Chapter 27
Use of Saliva to Better Understand the Daily Experience of Adulthood and Aging

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Abstract This chapter reviews the use of saliva to assess age-related changes in important biological systems, describes how saliva can be used to assess naturally occurring fluctuations of biomarkers in adults' daily lives, and offers cutting-edge statistical approaches that can help answer research questions that involve these multivariate and dynamic phenomena. This chapter highlights the use of saliva to assess day-to-day variability in biological markers across adulthood. Salivary biomarkers offer a unique and innovative window into investigating the daily experiences of midlife and older adults. Using findings from multiple daily diary studies where participants provide multiple saliva samples each day, we describe within-and across-day patterns of cortisol, dehydroepiandrosterone sulfate (DHEA-S), and salivary alpha-amylase (sAA). Using multilevel and latent state-trait modeling, we show differentiated patterns in each of these biomarkers across the day. Specific attention is paid to age differences in the daily patterning of these salivary biomarkers as well as their links to stressful events. The chapter also reviews recent research that links daily salivary biomarkers to long-term health and well-being. Recommendations for the design, collection, and statistical modeling of daily assessments of salivary biomarkers are also provided.

Keywords Cortisol · Dehydroepiandrosterone sulfate (DHEA-S) · Salivary alpha-amylase (sAA) · Daily stress · Multilevel modeling · Latent state-trait modeling

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27.1 History of Salivary Bioscience in Aging

Saliva plays a critical role in maintaining health and can provide insight into underlying physiological processes. Composed primarily of water, saliva also contains several other compounds, including electrolytes and proteins, that help to ensure proper oral health and initiate digestion (Dodds, Johnson, & Yeh, 2005). Healthy individuals produce approximately 1–1.5 L of saliva per day. Alterations in saliva, whether by volume loss or compositional changes, have the potential to negatively impact oral health, which, in turn, has implications for overall systemic health (Varga, 2012). Aging is one factor that has been linked with both decreased salivary flow (Smith et al., 2013) and alterations in its constituent parts (Nagler & Hershkovich, 2005), even when not accompanied by other risk factors, such as medication use (Wolff et al., 2017) and chronic health conditions (Mortazavi, Baharvand, Movahhedian, Mohammadi, & Khodadoustan, 2014; Zhang et al., 2016).

Importantly, the relationship between saliva and aging is not static: research indicates that saliva flow rate (Affoo, Foley, Garrick, Siqueira, & Martin, 2015) and the composition of biological markers found in saliva (for review, see Piazza, Almeida, Dmitrieva, & Klein, 2010) fluctuate across daily life. Thus, for research scientists interested in aging, saliva provides a critical window into processes that until relatively recently could only be examined using invasive means. The goal of this chapter is threefold. First, we review the use of saliva to assess age-related changes in important biological systems, including the hypothalamic–pituitary–adrenal (HPA) axis and the sympathomedullary (SAM) pathway. Second, we describe how saliva can be used to assess naturally occurring fluctuations of biomarkers in adults' daily lives by summarizing findings from two research projects that collected and assessed multiple samples of saliva across a series of days. Third, we describe cutting-edge statistical approaches that can help answer research questions that involve these multivariate and dynamic phenomena.

Age, Saliva, and Underlying Biological Processes For years, salivary flow has been examined as a marker of aging (e.g., Affoo et al., 2015; Smith et al., 2013). Scientific developments, however, have enabled researchers to expand their inquiry into identifying components of saliva that are indicative of underlying physiological processes. Two systems that have been widely examined in conjunction with saliva and aging are the HPA axis and the SAM pathway. Both systems are critical for engaging the body's stress response, with the SAM pathway providing an immediate response to a stressor and the HPA axis providing a longer term hormonal response. Both systems also show several age-related changes, many of which mimic those that occur with increased stressor exposure (for review, see Piazza et al., 2010). Salivary biomarkers of the HPA axis commonly used in research include the hormones cortisol and dehydroepiandrosterone sulfate (DHEA-S), and for the SAM pathway, the digestive enzyme salivary alpha-amylase (sAA).

