliva samples. In addition, participants were asked questions related to alcohol and medication usage, smoking, and whether or not they ate, drank, or brushed their teeth within thirty minutes to an hour of providing a sample. Participants were also asked questions related to their health including medication taken over the last 48 hours.

Adherence

At the follow-up visit, participants were asked to numerically (0-10, with 10 indicating following the saliva protocol 100% of the time) assess their level of adherence to the saliva protocol. In order to ensure participants reported their adherence as accurately as possible, study staff provided the adherence measure after participants were given their study compensation. Study staff were not present, and participants returned their completed forms in a sealed box.

Saliva Collection

Participants collected samples from awakening, +30 minutes after waking, mid-day, and evening over three days (12 samples per participants).

Data Analyses

Analyses were conducted using SPSS v. 27 (Corp., 2020). Sample characteristics on demographics such as age, sex, and race were analyzed. The sAA samples were assessed for skewness and outliers ±3SD from the mean were removed. The distribution of daily hassles were transformed to fit a negative binomial distribution by dividing numbers by 10 and rounding to

the nearest whole integer. Multilevel modeling was used to examine if daily hassles predicted greater sAA levels. Restricted maximum likelihood estimation was to be applied to handle missing sAA data.

The first model tested hypothesis I: Daily hassles will significantly predict greater sAA levels. A random intercept only model and random slope model was run to see whether the level two units differ from each other on different days. The random variance ($u_{0i \text{ and }} u_{1i}$) from the models provided person-level average and person-level variability in daily hassle effects. The fixed effect represented the impact of daily hassles. Then a multilevel model was estimated to determine if daily hassles predicted greater sAA level.

A second analysis tested hypothesis II: Pearson *r* correlational statistics was used to determine the bivariate relationship between the number of life stressors and average sAA output.

The third model tested hypothesis III: Those with a chronic medical condition will have greater sAA output compared to those without a chronic medical condition. An independent sample t-test was run to see whether there was a difference in sAA output based on presence or absence of a chronic medical condition. Chronic medical conditions were dummy coded to represent presence (1) or absence (0) of CMC.

Exploratory analyses examined how different types of stressors impact the diurnal response profile of sAA, independent samples t-tests were conducted to determine whether there were differences in diurnal sAA patterns in participants with greater life stressors and in participants with a CMC. The sample was divided based on high and low stress. The cut-off value for these two groups was informed by previous research on one type of stressor, adverse childhood experiences (ACEs). The seminal ACEs study (Felitti et al., 1998) showed that

individuals with four or more ACEs demonstrated a four-12 fold increase in health risks. As such, the high stress group included participants with ≥4 stressors (n=36) and low stress included participants with ≤3 stressors (n=46). Each participant had three samples for each timepoint (e.g., three awakening samples across three days), and the daily average sAA values were used for all four timepoints. The sAA values across 3 days for each of the four timepoints were significantly correlated (*r* range .45-.74) indicating intra-individual stability over time (Veen et al., 2011).

Results

Data were consistent with normality and assumptions necessary for multilevel modeling. Out of the 88 participants recruited, three participants did not have baseline data, and two sAA outliers (±3SD from the mean) were removed to reduce the influence of extremely high or low values with a final sample of 83 participants. Fifteen participants in the final sample had partial data with a total of 20 missing samples among them - there were 13 missing samples, three duplicate samples that were averaged, one sample where the quality of the sample was not sufficient to obtain sAA level and three samples were below the limit of sensitivity (0.4 U/mL) as determined by Salimetrics. Saliva adherence ranged from 4-10 (M=7.73, SD=1.4). Correlation between main variables of interest are included in Table 2. A correlation, t-test, and ANOVA were used to determine if sAA and life stressors differed on all demographic variables. Life stressors were signifigantly different based on race ($F_{4, 77} = 2.825$, $p < 0.05$), however, life stressors and average sAA did not significantly differ on all other demographic varibles.

Multilevel modeling was used to test the relationship between daily hassles and sAA. The relationship between daily hassles and sAA was not significant $F(1, 50.59) = 0.014$, $p = .907$ (Table 3). In order to maximize power to estimate random effects, the random intercept alone was tested. While results remained nonsignificant (p=.840) (Table 4), results demonstrated

variability from participant to participant on average sAA. The centered independent variable, daily hassles, was then disentangled to examine person level deviation and person level average across days ($F(1, 71.55) = 1.186$, $p = .280$) (Table 5).

The majority of the sample (96%) endorsed at least one life stressor with almost half of the sample reporting having someone close to them die. Life stressors ranged from one stressor to 11 stressors. Person correlation was used to understand the relationship between exposure to more life stressors and average sAA output. The number of life stressors was significantly correlated with average sAA output (r=.226, p=.041).

Approximately half of the sample endorsed having at least one CMC and the most common CMCs were allergies and obesity. Although the group with a CMC had higher sAA levels (M=93.00, SD=52.86) compared to those without a CMC (M=85.73, SD=55.35), this difference was not significant (t=-.604, p=.548). Participants with a chronic medical condition were not more likely to be taking medication ($X^2(2) = 1.75$, $p = .186$).

Diurnal sAA patterns were compared in participants with high and low stressors (Figure 1). There was a significant different in sAA levels 30 minutes after waking (t=-2.94, p=.025) and in the evening (t=-2.14 p=.049) between the high and low stressors group (Table 6). There was not a significant difference when comparing other timepoints (awakening and afternoon levels) (Table 6). Regarding diurnal patterns amongst participants with and without CMCs (Figure 2), there was not a significant difference in sAA levels at any timepoint (Table 7).

Discussion

The present longitudinal study is one of the first to our knowledge to examine sAA in the context of multiple levels of stressors in young adults. Our study explored the impact of daily hassles, life stressors, and chronic medical conditions on sAA levels in young adults. These

findings contribute to the limited literature on the neuroendocrine response of sAA by providing an introductory understanding of how sAA behaves when considering different types of stressors. Surprisingly, although daily hassles and CMCs pose more immediate stress, these did not influence sAA levels. Notably, there was a significant relationship between the number of life stressors and sAA. Finally, diurnal patterns were examined. Overall, these patterns seemed to follow the expected trend of a decrease in sAA levels after waking following by a gradual increase throughout the afternoon to evening. There were significant differences in sAA levels in which those with higher life stressors showed significantly different levels 30 minutes after waking and in the evening.

There was a significant relationship between the number of life stressors and average sAA output, in which average sAA output increased as the number of life stressors increased. Similarly, there were significant differences between the high stressors group and low stressors group in sAA levels 30 minutes after waking and in the evening (Table 5 & Figure 1). These results replicate findings from a previous study which demonstrated a positive correlation between sAA activity and the number of life stressors (Bosch et al., 1998). In contrast, individuals who have experienced significant trauma have a blunted sAA response profile (Mielock, Morris, & Rao, 2017). The current study is a nonclinical sample and shows that more life stress can increase the sensitivity of the SAM system thus leading to greater sAA output, whereas experiencing significant trauma can decrease the sensitivity of the SAM system leading to lower sAA output. Allostatic load or the increasing wear and tear on the body that can result from chronic activation of stress system can lead to dysregulation of these systems (McEwen & Wingfield, 2003) and ultimately cause systematic physiological damage (Stewart, 2006). The allostatic load hypothesis can provide an explanation as to why there are alterations in the

sensitivity of these stress systems and help to provide support as to why we saw these differences in sAA as the number of life stressors increased. It is likely that there are a number of factors beyond the allostatic load hypothesis that predict the increase or decrease in sensitivity of the SAM system, however, these findings suggest that the number of life stressors contribute to the sensitivity of the SAM system.

