Psychoneuroendocrinology (2013) 38, 3167—3171

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SHORT COMMUNICATION

Diurnal profiles of salivary cortisol and alpha-amylase change across the adult lifespan: Evidence from repeated daily life assessments

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Received 11 April 2013; received in revised form 9 September 2013; accepted 9 September 2013

KEYWORDS
Aging;
Cortisol;
Lifespan;
Salivary alpha-amylase

Summary Salivary cortisol and alpha-amylase are known to have distinctive diurnal profiles. However, little is known about systematic changes in these biomarkers across the adult lifespan. In a study of 185 participants (aged 20—81 years), time-stamped salivary cortisol and alpha-amylase were collected 7 times/day over 10 days. Samples were taken upon waking, 30 min later, and then approximately every 3 h until 9 pm. Multilevel models showed that older age was associated with increased daily cortisol secretion as indicated by greater area under the curve, attenuated wake-evening slopes, and more pronounced cortisol awakening responses. Further, older age was related to greater daily alpha-amylase output and attenuated wake-evening slopes. No age differences were observed regarding the alpha-amylase awakening response. Our findings may contribute to a better understanding of age-related differences in functioning of stress-related systems.

1. Introduction

As individuals grow older, age-related processes accumulate to exert their influence on physical functioning (Seeman and Gruenewald, 2006). The psychobiological mechanisms that are thought to underlie age-related alterations are closely tied to the hypothalamus—pituitary—adrenal (HPA) axis and the autonomic nervous system (ANS). The HPA and ANS interact with each other and exert numerous effects throughout the body. These effects have important implications for daily functioning, as in the example of HPA and ANS effects on the central nervous system which may result in changes to cognitive functioning.

Most studies of age differences in cortisol have targeted diurnal rhythms to index basal HPA axis regulation. Among

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http://dx.doi.org/10.1016/j.psyneuen.2013.09.008

designs using at least 2 measurement points of plasma cortisol, age tends to be associated with higher cortisol levels: higher basal cortisol (Sherman et al., 1985), a 20—50% increase in 24 h-profiles of cortisol (Van Cauter et al., 1996), and increases in cortisol concentrations over the day using 24-h blood sampling (Deuschle et al., 1997). Age-related increases in basal salivary cortisol have also been found; over a two-day diurnal measurement period older individuals showed the highest concentrations (Nicolson et al., 1997), and higher levels of morning and evening cortisol levels with increasing age (Ice, 2005). Recently, results from a national sample of 1143 adults aged 33—84 who provided salivary cortisol samples four times per day for four days showed higher morning cortisol variability (Almeida et al., 2009) and increased overall cortisol output (Piazza et al., 2013) in older men but not in women.

Age-related findings on ANS regulation, in contrast, are relatively scarce. For example, there is evidence from norepinephrine levels that increased tonic activity of the sympathetic branch of the ANS may be prevalent in older individuals (Seals and Dinenno, 2004). This relative dearth in research on aging and ANS function may be because most ANS measures are difficult to obtain in the daily life of study subjects. Salivary alpha-amylase (sAA) which is an enzyme secreted from the salivary glands upon activation of the sympathetic and parasympathetic branches of the ANS however, is easy to measure (Nater and Rohleder, 2009). Very few studies have examined age-related differences in diurnal sAA secretion. The limited evidence available suggests higher sAA activity with older age based on a single measurement (Ben-Aryeh et al., 1990) and that compared to younger adults, older individuals displayed higher diurnal sAA activity (Strahler et al., 2010).

Taken together, the available evidence points to an overall increased HPA axis and ANS activity in old age. The following caveats need be addressed, however: (1) given the diurnal rhythm of cortisol and alpha-amylase, sample collection needs to adequately cover the inherent dynamics of both HPA axis and ANS. Although there are examples of high frequency sampling (Deuschle et al., 1997), most studies have collected samples at only a few or a single time point, rendering the results ''snap-shots'' in time. (2) Further, diurnal rhythms might differ from one day to the next because of situational factors. Situational factors are particularly concerning for the results based on low frequency sampling because at least 6 days of assessment are needed in order to get at reliable estimates of dynamic cortisol activity (Hellhammer et al., 2007). None of the above-mentioned studies used more than 4 days of measurement. (3) With a few exceptions (one which is a re-analysis of an aggregated data set, Van Cauter et al., 1996), and the NSDE analyses, the sample sizes of these studies have been small, which may reduce power to detect differences if they are present. (4) Except for one study (Strahler et al., 2010), none of these studies measured both HPA axis and ANS regulation within the same sample. Therefore, little information is available to directly compare the age-related trends in HPA axis and ANS. (5) It is well-known that non-adherence to collection guidelines may critically impact biological measures, thus compliance needs to be ensured by procedures such as time-stamped sample collection. While some studies (e.g., Strahler et al., 2010) used compliance control methods (such as electronic recording of saliva sample collection), most did not, making the findings difficult to interpret. (6) Finally, in order to test the impact of age on biological measures, the sample needs to adequately reflect a broad age range.

Our study attempts to overcome these limitations. We hypothesized that both cortisol (as an index of HPA axis regulation) and sAA (as an index of ANS regulation) levels are higher in older age. We test this hypothesis in an adult lifespan sample with similar representation of individuals across the spectrum of 20—81 year olds.

2. Materials and methods

2.1. Study design and data collection

Participants completed questionnaires including sociodemographic characteristics, height, and weight. They then entered a 10-day time-sampling phase during which they completed self-initiated questionnaires when waking up and 30 min later, as well as 5 daily prompted questionnaires approximately every 3 h. At each measurement point, participants provided saliva samples for cortisol and alpha-amylase assays. Tungsten T handheld computers were used to record collection time and numbers on saliva samples. Participants completed 79% of surveys within 30 min of the beep prompt.

2.2. Study group

The sample consisted of 185 adults from the Atlanta, GA, metropolitan area (*M* age = 49 years; age range = 20—81 years; 51% female; 74% Caucasian, 17% African American, 9% other; 84% completed some college education). Exclusionary criteria were pregnancy, breastfeeding, thyroid dysfunction, mental disorders such as PTSD, bipolar disorders, psychosis, eating disorders, alcohol/substance abuse, dementias such as Parkinson or Alzheimer, endocrine conditions such as Cushing or Addison, obesity, i.e., BMI $>$ 35, hormone-producing cancers. Further, participants were also excluded if it was a non-typical week (i.e., death in the family, surgery), if they were using anxiety or depression medications, or had schedules that would interfere with data collection (i.e., shift work). In order to ensure that participants could follow the protocol, participants were excluded from the study if they did not speak English in their homes. Five participants had to be excluded due to incomplete cortisol and amylase data. All procedures were carried out with the adequate understanding and written consent of the subjects.

2.3. Assessments

2.3.1. Cortisol

Saliva samples were collected using Salivettes (Sarstedt). Participants were instructed to chew on the cotton rolls for 1 min. All participants provided their first sample after awakening while still lying in bed and then 30 min later. Further samples were taken around 0900 h, 1200 h, 1500 h, 1800 h, and 2100 h, thus covering the full diurnal cycles of cortisol and sAA, respectively. Participants were given the option to