Cortisol and Age-Associated Changes Cortisol is a product of the HPA axis that is essential for regulating metabolism, moderating inflammation, and mobilizing

energy. It is released throughout the day in a pulsatile fashion, with increased pulses during times of acute stress (Tsigos, Kyrou, Kassi, & Chrousos, 2000). Release of cortisol in response to stress is a self-regulated, multistep process. When a stressor is perceived, the paraventricular nucleus of the hypothalamus triggers the release of corticotrophin-releasing hormone (CRH). Upon its release, CRH triggers the release of arginine vasopressin (AVP) and adrenocorticotropine hormone (ACTH). ACTH, which circulates to the adrenal glands, then stimulates the release of corticosteroids (e.g., cortisol) and other glucocorticoids (for review, see Klein & Corwin, 2007). After an optimal amount of corticosteroids have been released, the HPA axis restores itself through a negative feedback loop, whereby it dampens that release of CRH and ACTH, and, in turn, cortisol.

Cortisol exhibits a robust diurnal pattern: upon awakening, levels increase, reaching a peak between 30 and 45 min later, and then gradually decrease throughout the day until reaching a nadir in the evening hours (Kirschbaum & Hellhammer, 1989). Increasing age is associated with alterations in this pattern, including higher levels across the day, particularly in the evening. Older age is also associated with an attenuated awakening response, and an overall flatter diurnal pattern (for review, see Epel, Burke, & Wolkowitz, 2009). Research indicates that additional factors (e.g., stress) may exacerbate these age-associated changes (e.g., Piazza, Dmitrieva, Charles, Almeida, & Orona, 2018).

DHEA-S and Age-Associated Changes DHEA-S is another commonly assessed salivary biomarker of the HPA axis. Unlike cortisol, which is a catabolic hormone, DHEA-S is an anabolic hormone primarily secreted by the adrenal cortex, as well as ovaries and testes (for review, see Maninger, Wolkowitz, Reus, Epel, & Mellon, 2009). DHEA-S is protective against a number of conditions experienced more often in later life, including decline in cognitive functioning, atherosclerosis, cancer, and diabetes (Krug, Ziegler, & Bornstein, 2008). Levels of DHEA-S reach their peak in the morning and steadily decline across the remainder of the day (Klein et al., 2008). DHEA-S significantly declines across the lifespan. After the age of 40 years, plasma concentrations decline by 2% each year; for men, the decline ranges between 1% and 4% per year. DHEA-S reaches its nadir between the ages of 65 and 70, which is also the time at which health typically declines (e.g., Tannenbaum, Barrett-Connor, Laughlin, & Platt, 2003).

The SAM Pathway and sAA The SAM pathway, commonly referred to as the "fight or flight response," refers to the release of the catecholamines, epinephrine (EPI), and norepinephrine (NE) from the adrenal medulla upon activation of the sympathetic branch of the autonomic nervous system (for review, see Klein & Corwin, 2007). Although EPI and NE are found in saliva, direct assessment of the catecholamines is not recommended in field studies, due to the length of time it takes for them to transfer from blood to saliva and their stringent processing requirements (e.g., Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). Given these constraints, researchers have turned to the digestive enzyme salivary α-amylase (sAA) as a proxy of SNS activation (Nater & Rohleder, 2009). The justification for using sAA is that when catecholamines are released in response to SNS activation, they trigger

changes in salivary gland receptors, ultimately altering their activity (Nederfors & Dahlof, 1992). Although there is some conflicting evidence in the literature, several studies indicate that there is an association between sAA and the release of NE and EPI (e.g., Ditzen, Ehlert, & Nater, 2014; Thoma, Kirschbaum, Wolf, & Rohleder, 2012). As such, sAA has thus been used as a marker of SNS activity across a number of studies. sAA exhibits a diurnal pattern with levels moderately low upon awakening, dropping briefly at 30 min post awakening, and gradually increasing throughout the day (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). With age, there is greater sAA output, but an attenuation of slope (e.g., attenuation of the diurnal increase), although basal levels are higher (Nater, Hoppmann, & Scott, 2013).

Collection of Salivary Biomarkers Assessment of biomarkers found in saliva has several benefits over self-reported measures, as well as other objective measures of biomarker collection, such as blood draw. Compared to more invasive assessments, salivary biomarkers are easy to collect, less burdensome to participants and researchers, and can be used in field studies, where frequent assessment of biomarkers is warranted. They also provide an objective indicator of physical health, which is difficult to attain through self-reported measures alone. Moreover, self-reported measures and salivary biomarkers in conjunction have the potential to provide a more comprehensive picture of an individual's state of health and the factors that influence it. To this aim, Sect. 27.2 of the chapter shows how the saliva is used to gather information on within- day and across-day assessment of biological functioning during adulthood.