While distal stressors impacted SNS activation, proximal stressors did not in our study. Multilevel modeling analysis showed daily hassles did not significantly predict sAA levels. The neuroendocrine response system is activated immediately following the exposure to a stressful or threatening situation (e.g., (Het et al., 2009; Kang, 2010; Kirschbaum et al., 1993; Takai et al., 2004)) resulting in an immediate release of sAA. Even with the daily assessment of hassles there is no accurate way of knowing the time a participant took a saliva sample coincided with the experience of a hassle. For example, if a participant reported a hassle related to an academic deadline that occurred in the middle of the day but did not take their saliva sample until an hour after experiencing this stressor, their afternoon sAA level likely will not capture this specific hassle given the immediate activation of the SNS following a stressor.

Furthermore, regardless of the presence of absence of a CMC there was not a significant difference in sAA. Our study conceptualized a CMC as a stressor, however, there was a great deal of variability in the types of CMCs. It is likely that these CMCs do not uniformly affect the SAM system the same way from both a physiological and psychological perspective. There is some evidence to show certain CMCs influence SNS reactivity differently (Fu, 2012; Wolf, Nicholls, & Chen, 2008). For example, chronically stressed children with asthma have lower sAA levels compared to healthy controls (Wolf et al., 2008). Additionally, different CMCs require different levels of management (e.g., diabetes versus allergies) which could influence the

level of stressors participants experienced. Allergies and obesity were the most commonly reported CMCs with almost half of the sample (n=36) endorsing one of these CMCs. In comparison to other CMCs included on the list (e.g., sickle cell disease (n=1), cystic fibrosis (n=1), inflammatory bowel disease (n=1)), it is likely that allergies and obesity require less daily management and subsequently result in less stress compared to these other diseases. This great variability in conditions could help to explain why we did not see a significant difference in sAA output between those with and without a CMC.

Commented [CJ1]: Should you be using the stressors language here consistent with the conceptualization in your introduction?

It is likely that certain medications influence sAA levels though there is very little literature on the impact of medication on sAA. Only two studies on sAA to our knowledge have excluded participants based on medication usage. One study (Breines et al., 2015) excluded participants on psychoactive drugs, beta-blockers, gonadal steroids (hormonal contraceptives), or glucocorticoids. Similarly, another study (Almela et al., 2011) excluded participants on medication related to emotional or cognitive functioning or medication that could impact hormonal or sAA levels (e.g., psychotropic medication, beta-blockers, benzodiazepines, etc.). However, the majority of studies on sAA did not preclude participation based on medication usage. Approximately half of our sample was taking a hormonal contraceptive or stimulant medication and it is possible that these medications could impact sAA response in ways that we do not know yet given the paucity of literature in this area.

In our primary analyses, we examined average sAA output amongst participants as opposed to examining specific timepoints due to the lack of research in this area. However, there is some preliminary work (Feldman et al., 2013; Kinney et al., 2021; Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007; Wolf et al., 2008) which helped to guide our exploratory analyses in order to better understand diurnal patterns of sAA. Consistent with previous findings, the

diurnal pattern showed decrease in sAA after waking followed by a progressive increase throughout the day. Results showed significant differences in sAA levels in the high stressors group compared to the low stressors group 30 minutes after waking and in the evening. These findings map onto current research which has shown adults who have experienced chronic stress or childhood trauma tend to show altered daily sAA patterns of secretion (Kinney et al., 2021; Nater et al., 2007; Wolf et al., 2008). Interestingly enough, children with PTSD show lower sAA levels compared to children without PTSD (Feldman et al., 2013). There are differences in SNS responses in adults and children who have experienced trauma or chronic stress but little is known as to what contributes to these discrepant findings (Keeshin, Strawn, Out, Granger, & Putnam, 2015).

Limitations and Future Directions

There are several limitations and subsequent future directions that should be noted. Given that life stressors were signifigantly different based on race, future work with a more robust sample should control for race to limit the impact of potentially confounding variables. The sample size (N=83) is small and limited our ability to examine across and within participant sAA levels. Saliva samples were collected four times a day over the course of three days which is consistent with recommendations in the literature regarding repeated measurements for saliva collection (Nater, Hoppmann, & Scott, 2013). However, there is evidence to suggest there is a great deal of inter-individual variability in sAA level (Strahler, Skoluda, Kappert, & Nater, 2017) thus it might be more meaningful to examine a greater number of individual sAA levels. Future work should focus on collecting samples across more days in order to better examine trends in sAA to eventually guide in determining sAA parameters and cutoffs. Additionally, there are limitations related to measurement and opportunities to further improve the ways in which we

examine these three stressors. Overall findings from the study revealed stressors specifically greater life stressors significantly impact SNS activity highlighting the sensitivity of the SAM system. It is likely that there are a number of factors that influence the sensitivity of the SAM system beyond the number of life stressors and future research should better understand the factors that contribute to these changes in SAM sensitivity. As previously mentioned, CMCs may differentially impact SNS activity and there are a number of physiological and psychological factors that could change the way in which individuals respond to stressors. The current study conceptualized a CMC as a stressor and dichotomized CMCs into presence or absence of a condition which limits our understanding of the nuanced ways in which different CMC influence SNS activity. Future research in medical settings should focus on understanding the ways in which different CMCs influence sAA levels.

In regards to measurement, daily hassles is not a well-researched construct and the BCSHS captured a rather narrow list of items and did not account for stressors (i.e., potentially traumatic events, mental or physical health concerns) that might have occurred during the two-week period when participants completed this measure. The BCSHS was also developed in 1991 and does not assess for hassles that might affect students currently. For example, the BCSHS does not assess for stress related to social media usage and the negative impact of social media on mental health in young adults is well-documented (Abi-Jaoude, Naylor, & Pignatiello, 2020; Bashir & Bhat, 2017; Braghieri, Levy, & Makarin, 2021; Mishna et al., 2018). Other relevant stressors that students might have faced during the time the study was conducted (2017-2018) are stressors related to changes in the sociopolitical climate, increased globalization, and inflation rates. Approximately one-third of the university's total undergraduate population is first generation and the BCSHS and LSC did not assess for stressors that first generation college

students might commonly experience, such as academic acculturation or stress associated with acculturating to an academic setting (Cheng & Fox, 2008). While life stressors and daily hassles were correlated ($r = .255$, $p < .05$), controlling for these variables were beyond the scope of the current study and future work should analyze these variables jointly

One methodological limitation is related to adherence. Participants were instructed on how to take their saliva samples during their in-person baseline visit. They were given clear directions with photos to explain how to provide their samples and asked to avoid doing certain things (eating large meal, drinking, smoking) prior to providing a sample. Potential lack of adherence to the saliva protocol likely impacted the accuracy of saliva samples received. If participants did not collect saliva samples at the correct time each day (awakening, 30 minutes after waking, mid-day, and evening) then the trends we observed might not accurately reflect the diurnal response. Approximately half the sample (51%) reported an adherence level of ≥8; however, there are limitations to self-report measures specifically when assessing for adherence so it is possible that these values might even be an underestimate of true adherence to the protocol.

Conclusion

This is one of the first longitudinal studies to examine sAA in young adults in the context of both proximal and distal stressors. While daily hassles and CMCs did not impact sAA levels, life stressors are significantly related to sAA output and there are significant differences in sAA diurnal patterns (30 minutes after waking and evening level) when comparing participants with high life stressors and low life stressors. Greater life stressors are predictive of increased SNS activity thus highlighting the need to understand factors that contribute to SAM activity and the implications of over or underactivity of the SAM system. Additionally, future research should

focus on conducting similar research with a more robust sample size and a greater number of sAA observations to help in guiding parameters for understanding sAA levels.

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Table 1.