27.2 State of the Knowledge

The collection of saliva has played a critical role in understanding how the daily lives of adults change as they age and how daily experiences shape health and well-being. These have been the overarching objectives of the National Study of Daily Experiences (NSDE) and the Daily Stress and Health study (DaSH). Both of these projects combine self-reports of daily experiences such as mood, physical symptoms, and stressors with multiple assessments of saliva to better understand the daily experiences of adulthood. The use of saliva in both projects is described below.

27.2.1 NSDE: Saliva as a Window into Daily Stress of Adulthood

The NSDE is one of the projects of the Midlife in the United States study (MIDUS). The protocol involves two measurement bursts collected 10 years apart. Each burst consists of eight consecutive days of telephone interviews of daily stressors and well-being combined with multiple assessments of daily salivary cortisol

(4 occasions × 4 days). Consisting of over 25,000 daily interviews from 2022 adults ranging in age from 24 to 85, the NSDE is the largest longitudinal diary study of daily health and well-being in the USA. Early findings from the documented age and sociodemographic patterns of self-reported daily experiences include stressful experiences, mood, and physical health (Almeida, 2005; Almeida, Neupert, Banks, & Serido, 2005). The inclusion of saliva collection multiple times a day on multiple days has offered a multitude of opportunities to enhance this line of research. We first provide a description of how we collected saliva and then review only a few of these opportunities as they relate to aging research using salivary cortisol as an example.

Saliva Collection Protocol and Compliance All of our saliva was self-collected by participants in their homes. As part of recruitment, respondents received a Home Saliva Collection Kit one week prior to their initial phone call, containing 16 numbered and color-coded salivettes, as well as a detailed instruction sheet and a compact disk with video instructions. In addition to written and video instructions, telephone interviewers review the collection procedures and answer any questions the evening prior to the initial collection day. Respondents provide four saliva samples per day on days 2–5 of the eight-day period to be assayed for cortisol and sAA. To maximize compliance, our collection procedures are designed to be as convenient as possible. On saliva collection days, respondents will produce four saliva samples throughout the day: upon awakening, 30 min after getting out of bed, before lunch, and at bedtime. Data on the exact time respondents provide each saliva sample will be obtained from the nightly telephone interviews and from a form sent with the collection kit.

Compliance to the saliva collection protocol has been validated in a series of papers (Almeida, McGonagle, & King, 2009; Almeida, Piazza, & Stawski, 2009; Stawski, Cichy, Piazza, & Almeida, 2013). The correlations of self-reported times across collection occasions were all above 0.9. The correlations between self-reported times and times obtained from the "smart box" (a box provided to 25% of the sample that recorded when they placed their salivettes into the box) ranged from 0.75 for the evening occasion to 0.95 for the morning occasion. Of the 2022 respondents from the second wave of MIDUS Diary Project, 1736 participated in saliva collection (86%). Of the 27,776 possible saliva samples (1736 participants × 16 samples), there were 874 missed or unreliable samples, samples that could not be linked to a specific day, or samples with insufficient volume to detect cortisol, which resulted in 97% usable samples (26,902).

Using Saliva to Assess Daily Stress in Adulthood Most studies of psychosocial stress and physiology demonstrate elevated cortisol levels in response to laboratory-controlled acute psychological stressors (Dickerson & Kemeny, 2004). Less is known about the relationship between naturally occurring stressors and cortisol responses (Dettenborn et al., 2005; Polk et al., 2005). NSDE is the largest study to investigate the relationship between naturally occurring stressors and cortisol. Examining patterns of cortisol throughout the day (collected through saliva) has enabled us to determine HPA axis responses to external environments. For example,

failure to activate the HPA axis in the morning and deactivate it in the evening may indicate difficulty disengaging from external demands, leading to inhibition of restoration and recovery processes (Sapolsky, Krey, & McEwen, 1986).

An initial set of analyses examined age and gender differences in diurnal cortisol. The entire daytime cortisol trajectory (including waking, peak, and nadir values and AUC) was higher in older than in younger participants and in men than in women (Almeida, Piazza, Stawski, & Klein, 2011; Karlamangla et al., 2018). There was a clear dose response with age, with the oldest group having significantly higher mean levels and total concentrations assessed by area under the curve across the day (AUC) than the middle group (ages 50–64 years), and the latter having significantly higher mean levels (and AUC) than the youngest group. The male–female differences in mean nadir and AUC were comparable to corresponding differences between the mid-age and youngest age groups (Karlamangla et al., 2018).

Integrating Psychosocial and Biological Stress Our dynamic assessment of diurnal cortisol in relation to both daily and chronic stress processes has resulted in many important findings (Almeida, McGonagle, et al., 2009; Almeida, Piazza, et al., 2009). Thus far, our work has linked elevated diurnal cortisol rhythms and cortisol levels to increasing age (Almeida, Piazza, et al., 2009), daily stressors (Stawski et al., 2013), negative affect (Piazza, Charles, Stawski, & Almeida, 2013), social strain (Friedman, Karlamangla, Almeida, & Seeman, 2012), poor cognitive performance (Stawski et al., 2011), early life adversity (Taylor, Karlamangla, Friedman, & Seeman, 2010), and cancer survivorship (Costanzo, Stawski, Ryff, Coe, & Almeida, 2012). We have also examined diurnal cortisol patterns to understand the day-to-day physiological effects of social role transitions, including widowhood (Ong, Fuller-Rowell, Bonanno, & Almeida, 2011), nonnormative parenting (Barker, Greenberg, Mailick Seltzer, & Almeida, 2012; Seltzer et al., 2010), and early and late retirement (Almeida & Wong, 2009). In contrast, positive psychosocial resources, such as perceived partner responsiveness among married couples (Slatcher, Selcuk, & Ong, 2015) and daily positive events (Sin, Graham, & Almeida, 2015), are associated with adaptive diurnal cortisol profiles.

Current analyses have focused on a specific diurnal rhythm characterized by both a low peak following waking and a failure to lessen cortisol output throughout the day, which we have identified as compressed dynamic range (CDR; Karlamangla et al., 2018). CDR is an innovative potential marker for chronic stress and is linked to lower education, minority status, and chronic health conditions (Dmitrieva, Almeida, Dmitrieva, Loken, & Pieper, 2013; Karlamangla et al., 2018).

Other recent work also reveals exciting linkages of daily experiences with psychophysiology, neuroscience, and biomarker assessments. New findings from the MIDUS Neuroscience Project show that sustained activity in the striatum and dorsolateral prefrontal cortex to positive stimuli is linked to better cortisol regulation and higher well-being (Heller et al., 2013).

Researchers have linked high levels of cortisol to a number of health problems, yet it remains unclear how or why daily patterns of cortisol change over time, how diurnal cortisol rhythms are influenced by changes in psychosocial factors, or the

mechanisms whereby cortisol patterns influence long-term health outcomes. Our team and others are currently addressing these issues. For example, early life adversity appears to leave a long-term imprint on cortisol secretion dynamics, reducing diurnal dynamic range without increasing total secretion. This points to the importance of examining the adaptation capacity of physiological systems when studying the impact of early life and chronic stresses on adult health (Karlamangla et al., 2018). Another recent paper documented that older participants with dysregulated profiles across all interview days (i.e., all days elevated, flattened, or a combination of elevated and flattened) showed greater concurrent inflammation risk burden indexed by Il6 and CRP as well as more functional limitations 10 years later (Piazza et al., 2018).

In addition to cortisol, we have been assaying salivary alpha-amylase (sAA), which is a minimally invasive and easily obtainable surrogate marker of individuals' chronobiology and sympathetic nervous system (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Keenan, Licinio, & Veldhuis, 2001). Studies suggest that sAA levels increase in response to stressors such as extreme exercise, heat and cold stress, written examinations (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Chatterton, Vogelsong, Lu, & Hudgens, 1997; Skosnik, Chatterton, Swisher, & Park, 2000), and laboratory-based stressors (Gordis, Granger, Susman, & Trickett, 2008; Nater et al., 2005, 2006). We have found that perceptions of and affective reactions to daily stressors were associated with vagal withdrawal and increased sympathetic predominance, as indexed by heart rate variability (Sin et al., 2015). The inclusion of salivary cortisol and sAA provides an integrated perspective on biological stress functioning by examining both HPA axis and SNS response (Granger et al., 2007; Lovallo & Thomas, 2000; McEwen, 2000). These systems interact with one another, leading to dynamic, synergistic effects on the body. Both sAA and cortisol follow diurnal patterns that adjust in response to stressful events (Granger et al., 2003; Nater et al., 2005) and may change from young adulthood to older ages (Nater et al., 2007).