Participant Characteristics (N=83)

		m	*sd*	n	%
Age	(18-24 years)	19.7	1.6		
Sex					
	Male			30	36.1
	Female			53	63.9
Gender Identification					
	Male			30	36.1
	Female			53	63.9
Race					
	White			45	55.4
	Black or African American			4	4.8
	Asian or Asian American			15	18.1
	Native Hawaiian or Pacific Islander			1	1.2
	Other			17	20.5
Ethnicity					
	Hispanic			22	26.5
	Non-Hispanic			61	73.5

Table 2.

MLM Estimates of Daily Hassles Predicting sAA

Variables	1	2	3	4	5	6	7
1. Hassles Day 1	1	.805**	.729**	-.014	-.010	-.012	.255*
2. Hassles Day 2	--	1	.702**	-.045	.077	.147	.297*
3. Hassles Day 3	--	--	1	-.023	-.048	-.089	.169
4. Average sAA Day 1	--	--	--	1	.790**	.728**	.187
5. Average sAA Day 2	--	--	--	--	1	.826**	.229*
6. Average sAA Day 3	--	--	--	--	--	1	.176
7. LSC total score	--	--	--	--	--	--	1

Note. **$p < .01$, *$p < .05$

Table 3.

MLM Estimates of Daily Hassles Predicting sAA

	Parameter	Estimate	S.E.	*t*(df)/z
Fixed Effects	Intercept	98.49	6.28	15.66(130.53)
	Hassles Centered	0.68	5.79	0.12(50.59)
Random Effects	Intercept	1976.40	676.07	3.33*
	Residual	2249.84	676.07	4.08**

Note. * $p = .001$; ** $p < .0001$

Table 4.

MLM Estimates of Daily Hassles Predicting sAA with Random Intercept Only

	Parameter	Estimate	S.E.	*t*(df)/z
Fixed Effects	Intercept	99.31	8.97	11.07(73.53)
	Hassles Centered	0.10	0.49	0.20(119.99)
Random Effects	Intercept	5482.84	983.14	7.74**
	Residual	1139.51	147.30	5.58**

Note. * $p = .001$; ** $p < .0001$

Table 5.

MLM Estimates of Daily Hassles Predicting sAA with Daily Hassles Disentangled

	Parameter	Estimate	S.E.	*t*(df)/z
Fixed Effects	Intercept	68.45	29.81	2.30(72.02)
	Hassles Mean	0.90	0.83	1.90(71.55)
	Hassles Deviation	0.10	0.50	0.20(71.55)
Random Effects	Intercept	5470.29	148.60	7.71**
	Residual	1145.59	987.49	5.54**

Note. ** $p < .0001$

Table 6.

Diurnal sAA Levels Between High and Low Stressors Groups

Time of Day	Low Stressors (n=46)		High Stressors (n=36)		*p*
	Mean	SD	Mean	SD	
Awakening	64.93	50.77	87.63	59.87	0.306
+30 Minutes After Waking	41.80	30.95	69.49	53.54	0.025
Afternoon	108.93	68.02	142.18	91.61	0.202
Evening	83.55	56.84	117.21	84.99	0.049

Table 7.

Diurnal sAA Levels Between Participants with and without a CMC

Time of Day	No CMC (n=41)		CMC (n=40)		*p*
	Mean	SD	Mean	SD	
Awakening	78.18	55.31	73.25	56.45	0.833
+30 Minutes After Waking	52.37	49.53	56.72	38.69	0.91
Afternoon	120.30	80.58	128.84	81.12	0.939
Evening	90.65	66.76	107.80	77.25	0.306

Figure 1.

Diurnal sAA Profile of Participants with High (≥4) and Low (≤3) Stressors

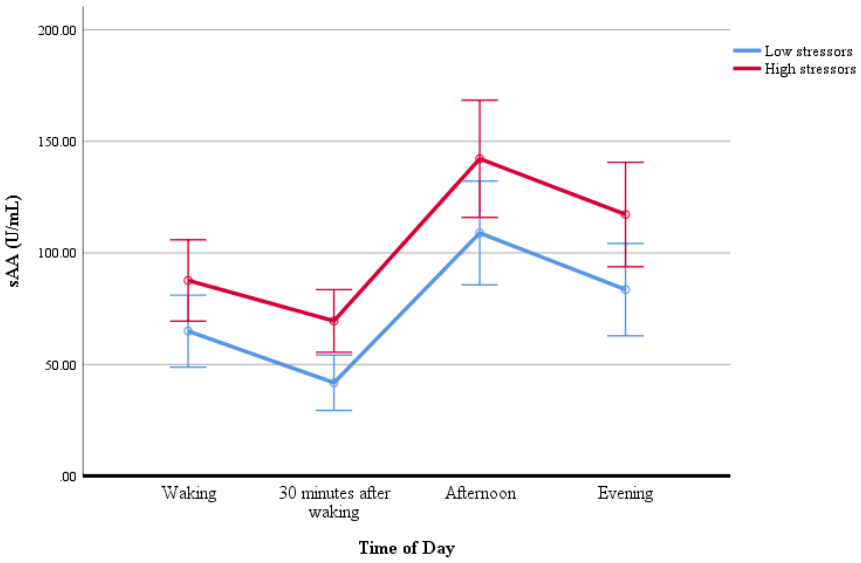
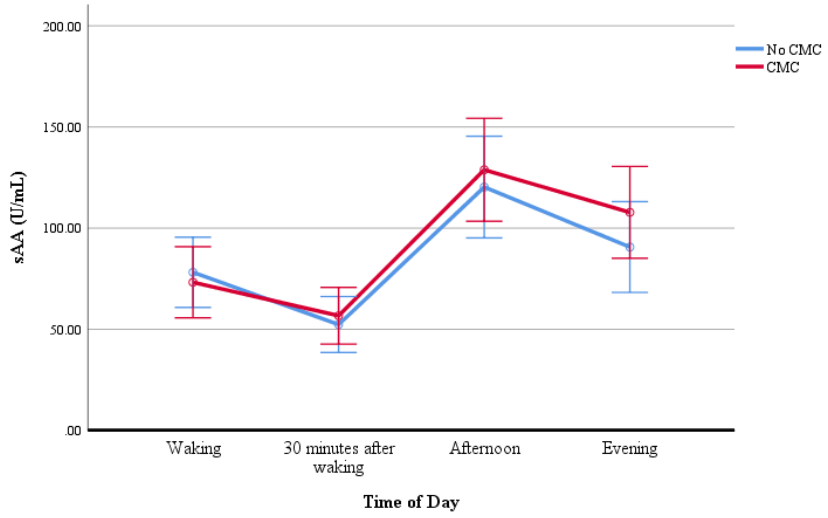


Figure 2.

Diurnal sAA Profile of Participants with and without a CMC



Appendix A

Extended Literature Review

Stress is a natural phenomenon that occurs in the presence of perceived threat or danger. Though stress is highly prevalent, it is complex, variable in intensity and impact, and often occurs in the context of many other factors. For instance, a history of significant life stress, experiencing a high number of daily hassles, or living with a chronic medical condition could potentially worsen the psychological and physiological effects of stress on the body. Exposure to these stressors during a vulnerable period of development, young adulthood, could also worsen the effects of these stressors. Examining how biomarkers of stress, such as sAA, function against the backdrop of these different stressors can help to explain how the body responds to stressors. Therefore, the current study aims to explore the impact daily hassles, life stressors, and chronic medical conditions have on sAA in young adults.

The terms “stress” and “stressors” are defined inconsistently in the literature. However, experts in the field have conceptualized and defined these constructs in a way that accounts for both the practical and theoretical significance. Across the literature, stress is commonly defined using two different models: the stimulus model and the transactional model of stress. The stimulus model, according to Grant and colleagues (2003), defines “stressors” as the actual environmental experiences that negatively impact the individual whereas “stress” is an all-encompassing term that refers to the environmental events and accounts for the consequences of exposure to the stressor (Grant et al., 2003). This definition looks at stress objectively without taking into account the valence or impact of the stressor. Conversely, the transactional model of stress is the interaction between the person and the environment focusing more on the interpretation of the event rather than the event itself (Lazarus & Folkman, 1984). The model

suggests that there are two stages of appraisal before responding to the stress. The first stage of appraisal is to interpret the event as threatening or nonthreatening and the second stage is to evaluate the ability or inability to cope with the stressor. For the purpose of this study, we will be examining stress from both stimulus model and the primary appraisal portion of the transactional model of stress.