27.2.2 DaSH Findings: Saliva as a Window into Caregiving

Biomarkers obtained from saliva can also be used in the study of a variety of problems in adulthood that involve exposure to stressors and in interventions designed to mitigate the effects of stress. One area where there is a growing interest in the use of biomarkers is family caregiving (Lovell & Wetherell, 2011; Von Känel et al., 2012). Family caregiving to individuals with dementia (IWDs), which usually is sustained for years, is both physically and emotionally challenging (Aneshensel, Pearlin, Mullan, Zarit, & Whitlatch, 1995). Caregiving to IWDs has been found to be more challenging compared to other types of chronic conditions, as consequence of the degenerative nature of the disease and the frequency that caregivers must contend with behavioral, cognitive, and emotional problems (Pearlin, Mullan, Semple, & Skaff, 1990). Thus, it is not surprising that dementia family caregivers are at the

highest risks for adverse health changes compared to caregivers of persons with other types of disabilities (Vitaliano, Zhang, & Scanlan, 2003). Although various types of interventions to promote caregiver health have been tested, their effects have typically been modest (Sörensen, Pinquart, & Duberstein, 2002).

One promising approach for caregivers of IWDs is the use of adult day services (ADS). ADS and other types of respite care provide family caregivers time away from the IWD, thereby reducing their exposure to care-related stressors and giving them time to engage in other activities (Zarit et al., 2011). Building on this finding, the Daily Stress and Health (DaSH) study was designed to explore the effects of high and low stress days on caregivers' salivary stress biomarkers (Klein et al., 2016; Zarit, Kim, Femia, Almeida, & Klein, 2014). Participants were primary caregivers of IWDs and were using at least 2 days of ADS a week. Following an initial in-home interview, caregivers provided five saliva samples each day (before getting out of bed, 30 min after getting out of bed, before lunch, before dinner, and before bed) for eight consecutive days and completed brief telephone interviews at the end of each day that obtained information about daily stressors and affect and also addressed any problems associated with saliva collection. The 8-day period was selected in order to obtain data on both some high stress days when caregivers provided all the care and on low stress days when the IWD attended ADS. The comparison across multiple days functioned in effect as a classic treatment reversal design, where treatment benefits would be expected in the presence of an intervention (ADS use days) and not when the intervention was withdrawn. Saliva collection and assay followed standard and validated procedures for salivary cortisol, dehydroepiandrosterone sulfate (DHEA-S), and salivary alpha-amylase (sAA) as biomarkers of the hypothalamic–pituitary–adrenal (HPA) axis and autonomic nervous system (ANS), respectively. Follow-up interviews with participants were conducted at 6 and 12 months, and obtained information about affect and health, as well as transitions regarding caregiving (institutionalization and/or death of the IWD).

Initial analyses confirmed that the intervention had its intended effect in lowering caregivers' exposure to care-related stressors on days the IWD attended ADS, compared to days when caregivers provided all the care. Turning to HPA markers, caregivers had improved regulation of cortisol on ADS days and of DHEA-S on days following ADS use (Klein et al., 2016; Liu, Almeida, Rovine, & Zarit, 2016; Zarit et al., 2014). Typical of other chronic stress situations, many of the caregivers had blunted cortisol awakening responses (CAR) on days they provided all the care and increased CAR when the IWD attended ADS (Liu, Kong, Bangerter, Zarit, & Almeida, 2018). Likewise, caregivers had relatively low daily levels of DHEA-S, which is typical of other chronic stress situations (e.g., Lennartsson, Theorell, Kushnir, Bergquist, & Jonsdottir, 2013), but output increased on days following ADS use.

Additionally, ADS use impacted ANS regulation as indicated by diurnal trajectory of sAA. Previous laboratory studies had found that ANS dysregulation and poor sAA recovery from pre- to post-stress conditions relate to health problems such as fatigue and frequency of illness among children (Granger et al., 2007). Findings from the DaSH study showed that, controlling for daily ADS use, greater ADS use

across 8 days was associated with a more prominent rise between 30 min after wake-up and before lunch, and a more prominent decline between before lunch and late afternoon, whereas fewer ADS days were associated with a more flattened and blunt sAA diurnal rhythm (Liu et al., 2017).