Stressors can disrupt the homeostatic setpoint and lead to psychological and physical consequences (Goldstein & McEwen, 2002). There are many different mechanisms involved in keeping the body's internal environment balanced and the slightest disturbance can disrupt this balance. The hypothalamic pituitary axis (HPA) and the autonomic nervous system specifically the sympathetic-adrenal-medullary system (SAM) are activated following exposure to stress. Activation of the hypothalamic-pituitary-adrenal (HPA) axis occurs in response to stress under normal homeostasis. The hypothalamus releases corticotrophin-releasing factor (CRF) which binds to the pituitary gland resulting the release of adrenocorticotropic hormone (ACTH) releasing from the anterior pituitary (Alink, Cicchetti, Kim, & Rogosch, 2012). ACTH acts on the adrenal cortex to release cortisol. The HPA axis is a negative feedback system where the cortisol binds to glucocorticoid receptors signaling the hypothalamus and pituitary, which then prevents continued activation of the HPA axis. However, when stress continues and the individual has trouble dealing with stressors, sensitivity of the HPA axis may increase or decrease which in turn affects the production of cortisol (Alink et al., 2012).

The nervous system has two divisions, the central nervous and peripheral nervous system. The autonomic nervous system is directly involved in the stress response and is divided into the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). When the body responds to stress, the SNS activates a "fight, flight or freeze" response and the SAM is

activated. The SAM is the pathway where the sympathetic branch of the autonomic nervous system is activated in response to stress. When the body interprets a situation as threating, the amygdala is activated and sends a signal to the hypothalamus which then activates the SNS. This then causes pathways connected to the adrenal glands to release adrenaline and noradrenaline. The sudden increase of these catecholamines in the bloodstream prepares the body for the "fight, flight or freeze" response which leads to physiological changes such as decreased digestion, increased heart rate, and dilated pupils. Once the stressor or threat is removed, the PNS helps the body return back to a relaxed state. However, when individuals experience repeated stress, the body's SNS remains activated and does not have a chance to return to its relaxed state. The balance between the PNS and SNS is crucial in maintaining homeostasis and when this is compromised, the body suffers both physiological and psychological consequences.

Neuroendocrine research examining stress primarily focuses on HPA axis activity and its corresponding stress hormone, cortisol, and consequently salivary cortisol is considered the optimum biomarker of stress. However, the focus on this one stress hormone limits our understanding of how other systems function in response to stress. The SNS, in addition to the HPA axis both play critical roles in stress response. While there are several ways to measure SNS activity such as measuring norepinephrine level in cerebrospinal fluid or epinephrine in urine samples, salivary alpha amylase (sAA) offers a much less invasive approach to measure SNS activation as it can be examined via saliva. SAA is considered to be a noninvasive biomarker of the SNS (Yoon & Weierich, 2016) and emerging research provides support for sAA as a biomarker of stress (e.g., (Kirschbaum et al., 1993; Nater et al., 2005; Yoon & Weierich, 2016)).

Alpha-amylase is one of the major proteins in saliva and has two main functions 1) digestion of carbohydrates and (Zakowski & Bruns, 1985) 2) prevention of growth bacteria to allow for bacterial cleanse (Bosch et al., 2003). Under standard conditions, sAA is released by the acinar cells of the salivary glands (Baum, 1993), which make up the majority of cells in the salivary glands (Castle & Castle, 1998). Both the PNS and SNS innervate the acinar cells to produce sAA (Proctor & Carpenter, 2007). SAA is sensitive to physical and psychological stress leading to a rapid rise in sAA level. Recent literature demonstrates a significant increase in sAA in response to stressful situations (Nater et al., 2005). The SNS's response to stress shows an immediate activation of sAA followed by an immediate recovery profile.

Effect of Stress on Salivary Alpha Amylase

Promising findings have identified sAA as a biomarker for stress in the autonomic nervous system. Laboratory-based tasks have been used to elicit specific responses (e.g., stress) and observe the psychoneuroendocrine response. The Trier Social Stress Test (TSST) is common tool used in laboratory settings to understand the psychobiological aspects of the stress response. The format of the TSST includes a 10-minute anticipation period followed by a 10-minute test period in which participants deliver some sort of free speech. During the last part of the test, participants are asked to complete a set of arithmetic problems in front of an audience (Kirschbaum et al., 1993). Findings using the TSST have demonstrated immediate activation in sAA concentration following exposure to the TSST whereas the control group (placebo TSST) showed a marginal increase in sAA concentration (Het et al., 2009). Results from other lab-based studies have provided similar evidence for the impact of stress on sAA. For instance, participants demonstrated a significant increase in sAA when viewing a stressful video compared to the relaxing video (Takai et al., 2004); and when viewing a graphic surgical video, presumably the

most stressful condition, compared to completing a memory task or a control group (Bosch et al., 2003). These lab-based tasks consistently show that sAA is elevated in response to stress.

Lab-based studies conducted under controlled conditions have limitations with respect to external validity; however, similar findings have been replicated in real-world settings. The following research examined the effects of stressful events outside the lab on sAA. Stroud et. al (2006) examined sAA levels in response to peer rejection where participants demonstrated a spike in sAA following a peer rejection scenario. Marked increases in sAA levels have also been reported in response to various academic stressors. Participants demonstrated significant increases in sAA following an academic test compared to controls (Kang, 2010). Medical trainees showed an elevation in sAA following administration of a surgical procedure (Yamakage, Hayase, Satoh, & Namiki, 2007). Undergraduates demonstrated an elevation in sAA following an oral examination (Schoofs, Hartmann, & Wolf, 2008). Research has also provided support for the immediate activation of sAA from simply observing a stressful situation. In a task simulating a real-life scenario, mothers showed immediate elevation in sAA when observing their child participating in a stressful task (Granger et al., 2006). Findings across studies from both laboratory and real-life settings routinely show elevations shortly after exposure to the stressor. The immediate activation illustrates the fast-acting response of the SNS.

Life Stressors and sAA

Life stressors are a heterogenous category of potentially traumatic events and include events such as experiencing a serious accident, financial difficulties, sudden loss of a loved one and abuse (Dohrenwend, 2006). While the literature on the effect of discrete lab-based stressors and limited real-life stressors on sAA is clear, the effect of major life stressors is less known. Given the many contextual variables, it is likely that there are several factors which might cause

this response to behave differently. For instance, individuals with a history of abuse may be continually exposed to stressful situations, which could lead to an adaption of the stress response and possibly eventual disruption of the homeostatic setpoint. Consequently, this results in less pronounced response and recovery profile or an attenuated stress response (Bosch et al., 1995) as observed in the study conducted by Gordis and colleagues. Compared to the literature available on lab-based tasks and sAA response, there is only one study to the author's knowledge that examined different profiles between individuals with and without significant life stressors. Gordis et al. (2006) utilized a modified version of the TSST (adolescents M_{age}=12.2 years old) examining maltreated and comparison youth. The response profile of sAA revealed a similar activation and recovery profile for both groups; however, the comparison group had greater levels of sAA pre- and post-TSST compared to the maltreated group. The results suggest a potentially reduced stress response in the maltreated youth group. It is possible that the severe trauma could desensitize the SNS response thus resulting in a less pronounced profile (Morris & Rao, 2013).