The DaSH study also explored the potential effects of the stress biomarkers on health over a 1-year period (Liu, Almeida, Rovine, & Zarit, 2017). During that time, some caregivers experienced major transitions, including placing the IWD into a residential care setting or turning caregiving over to another family member. Since those transitions alter stressor exposure, they were taken into account in the analyses. Among caregivers who transitioned out of the role, and who used fewer than average ADS days per week at baseline, lower daily cortisol total output and lower daily sAA total output were associated with increasing functional limitations over 12 months. Caregivers who experienced a transition but had more than average ADS days per week did not show such patterns of association. Among caregivers not experiencing a transition, functional health was stable and showed no association with baseline levels of any of the biomarkers. These findings suggest that ADS use may play a protective role in health outcomes (Liu, Almeida, et al., 2017).

27.3 Issues and Challenges: Assessing Daily Salivary Biomarkers in the Field

Conducting daily diary studies that incorporate saliva collection presents unique challenges due to the respondent burden and lack of investigator control in collecting salivary biomarkers. The next section provides evidence of the feasibility, reliability, and validity of the use of saliva in daily studies.

27.3.1 Challenge 1: Feasibility of Collecting Saliva in Field Studies

Given the requirements of the daily designs that involve multiple interviews and multiple saliva collections throughout the day, a key issue is the feasibility of implementing this approach in large social surveys. Findings from the telephone-based NSDE, DaSH, and other studies suggest that overall, respondents are willing to participate and complete the protocol. Participants overwhelmingly participated in the saliva collection protocol. Of the 2022 respondents who completed the second wave of the NSDE, 1736 provided saliva samples (86%). Perhaps more remarkable was the rarity of missed saliva collections—approximately 3%. Rates of retention and successful saliva collection were similar in the DaSH study, despite the levels of stress experienced by the sample (Klein et al., 2016). The NSDE protocol has been adapted by other social surveys that include samples of elderly couples, mothers of

children with autism, and hotel workers, their spouses, and children (Yorgason, Almeida, Neupert, Spiro, & Hoffman, 2006; O'Neill et al., 2009; Seltzer et al., 2010). Although the sample and protocol are somewhat different, the recruitment and retention rates are similar to the NSDE.

27.3.2 Challenge 2: Reliability

If respondents agree to participate in daily saliva studies, will they be compliant? Adherence to the saliva collection protocol is critical in obtaining reliable assessments of diurnal cortisol. We gauged compliance by contaminated samples and timing of collection. Of the 27,776 possible saliva samples (1736 × 16 samples), there were 874 missed or unreliable samples, samples that could not be linked to a specific day, or samples with insufficient volume to detect cortisol (~3%). These data resulted in final cortisol analyses based on 97% usable samples ($N = 26{,}902$).

Data on the exact time respondents provide each saliva sample were obtained from the nightly telephone interviews and on a paper-and-pencil log sent with the collection kit. In addition, approximately 25% of the respondents ($N = 430$) received a "smart box" to store their salivettes. These boxes contained a computer chip that recorded the time respondents opened and closed the box. The correlations of self-reported times across collection occasions were all above 0.9. The correlations between self-reported times and times obtained from the "smart box" ranged from 0.75 for the evening occasion to 0.95 for the morning occasion. Assessing diurnal rhythm also requires careful timing of collection. The biggest challenge we faced was collection of the second sample of the day (30 min after waking). Missing this time window could alter the assessment of the cortisol awakening response (CAR) parameter of the diurnal rhythm. On approximately 10% of our collection days, respondents either provided the sample too early or too late to capture the CAR. Additional protocols could be implemented to increase adherence to this critical time window, including alarm clocks, electronic time stampers, and additional instructions. Indeed, our team at Penn State recently produced an instructional video in collaboration with our local public television affiliate on how and when to collect saliva (a copy is available upon request to the first author). Future data collections will include test instructions on a DVD in the saliva collection kits.

27.3.3 Challenge 3: Validity

Lack of control over saliva collection in social surveys poses risks to the interpretation of cortisol. We used NSDE data to assess the validity of field assessments of diurnal cortisol. We compared components of the diurnal rhythm of cortisol in the NSDE with smaller samples in more controlled research settings and with more within-day assessments (Almeida, McGonagle, et al., 2009). We compared our

cortisol values for the CAR to the findings of four studies combined and presented by Wüst et al. (2000).