The literature also includes mixed findings regarding different biomarkers of SNS activity. Elevations in norepinephrine levels have been shown in in individuals with a history of PTSD indicating increased SNS activity (Kosten, Mason, Giller, Ostroff, & Harkness, 1987). Similarly, Vietnam combat veterans with a history of PTSD showed elevations in norepinephrine and epinephrine compared to healthy controls and norepinephrine was predictive of PTSD severity (Yehuda, Southwick, Giller, Ma, & Mason, 1992). In contrast to these findings, one study found no difference in SNS activity in individuals with and without a history of PTSD (Blanchard, Kolb, Prins, Gates, & McCoy, 1991), and another suggested that those with a history of PTSD might show a blunted response to perceived threat (Morris & Rao, 2013).

Other psychosocial variables can also impact the stress response. Past research has indicated that it is not a single variable that can impact the stress response but rather a combination of these psychosocial variables that mediate the stress response (Vedhara, Shanks, Anderson, & Lightman, 2000). A combination of psychosocial factors including coping style, self-concept, and social support have been shown to influence the stress response (Vedhara et al., 2000). In addition, factors such as self-compassion, treating oneself with kindness and understanding, serves as a protective factor and findings indicated an inverse relationship between sAA and self compassion where lower levels of sAA reactivity were observed in individuals with higher levels of self-compassion (Breines et al., 2015). When one considers all these different factors, type of trauma, severity, and other psychosocial variables, it underscores the sensitivity of the SNS and the need to further examine how these different factors impact the neuroendocrine response.

Hassles and sAA

Lazarus (1986) defined daily hassles as, "Experiences and conditions of daily living that have been appraised as salient and harmful or threatening to the endorser's well-being." Hassles provide insight into the daily variability in stress that might not be captured by other related variables that focus on single events (e.g., life events and potentially traumatic events). Understanding the day-to-day fluctuation in stress is important in understanding the stress response (DeLongis et al., 1982). In addition, the stress response is very sensitive to minor disruptions therefore utilizing a measure that assesses daily stress will account for the variability of stressors.

Daily hassles have a great deal of conceptual overlap with many other constructs including chronic stressors (Hahn & Smith, 1999). Chronic stressors occur at a high frequency

and are described as “frustrations encountered in daily living” (D'Angelo & Wierzbicki, 2003) that range from low to high levels of intensity. Similarly, daily hassles can also occur at a high frequency and low intensity. However, the main differentiating feature is that daily hassles are typically described in the literature as stressful events or minor events (Aneshensel, Phelan, & Bierman, 1999) of relatively low intensity (Holahan & Holahan, 1987) whereas chronic stressors can range in level of intensity.

Though there are no studies to our knowledge assessing the impact daily hassles have on the neuroendocrine response, it is likely that there would be an observable increase in sAA in response to these disruptions in daily living. Laboratory based studies designed to elicit a stressful response have demonstrated elevations in sAA as soon as 10 minutes after the onset of the stressor (Stroud et al., 2006). However, given data across studies demonstrating elevations in sAA years after stressors (Gordis et al., 2008), it is very likely that an overall increase in sAA levels would be observed in response to daily hassles. Therefore, we would expect that after increased daily hassles we would see an increase in sAA.

Chronic Medical Conditions and Stress

Individuals with a chronic medical condition (CMC) often experience stress related to management and maintenance of their condition. Living with a life-long illness requires a great deal of ongoing care many of which involve daily demands. For example, living with Type 1 diabetes mellitus involves daily treatment including checking blood sugars, insulin injections, and maintaining a healthy diet and exercise. Because it is likely that baseline levels of stress are already elevated in individuals with a CMC, additional stress unrelated to a CMC potentially places this population at increased risk for stress-related physiological consequences. When one considers this significant stressor in the context of a critical period of development where these

youth are likely learning how to independently manage their CMC for the first time, it makes for a very complicated profile.

The complex relationship between stress and disease is best conceptualized as a bidirectional relationship where stress is a consequence of a CMC and stress can negatively impact health. Because having a CMC requires constant management, it is possible that there is a continuous activation of the SNS stress response which could lead to more wear and tear on the body, commonly referred to as allostatic load, where the body learns to adapt to the negative psychological and physiological effects (McEwen & Wingfield, 2003). By understanding how sAA behaves in this group, will help to provide a clearer picture how certain CMCs affect SNS activity. It is also important consider the impact different CMCs might have on sAA activity. Chronically stressed children with asthma have lower sAA levels compared to healthy controls (Wolf et al., 2008) with chronic stress suggesting certain CMC are associated with lower SNS activity thus lower sAA levels. Though the research on sAA and CMC is limited, increasing evidence demonstrates a relationship between certain CMCs and either over or underactivity of the SNS which presumably means higher or lower sAA levels (Fu, 2012). These findings add to the complicated nature of exploring the relationship between CMCs and sAA, however stress and CMCs are inextricably linked, and it is necessary to understand how CMCs impact biomarkers of stress.

Age and Stress

Young adulthood, where college-aged students fall, is a critical developmental period where health behaviors are formed. College is a significant transitional period that comes with a new level of independence (e.g., financial independence and independent living) often coupled with additional stressors. Many of these stressors are new and students might not be equipped

with coping skills to handle these stressors leaving them vulnerable to negative consequences of stress (Coiro et al., 2017). During this critical period, mental health disorders are at their highest in with 75% of youth experiencing their first clinical diagnoses during this time (Kessler et al., 2007). Over the years, the severity of psychopathology has also increased significantly with 88% college counseling directors reporting an increase in severity of disorders (Gallagher, Gill, & Sysko, 2000).

Young adulthood is a stable point in physical development where SNS activity is not influenced by age. The literature on age and sAA specifies certain periods of development (infancy and older adulthood) (Almela et al., 2011) where sAA might be impacted. SAA is absent in neonates and the onset of sAA activity coincides with the introduction of solid foods as sAA assists in digestion of carbohydrates (O'Donnell & Miller, 1980). An investigation on age and sAA demonstrated greater levels of sAA in older adults in response to stress (M_{age}=61.8) (Almela et al., 2011). Because the current study examines participants from a narrow age range outside of these sensitive ranges, it is unlikely that variability in sAA would be attributed to age. Young adulthood is an important stage of development as it represents a convergence of many different changes including academic, social, psychological, and financial changes; thus, it underscores the importance of understanding how the stress response is impacted.

Rationale

There are a number of factors that affect the stress response including severity of trauma, psychosocial factors, and the number, types, and frequency of stressors. These factors do not occur in isolation and often occur in the context of many other factors which creates a complex profile. Given the paucity of research on sAA as a biomarker of stress, it is difficult to predict how these different factors, individually and when considered together, might affect sAA output. It is evident that all these factors influence the stress response in some capacity but there is

limited research understanding the extent to which they impact SNS biomarkers. In addition, less is known about how all these different factors affect young adults. Young adulthood is a particularly vulnerable age group associated with changes psychologically, socially, and physically. This period of development comes with additional stress as these youth navigate increasing autonomy. Daily hassles are a plausible consequence of these changes during this time and it is likely that SNS response will be impacted thus resulting in increased levels of sAA. In addition, significant life stressors can complicate this profile and given the aforementioned research on life stress and the stress response, it could result in greater sAA output. Having a CMC during this challenging period of development will likely create additional stress or even exacerbate current symptoms related to a CMC. Thus, the current study helps to fill an important gap by examining the impact daily hassles, life stressors, chronic medical conditions have on sAA output.

Aims and Hypotheses

The first aim of the study evaluated the impact of daily hassles on sAA response profiles.

Hypothesis I. With regard to aim 1 it is hypothesized that participants' daily hassles will significantly predict greater sAA levels.

The second aim of the study examined the sAA response profile of adolescents with a history of life stressors.