Mean cortisol levels in these published studies were very similar to the NSDE for both awakening cortisol and for cortisol measured 30 min after awakening. We also compared the daily decline slopes from the NSDE with four studies reviewed in Stone et al. (2001). It is important to note that these previous studies had more control over the study protocol such as face-to-face instruction and telephone reminders for collection. Despite the differences between these four studies and NSDE in the number of participants, the number of saliva collections throughout the day, and the number of days assessed, values for the slopes are remarkably parallel (Almeida, McGonagle, et al., 2009; Almeida, Piazza, et al., 2009).

27.3.4 Challenge 4: Costs

The average cost of the NSDE and DaSH protocol was approximately $350 per respondent. The collection kits including salivettes, packaging materials, boxes, and postage cost $44. The cortisol assaying was conducted in the Biological Psychology Laboratory at the Technical University of Dresden at a cost of approximately $96 ($6 per sample × 16 samples). The interviewing cost via Penn State Survey Research Center was approximately $160 per person ($20 per interview × 8 interviews). Finally, the participants were given $50 as incentive to finish the protocol.

27.4 Future Directions in Analyses

Salivary stress biomarkers that are collected intensively within a day and repeatedly across multiple days have mostly been modeled using univariate approaches. However, the field has also seen some innovative models in recent years taking multivariate approaches, where more than one type of biomarkers either from the same person or spouses are modeled simultaneously. We will next contrast these varying modeling approaches to daily biomarkers.

The univariate approach entails modeling one and only one type of biomarker as the outcome, and there are three general approaches. The first approach utilizes a daily summary or composite score, which congregates one type of repeatedly measured biomarkers within a day as one daily measurement. Some methods include the average score within the day, the daily total outputs commonly calculated as the diurnal area under the curve with respect to ground (AUCg) (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003), and the difference score between specific samples within a day such as the cortisol awakening response (CAR). The CAR can be calculated as the increase (CARi) in cortisol levels using the first two morning samples (Chida & Steptoe, 2009; Pruessner, Hellhammer, Pruessner, & Lupien, 2003), or more recently, as the rate of change in cortisol concentration that occurs

during the first 30 min after waking up, which we will explain next (Leggett, Liu, Klein, & Zarit, 2016).

The second univariate approach aims to define the diurnal slope as the linear rate of change in biomarker levels across the day. This approach is most useful for modeling diurnal trajectories of salivary biomarkers that demonstrate circadian rhythms such as diurnal cortisol and salivary alpha-amylase. It typically utilizes each single, repeatedly measured biomarker sample within- and across days as the outcomes. Although this approach entails modeling more than one measurement within the day as the outcome, it is still considered as the univariate approach because only one type of biomarker is considered in the model. Multilevel growth curve modeling based on the linear mixed models, and specifically spline growth curve models, can be used to model these intensively measured biomarkers within a day and across days. Various diurnal slopes have been defined, including the cortisol awakening response (CAR) slope, the cortisol diurnal decline slope, and the salivary alpha-amylase morning decline and diurnal rise slopes. Both of the aforementioned univariate approaches are useful tools for understanding the impact of daily experiences and other personal traits on the daily physiology of one type of biomarker. Alternatively, the composite scores and diurnal slopes can also serve as daily physiological predictors of long-term physical health as detailed in some of the DaSH studies we mentioned earlier (Leggett et al., 2016; Liu, Granger, et al., 2017).

However, studies also show a major issue for these daily metrics of physiology. Specifically, they may fail to capture any stable individual differences in cortisol functioning over any period greater than 1 month. For example, the stability estimates were the lowest for CAR, moderate for diurnal slopes, and inconsistent for the AUCg across 8 months to 6 years (Doane, Chen, Sladek, Van Lenten, & Granger, 2015). Thus, the third univariate approach applies the latent state-trait modeling to diurnal cortisol levels to specifically capture the underlying individual differences by the latent trait cortisol (LTC) factor, as well as any state-specific situational influences and random errors by the latent state cortisol factor (Steyer, Mayer, Geiser, & Cole, 2015). In the first LTC study, Kirschbaum et al. (1990) showed that the variability in morning cortisol levels was largely accounted for by a latent trait factor, whereas the afternoon levels were largely accounted for by the latent state factor. Doane et al. (2015) further examined the reliability, validity, and stability of the LTC based on a study where salivary cortisol was repeatedly measured five times a day, over 3 days, and across three waves over 9 months. They used the first two morning samples to derive the LTC factor within and across waves. The study showed that the LTC was distinct from the CAR, differentially predictive of varying components of the diurnal slopes, and stable across the assessment waves (Doane et al., 2015).