Hypothesis II. With regard to aim 2, it is hypothesized that those with exposure to a greater number of life stressors will have greater average sAA output.

The third aim of the study conducted exploratory analyses to examine to sAA response output in those with a chronic medical condition compared those without.

Hypothesis III. With regard to aim 3, it is hypothesized that those with a chronic medical condition will have greater sAA output compared to those without a chronic medical condition.

The fourth aim of the study conducted exploratory analyses to examine how different types of stressors (CMC and life stressors) impact the diurnal response profile of sAA.

Hypothesis IV. With regard to aim 4, it is hypothesized that participants with greater life stressors (≥4 stressors) will demonstrate differences in diurnal sAA patterns compared to participants with fewer life stressors (≤3 stressors). Specifically, those with greater life stressors will show significantly elevated levels 30 minutes post waking compared to participants with fewer life stressors.

Hypothesis V. With regard to aim 4, it is hypothesized that those with a CMC will demonstrate differences in diurnal sAA patterns compared to participants without a CMC.

Extended Methods

Participants

265 undergraduates were recruited as part of a longitudinal study. Inclusion criteria included the following: 1) between the ages of 18-24 at the time of consent; 2) currently enrolled at the university; 3) fluency in English; 4) a mobile phone with unlimited text-message capabilities in order to participate in the daily assessments. Exclusion criteria included: 1) illiterate or inability to provide informed consent; 2) involvement in varsity athletic team. A subset of this sample (N=95) consented to participate in an optional saliva portion of the study in addition to participating in the full study.

Design

Participants were recruited through flyers posted around the university campus and recruitment information was distributed in classes and student organizations. The longitudinal study included an in-person baseline assessment followed by daily tracking over a two-week

period. At the conclusion of the two-week period, participants completed an in-person follow-up assessment. Participants were given the option to participate in a saliva portion of the study where they provided four saliva samples every day for three consecutive days during the two-week period between the baseline and follow-up assessment. For the purpose of this study, data was examined from baseline, daily tracking, and saliva collection.

Procedure

Baseline Assessment

Study staff reviewed the consent document with undergraduates which included explaining the optional saliva protocol of the study. Once participants consented, they completed a 60-minute battery of questionnaires through Qualtrics on a computer or iPad in a university psychology lab space. After completing the questionnaires, participants provided their cell phone number in order to receive daily text message reminders to complete daily surveys. Participants received $15 compensation after completion of the baseline assessment.

Daily Assessment

Participants received daily text message reminders in the evening to complete daily surveys assessing daily hassles. Participants earned $5 compensation each day they completed the survey with an opportunity to earn up to $70 for completing all 14 days of surveys.

Saliva

Participants had the opportunity to enroll in an optional saliva (passive drool) portion of the study. For the participants that consented to this portion of the study, they were provided with a saliva collection kit as well as verbal and written instructions on how to correctly provide saliva samples. Participants earned $15 compensation each day they complete the four samples with an opportunity to earn up to $45.

Schedule and Instructions

Participants were provided a study-developed schedule which indicated what days of the week participants provided their saliva samples. In addition, clear directions with photos were included to explain how to provide saliva samples. Participants were asked to avoid brushing teeth, eating a large meal, smoking and drinking thirty minutes to an hour prior to providing sample.

Saliva samples

The saliva kits were included in an insulated lunch bag that contained an ice pack in order for the sample to remain stable. In each bag there were three-gallon sized bags that each contained four individual bags of saliva collection items including a straw and a 2mL salivette. Participants were instructed to pool saliva in their mouth and use the straw to guide the saliva into the salivette. Participants were asked to provide four saliva samples each day for three consecutive days for a total of 12 samples. Participants returned their saliva kit at their follow-up appointment. Once, the study staff received the salivettes, they were frozen in a freezer at -20°C until the samples were shipped for analyses. Samples were shipped and assayed at the Salimetrics SalivaLab (Carlsbad, CA) using the Salmetrics Salivary Alpha-Amylase Assay Kit (Cat. No. 1-1902), without modifications to the manufacturer's protocol.

Measures

Background Information (Baseline)

Demographic information including age, sex, race, and ethnicity were obtained.

Physical Health Information Form (Baseline)

A study-developed questionnaire assessing the presence of chronic medical conditions and recurrent physical symptoms (e.g., asthma, diabetes, chronic pain, and chronic headaches).

Life Stressors Checklist Revised (Baseline).

The Life Stressors Checklist Revised (LSC-R) is a self-report measure that assesses 30 life stressors and other potentially traumatic events (e.g., financial difficulties, parental divorce, family member incarcerated, sexual abuse etc) (Wolfe et al., 1996). The checklist maps onto the DSM-IV Post Traumatic Stress Disorder Criterion A as it measures exposure to the event as well as experiencing intense helplessness, fear, or horror. Participants were given the option to opt out of answering questions if they did not want to. In addition, the LSC-R evaluates the timing of the event ("How old were you when it happened") and when the event ended; however, not all events have an endpoint. For example, a participant who has a family member who has been incarcerated multiple times or has experienced ongoing abuse, might not be able to accurately identify an endpoint thus this will be taken into consideration in the analyses of this measure. In addition, the LSC-R examines threat or potential death ("At the time of the event did you believe that you or someone else could be killed or seriously harmed?") and the relative impact ("How much is [sic] this affected your life in the past year?") based on a 1-5 score. There are several different methods for scoring the LSC-R. For the purpose of the study, two out of the three possible scoring methods will be used. One method includes scoring one point for each positively endorsed life stressor to derive an overall life stressor score ranging from 0-30. The second method assigns a weight to the positively endorsed life stressor based on the relative impact score ranging from 1-5. The scores range from 0-150, with higher scores indicating greater impact.

Hassles (Daily)

The 20-item Brief College Student Hassles Scale (BCSHS) is a self-report measure assessing the frequency of commonly reported hassles experienced by college students (e.g.,

academic, financial, and social stress). Participants rated their hassles on a 7-point Likert scale ranging from (1) "No hassle, not persistent at all" to (7) "High occurrence; extremely persistent, high frequency or duration." Initial validation of the BCSHS demonstrated good internal consistency based on total scores (Cronbach's $\alpha = 0.81$) (Blankstein & Flett, 1992; Blankstein et al., 1991). A total mean score is derived with higher scores indicating greater hassles.

Saliva Tracking Log

A study-developed saliva tracking log was filled out by participants on each saliva collection day. Participants were asked to include the time of day they took the four saliva samples. In addition, participants were asked questions related to alcohol and medication usage, smoking, and whether or not they ate, drank, or brushed their teeth within thirty minutes of providing a sample. Participants were asked to provide a subjective rating of their health ("compared to others your age rate your health for today") on each saliva collection day based on a 5-point Likert scale ranging from (1) Excellent to (5) Poor. Participants were also asked questions related to their health including medication taken over the last 48 hours. Because saliva is affected by hormone levels, females will be asked a separate list question. For example, "are you pregnant?" and "are you currently using contraceptives?"

Adherence

At the follow-up visit, participants completed a one-item questionnaire where they were asked to numerically (0-10) assess their level of adherence to the saliva protocol. In order to ensure participants assessed their adherence as accurately as possible, study staff provided the adherence measure after participants were given their study compensation. In addition, study staff were not present and participants returned their completed forms in a sealed box.

Saliva Collection

Participants collected samples from awakening, +30 minutes after waking, mid-day, and evening over three days (12 samples per participants).

Data Analyses

Analyses were conducted using SPSS v. 27 (Corp., 2020). Sample characteristics on demographics such as age, sex, and race were analyzed. The sAA samples were assessed for skewness and outliers ±3SD from the mean were removed. The distribution of daily hassles were transformed to fit a negative binomial distribution by dividing numbers by 10 and rounding to the nearest whole integer. Multilevel modeling was used to examine if daily hassles predicted greater sAA levels.

The first model tested hypothesis I: Daily hassles will significantly predict greater sAA levels. A random intercept only model and random slope model was run to see whether the level two units differ from each other on different days. The random variance ($u_{0i \text{ and }} u_{1i}$) from the models provided person-level average and person-level variability in daily hassle effects. The fixed effect represented the impact of daily hassles. Then a multilevel model was estimated to determine if daily hassles predicted greater sAA level.

A second analysis tested hypothesis II: Pearson *r* correlational statistics was used to determine the bivariate relationship between the number of life stressors and average sAA output.

The third model tested hypothesis III: Those with a chronic medical condition will have greater sAA output compared to those without a chronic medical condition. An independent sample t-test was run to see whether there was a difference in sAA output based on presence or absence of a chronic medical condition. Chronic medical conditions were dummy coded to represent presence (1) or absence (0) of CMC.

For exploratory analyses examining how different types of stressors impact the diurnal response profile of sAA, two one-way repeated measures multivariate analyses of variance (MANOVAs) were conducted to determine whether there were differences in diurnal sAA patterns in participants with greater life stressors and in participants with a CMC. For the first MANOVA, the sample was divided based on high and low stress. The cut-off value for these two groups was informed by previous research on one type of stressor, adverse childhood experiences (ACEs). The seminal ACEs study (Felitti et al., 1998) showed that individuals with four or more ACEs demonstrated a four-12 fold increase in health risks. As such, the high stress group included participants with ≥4 stressors (n=37) and low stress included participants with ≤3 stressors (n=46). Each participant had three samples for each timepoint (e.g., three awakening samples across three days). For both MANOVAS, average sAA values were used for all four timepoints. The sAA values across 3 days for each of the four timepoints were significantly correlated indicating (*r* range .45-.74) intra-individual stability over time (Veen et al., 2011).

Table 7.

Chronic Medical Conditions Reported in Current Sample (N=83)

Chronic Medical Conditions	N	%
Allergies	25	30.1
Obesity or Overweight	11	13.3
Asthma	4	4.8
Migraine Headaches	5	6.0
Food Allergies	4	4.8
Chronic Back or Neck Pain	4	4.8
Chronic Headaches (non- migraine)	3	3.6
Other	3	3.6
Hearing Impairment	2	2.4
Congenital Heart Disease	1	1.2
Celiac Disease	1	1.2
Cerebral Palsy	1	1.2
Cancer	1	1.2
Cystic Fibrosis	1	1.2
Diabetes	1	1.2
Ehlers Danlos Syndrome	1	1.2
Epilepsy or Seizure Disorder	1	1.2
Fibromyalgia	1	1.2
Hypertension/High Blood Pressure	1	1.2
Inflammatory Bowel Disease (Crohn's or Ulcerative Colitis)	1	1.2

Irritable Bowel Syndrome	1	1.2
Multiple Sclerosis	1	1.2
Recurrent Abdominal Pain	1	1.2
Sickle Cell Disease	1	1.2
Arthritis	-	-

Note. The other category included: exercise induced bronchial spasms, eczema, polycystic ovarian syndrome. The following health/mental health conditions were removed from the list: attention deficit hyperactivity disorder, anxiety, autism spectrum disorder, depression, eating disorder.

Table 8.

Prevalence of Stressors Reported on LSC-R.

Life Stressors	**N**	**%**
Someone close to you died (not unexpectedly)	46	53.5
Witnessed serious accident	31	36
Have any of the events mentioned above ever happened to someone close to you so that even though you didn't see it yourself, you were seriously upset by it?	31	36
Parental separation or divorce while living with them	25	29.1
Witnessed physical abuse (before age 16)	25	29.1
Experienced emotional abuse or neglect	21	24.4
Very serious physical or mental illness	16	18.6
Have you ever been bothered or harassed by sexual remarks, jokes, or demands for sexual favors by someone at work or school?	16	18.6
Someone close to you died suddenly or unexpectedly	15	17.5

Close family member sent to jail	13	15.1
Witnessed robbery, mugging or attack	10	11.6
Been in a Disaster	9	10.5
Involved in a Serious Accident	9	10.5
Serious money problems	9	10.5
Have you ever been robbed, mugged, or physically attacked by someone you did not know?	6	7
Before age 16, were you ever abused or physically attacked by someone you knew?	6	7
Responsible for taking care of someone close to you?	5	5.8
After age 16, were you ever touched or made to touch someone else in a sexual way because he/she forced you in some way or threatened to harm you if you didn't?	5	5.8
Other	5	5.8
After age 16, did you ever have sex (oral, anal, genital) when you didn't want to because someone forced you in some way or threatened to harm you if you didn't?	4	4.7
Had an abortion or miscarriage	3	3.5
Before age 16, did you ever have sex (oral, anal, genital) when you didn't want to because someone forced you in some way or threatened to hurt you if you didn't?	2	2.3
Have you been to jail	1	1.2
Foster Care or Adoption	1	1.2

Experienced physical neglect	1	1.2
After age 16, were you ever abused or physically attacked by someone you knew?	1	1.2
Have you been Separated or Divorced	0	0
Separated from a child against your will	0	0
Baby or child of yours ever had a severe mental handicap	0	0

Appendix B.

Measures

Chronic Medical Conditions

	Yes (1)	No (2)	No, but did in the past (3)
ADD/ADHD (B.PHIF1_1)	o	o	o
Allergies (B.PHIF1_2)	o	o	o
Anxiety (B.PHIF1_3)	o	o	o
Arthritis (B.PHIF1_4)	o	o	o
Asthma (B.PHIF1_5)	o	o	o
Autism/Autism Spectrum Disorder (B.PHIF1_6)	o	o	o
Congenital Heart Disease (B.PHIF1_7)	o	o	o
Celiac Disease (B.PHIF1_8)	o	o	o
Cerebral Palsy (B.PHIF1_9)	o	o	o
Cancer (B.PHIF1_10)	o	o	o
Cystic Fibrosis (B.PHIF1_11)	o	o	o
Depression (B.PHIF1_12)	o	o	o

Diabetes (B.PHIF1_13)	o	o	o
Eating Disorder (Anorexia, Bulimia, Binge Eating Disorder) (B.PHIF1_14)	o	o	o
Ehlers Danlos Syndrome (B.PHIF1_15)	o	o	o
Epilepsy or Seizure Disorder (B.PHIF1_16)	o	o	o
Fibromyalgia (B.PHIF1_17)	o	o	o
Food Allergies (B.PHIF1_18)	o	o	o
Chronic Back or Neck Pain (B.PHIF1_19)	o	o	o
Chronic Headaches (non- migraine) (B.PHIF1_20)	o	o	o
Hearing Impairment (B.PHIF1_21)	o	o	o
Hypertension/High Blood Pressure (B.PHIF1_22)	o	o	o
Inflammatory Bowel Disease (Crohn's or Ulcerative Colitis) (B.PHIF1_23)	o	o	o
Irritable Bowel Syndrome (B.PHIF1_24)	o	o	o
Migraine Headaches (B.PHIF1_25)	o	o	o
Multiple Sclerosis (B.PHIF1_26)	o	o	o

Obesity or Overweight (B.PHIF1_27)	o	o	o
Recurrent Abdominal Pain (B.PHIF1_28)	o	o	o
Sickle Cell Disease (B.PHIF1_29)	o	o	o
Other: (B.PHIF1_30)	o	o	o

Brief College Daily Hassles

Hassles are irritants that can range from minor annoyances to fairly major pressures, problems, or difficulties. They can occur few or many times. Directions: Please report hassles that you have personally experienced in the **previous 24 hours**. Please respond in terms of the persistence (frequency and duration) of experienced hassles. No hassle, not at all persistent