Progressing forward, one innovative and potentially important approach for diurnal biomarkers, however, is to use multivariate models to characterize the neuroendocrine and autonomic impact of daily experiences. Guided by the Allostatic Load theory (Juster, McEwen, & Lupien, 2010; Marin et al., 2011; McEwen, 2003, 2004; Miller, Chen, & Zhou, 2007; Miller, Cohen, & Ritchey, 2002; Rohleder,

Marin, Ma, & Miller, 2009; Zarit et al., 2011), optimum hypothalamic–pituitary–adrenal (HPA) axis and autonomic nervous system (ANS) functioning in response to stress entail coordination across these systems, which is regulated by the central nervous system (Bauer, Quas, & Boyce, 2002). Although the HPA axis and ANS are interconnected physiologically, their responses to stressors may become asymmetrical under aging and/or chronic stressor exposure, (Gordis et al., 2008) such as dementia caregiving. Thus, the HPA–ANS dissociation may reflect inefficient central coordination, which can become a form of allostatic load (Bauer et al., 2002; McEwen, 2004), and further impact individuals' affective well-being (McEwen, 2003).

The HPA–ANS synchrony is the simultaneous reactivity of these two systems under chronic stressor exposure. The HPA axis and ANS activities can be measured by cortisol and salivary alpha-amylase (sAA) levels, respectively. The HPA–ANS synchrony can be measured by the dynamic covariations between daily total outputs of these biomarkers collected intensively over time. Synchrony presents when cortisol and sAA levels covary positively in the process of stress regulation, whereas asynchrony or asymmetry is indicated by a relatively strong response in cortisol or sAA levels over the other (Gordis et al., 2008).

Another type of multivariate approach serves the dyadic research perspective and is best for modeling the co-regulation or synchrony of the physiological processes between various forms of dyads as dynamic systems. In such models, one type of biomarker is modeled simultaneously with measurements typically collected from both parties of one dyad such as husbands and wives. Generally, daily summary or composite scores will be calculated first from each of the dyads respectively, and multilevel cross-lagged models can be utilized to show how the physiology of one person in the dyads impact that of the other person, on the same day and/or on the next day; covariates of the synchrony can also be modeled. For example, one study showed that on days when one experienced faster or slower cortisol decline, the spouse also showed similar cortisol regulation. For CAR, such positive synchrony was only found in couples having high levels of marital strains. Further, couples reporting more spousal support had stronger stability in CAR within each individual (Liu, Rovine, Klein, & Almeida, 2013).

27.5 Conclusion

Use of saliva-based biomarkers has shown considerable promise for examining the immediate physiological consequences of daily stressors. The approach is more effective when observations can be made over multiple days, during which there are likely to be sufficient occurrences of stressors. Reactivity of these biomarkers to stressors within- and across days may be a more valuable measure of biological effects than average daily scores. Use with special populations experiencing high levels of stressors is also feasible.

Saliva-based biomarkers may also be used as outcome measures for treatment studies. Although they do not replace self-reports of affect and subjective stress, biomarkers provide a complementary perspective on the stress response, and may be useful for identifying pathways from daily experiences to health outcomes. Future research could combine daily saliva-based biomarkers with more traditional immune system and cardiovascular measures to explore how stress reactivity under varying circumstances might lead to subsequent illness.

In summary, we hope that this chapter encourages other scientists to incorporate saliva into their research. Saliva can help uncover important age-related changes in important biological systems, including the hypothalamic–pituitary–adrenal (HPA) axis and the sympathomedullary (SAM) pathway. Saliva can also be used to assess naturally occurring fluctuations of biomarkers in daily life. Indeed, this chapter provides some initial evidence of the scientific benefits of collecting multiple samples of saliva across a series of days. Finally, we acknowledge the practical and analytic challenges of incorporating saliva bioscience and offer some cutting-edge statistical approaches that can help address these issues. In our view, the future of salivary bioscience is multivariate and dynamic and we will need to continue to develop statistical approaches to keep pace with our ability to assess such naturally occurring biopsychosocial phenomena.

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