	(1) No hassle, not at all persistent	(2)	(3)	(4)	(5)	(6)	(7) extremely persistent hassle, high frequency and/or duration
Academic deadlines (B.BCSHS1)	○	○	○	○	○	○	○
Contact with girlfriend/boyfriend (B.BCSHS2)	○	○	○	○	○	○	○
Future job prospects (B.BCSHS3)	○	○	○	○	○	○	○
Relationship with people at work (B.BCSHS4)	○	○	○	○	○	○	○

Money for necessary expenses (B.BCSHS5)	○	○	○	○	○	○	○
Noise (B.BCSHS6)	○	○	○	○	○	○	○
Organization of time (B.BCSHS7)	○	○	○	○	○	○	○
Weight (B.BCSHS8)	○	○	○	○	○	○	○
Household chores (B.BCSHS9)	○	○	○	○	○	○	○
Family expectaton (B.BCSHS10)	○	○	○	○	○	○	○
Relationship with mother/father (B.BCSHS11)	○	○	○	○	○	○	○
Academic bureaucracy (B.BCSHS12)	○	○	○	○	○	○	○
Preparing meals (B.BCSHS13)	○	○	○	○	○	○	○
Exercise (B.BCSHS14)	○	○	○	○	○	○	○
Owing money (B.BCSHS15)	○	○	○	○	○	○	○
Job satisfaction (B.BCSHS16)	○	○	○	○	○	○	○

Financial security (B.BCSHS17)	○	○	○	○	○	○	○
Relationship with girlfriend/boyfriend (B.BCSHS18)	○	○	○	○	○	○	○
Relationship with brother/sister (B.BCSHS19)	○	○	○	○	○	○	○
College program requirements (B.BCSHS20)	○	○	○	○	○	○	○

Your Participation Schedule

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

Saliva Protocol Overview:

Day 1 of Saliva Collection: Complete "General Health Questions for Saliva Quality"

(on the next page) Put the ice pack in the freezer to use when transporting back to

campus.

Day 1, 2, & 3 of Saliva Collection: Follow "Instructions" below. If you forget a sample, you have an extra day before your follow-up to complete the missed sample. If you have questions about when to collect a sample, please call or email us at 773-325-5936 or healthyDPUundergradstudy@gmail.com

Day 1, 2, & 3 of Saliva Collection: Complete "Tracking Log" in this packet for every sample.

Instructions for Saliva Collection:

Step 1: Obtain the correct **large** Ziploc bag from your lunch bag (double check the day).

Step 2: Select the correct **small** Ziploc bag (double check the time).

Step 3: Open the **small** Ziploc bag and obtain the pre-labeled collection vial and the black straw.

Step 4: Allow saliva to pool in mouth for 5 seconds, then with head tilted forward, gently guide saliva through the straw into the vial. Fill to the 1.0mL line.

Step 5: Obtain the cap for the pre-labeled collection vial and screw the cap on the vial.

Step 6: Place the vial back in the **small** Ziploc bag.

Step 7: Immediately place small Ziploc with vial in refrigerator.

Step 8: Fill out "Tracking Log".

Day of Follow-Up Appointment:

- Collect all labeled samples from refrigerator.
- Place in insulated bag with frozen ice pack to keep cold.
- Bring to follow-up appointment in Byrne 553.

Things to Avoid Before Saliva Collection

Within 1 hour: No brushing teeth, eating a large meal, smoking, or drinking

Within 30 minutes: No eating or drinking (other than water)

Date:_____

General Health Questions for Saliva Quality – FILL OUT ON SALIVA COLLECTION DAY 1

Many things affect hormone levels in your saliva, so we need some information about your health.

1. Do you have any current dental problems? For example, cuts or sores in mouth, bleeding gums during brushing, or untreated cavities?
[ ] Yes
[ ] No

FEMALES ONLY – MALE PARTICIPANTS SKIP TO 7
2. Are you pregnant? [ ] Yes (SKIP TO 7) [ ] No [ ] I don't know 3. Have you had your period in the last three months? [ ] Yes [ ] No (SKIP TO 6) 4. Are you currently having your period today? [ ] Yes (SKIP TO 6) [ ] No 5. How many days ago did your period end? [ ]_____# of days [ ] I don't know 6. Are you currently using contraceptives (i.e. birth control or intrauterine device)? [ ] Yes → please provide the name of the contraceptive: _____ [ ] No

7. In the last 2 days (48 hours), have you taken any over-the-counter medicines or prescription medication (other than contraceptives)?
[ ] Yes
[ ] No

8. Please list each prescription medication or over-the-counter medicine you have taken in the last 2 days (48 hours). Please do not include contraceptives.

Medication Type/Drug Name	Do you take this medication every day or just when needed?
1.	
2.	
3.	
4.	
5.	

Saliva Tracking Log

	Example 1/1/18	Day One	Day Two	Day Three
Time Awake	**6** (AM)/PM	AM/PM	AM/PM	AM/PM
Time of Sample #1 (Waking)	**605** (AM)/PM	AM/PM	AM/PM	AM/PM
Time of Sample #2 (30 min after waking)	**635** (AM)/PM	AM/PM	AM/PM	AM/PM
Did you eat, drink, brush your teeth, or smoke before Sample 1 or 2?	Yes/(No)	Yes/No	Yes/No	Yes/No
Time of Sample #3 (2 PM)	**2** AM/(PM)	AM/PM	AM/PM	AM/PM
Did you eat, drink, brush teeth, or smoke within 30 min of Sample 3?	Yes/(No)	Yes/No	Yes/No	Yes/No
Time of Sample #4 (Right before brushing teeth/ going to bed)	**11** AM/(PM)	AM/PM	AM/PM	AM/PM
Did you eat, drink, brush teeth, or smoke within 30 min of Sample 4?	Yes/(No)	Yes/No	Yes/No	Yes/No
Did you smoke cigarettes today?	Yes/(No)	Yes/No	Yes/No	Yes/No
Did you smoke marijuana today?	Yes/(No)	Yes/No	Yes/No	Yes/No
Did you drink alcohol today?	Yes/(No)	Yes/No	Yes/No	Yes/No
Did you take any medications? Please list:	(Yes)/No **2 Tylenol extra strength**	Yes/No	Yes/No	Yes/No
Compared to others your age, rate your health for today: 1=Excellent 2=Very Good 3=Good 4=Fair 5=Poor	**3**			

Instructions for Saliva Collection

Supplies:

Frozen ice pack

Insulated bag

Saliva collection kits

Step 1: Obtain the correct **large** Ziploc bag from your lunch bag (double check the day).

Step 2: Select the correct **small** Ziploc bag (double check the time).

Step 3: **Open the small Ziploc bag and obtain the pre-labeled collection vial and the black straw.**

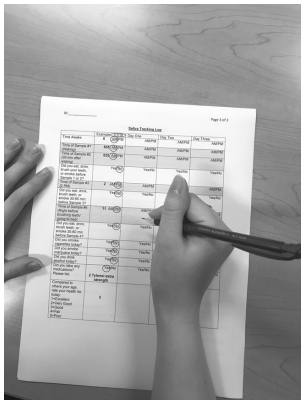
Step 4: Allow saliva to pool in mouth for 5 seconds, then with head tilted forward, gently guide saliva through the straw into the vial. Fill to the 1.0 mL line.

Step 5: Obtain the cap for the pre-labeled collection vial and screw the cap on the vial.

Step 6: Place the vial back in the **small** Ziploc bag.

Step 7: Immediately place small Ziploc with vial in refrigerator.

Step 8: Fill out "Tracking Log".



Adherence

How closely were you able to follow the saliva collection guidelines (e.g., on time, refrigeration, no eating)?

0	1	2	3	4	5	6	7	8	9	10
Not at all				Half of the time					100% of the time	