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

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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 minutes later, and then approximately every 3 hours until 9pm. 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.

Keywords

ageing; cortisol; lifespan; salivary alpha-amylase

1. Introduction

As individuals grow older, age-related processes accumulate to exert their influence on physical functioning (Seeman and Gruenewald, 2006). The psycho-biological mechanisms that are thought to underlie age-related alterations are closely tied to the hypothalamus-

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Conflicts of interest

The authors have no conflicts of interest and declare no financial interests.

Contributions

CAH contributed to the design of the study, wrote the protocol, and undertook the statistical analysis. UMN and CAH wrote the first draft of the manuscript. SBS prepared the data for analysis and provided valuable feedback to multiple iterations of the manuscript. All authors contributed to and have approved the final manuscript.

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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 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 24h-profiles of cortisol (Van Cauter et al., 1996), and increases in cortisol concentrations over the day using 24-hour 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 1,143 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 Dinunno, 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 timestamped 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 20–80 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 ten-day time-sampling phase during which they completed self-initiated questionnaires when waking up and 30 minutes later, as well as 5 daily prompted questionnaires approximately every three hours. 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 minutes 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 to 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, and shift work. 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 have a minimum of a high school education and 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

Cortisol—Saliva samples were collected using Salivettes (Sarstedt). Participants were instructed to chew on the cotton rolls for one minute. All participants provided their first sample after awakening while still lying in bed and then 30 minutes later. Further samples were taken around 0900h, 1200h, 1500h, 1800h, and 2100h, thus covering the full diurnal cycles of cortisol and sAA, respectively. Participants were given the option to deviate from the set time points to reduce scheduling conflicts (e.g. important meetings). Cortisol was analyzed using a commercial chemiluminescence immunoassay (IBL Hamburg, Germany). For this study we were particularly interested in three cortisol indices. Before computing the indices, we excluded all cortisol samples that deviated more than 3 standard deviations from the mean cortisol for the respective time of day. We also excluded all days that either did not have a morning value and/or that did not have at least 4 cortisol values. As a first measure, we estimated total daily cortisol secretion based on the “area under the curve” (AUC) (Pruessner et al., 2003). Second, we calculated daily cortisol slopes by subtracting the waking value from the evening value taking into account the respective time of day. Third, we estimated daily awakening responses by modeling cortisol values as a function of waking levels and time since waking.

Alpha-amylase—Alpha-amylase assays were based on the same saliva samples that were also used for cortisol assays. Alpha-amylase was analyzed using a commercially available

substrate reagent (alpha-amylase EPS Sys; Roche Diagnostics). Analogous to cortisol, we were interested in three indices (AUC, waking response, & daily slope). The same methods for treating outliers and missing values were used as described above. For cortisol; 762 days were excluded in total (this is 6.82% of the possible 11202 days for which data was available). For amylase, 960 days were excluded in total (this is 8.57% of the possible 11202 days for which data was available).

Control variables—We also controlled for the potential influence of body mass and sex (see e.g. Therrien et al., 2007).

2.4 Statistical Analysis

Both cortisol and sAA values were log-transformed due to positive skewness. Multilevel modeling was used to account for the hierarchical nature of this data set (MLM, Raudenbush et al., 2000). Specifically, we conducted models with two levels of nesting. The first level concerned daily assessments and the second level participants. Means, standard deviations, and intercorrelations among the central study variables can be obtained from Table 1.

3. Results

Age differences in diurnal cortisol profiles

As a first step, we examined the association between individual differences in participant age and the three different cortisol indices, always controlling for body mass and sex (see Table 2, first column). Findings indicate that age was positively associated with overall daily cortisol output as indicated by the AUC (*Model 1*). Furthermore, older adults displayed attenuated wake-evening slopes compared to middle-aged and young adults (*Model 2*). We further observed age differences in cortisol awakening responses with older adults displaying more pronounced morning rises (*Model 3*).

Age differences in diurnal alpha-amylase profiles

We then proceeded to examine the relationship between participant age and salivary alpha-amylase (Table 2, second column). Results show that older individuals displayed higher overall daily alpha-amylase output as measured by the area under the curve (*Model 4*). No age-differences were observed in the alpha-amylase wake-evening slopes and awakening response (*Models 5 & 6*).

4. Discussion

Using multilevel models we were able to show that older age was associated with higher diurnal cortisol secretion and higher daily alpha-amylase activity across multiple indices. Our findings of higher cortisol and sAA levels with older age are in accord with previous work suggesting age-related increases in activity of HPA axis and ANS (Aguilera, 2011; Seals and Dinunno, 2004) and extend them in important ways. While previous studies are based on a very limited observation period - 24 hours (Deuschle et al., 1997; Van Cauter et al., 1996) (but with higher frequency of sampling), one day (Strahler et al., 2010), two days (Nicolson et al., 1997), three days (Ice, 2005), or four days (Rueggeberg et al., 2012) -, we used the advantages of 7 daily assessments covering a 10 day measurement period. We were thus able to study the relationship between age and biological markers in a repeated daily assessment approach. It is conceivable that not all individuals will show the same pattern of HPA/ANS alterations over time; as it has been suggested by Lupien et al., there might be sub-groups of participants with increasing, decreasing, or stable HPA/ANS activity over time (Lupien et al., 1996). This poses the key question as to whether increased HPA/ANS activity is associated with increased intra- and inter-individual variability over time.

However, these hypotheses are only testable in longitudinal studies implementing several waves of intensive data.

Our findings of increased HPA axis and ANS activity with older age are highly relevant for better understanding negative health outcomes in older individuals. As an example, consequences of prolonged or frequently high exposure to glucocorticoids may increase vulnerability of glucocorticoid-sensitive neuronal structures, such as the hippocampus, which may ultimately affect key outcomes including later cognitive functioning (Aguilera, 2011). While we did not assess stress over lifetime, our observation of age-related increases in endocrine and autonomic functioning could reflect age-related accumulation of stress (Seeman et al., 2001).

Our findings provide confirmation for age-differences in HPA axis and an important contribution to the literature on age differences in ANS activity. It is important to note limitations of this study as well, specifically, our sample was likely selective as we examined individuals who were mentally and physically healthy. The present study demonstrated the feasibility of collecting HPA and ANS markers on a daily basis while monitoring compliance, therefore, similar designs should be used with population-based samples in order to understand HPA and ANS functioning across all levels of mental and physical functioning. This is particularly notable as it has been suggested that potential effects of age on both the HPA axis and the ANS may be in part explained by worse health occurring in older age (Almeida et al., 2011). Second, the stimulated saliva collected in this study may obscure sympathetic and parasympathetic contributions of sAA. Future studies should attempt to collect unstimulated saliva, as this may be more reflective of basal autonomic processes. Finally, it is not possible to conclude from our findings whether age-related changes might be due to altered biological stress reactivity (Otte et al., 2005). Future work should build from the design, attention to compliance, and sampling of the present study, and research should also examine HPA and ANS reactivity to acute stressors.

In sum, our findings are consistent with the notion that there is age-related wear-and-tear in biological stress systems. We extend previous findings of increased HPA axis activity in older individuals by adding compelling evidence that similar increased activity may be observed in the ANS. Importantly, this observation is based on data collected from real-life contexts, thereby maximizing ecological validity. Although our study may only constitute a first step towards an in-depth understanding of how age-related biological alterations occur, it paints a more detailed picture of age-related differences in functioning of stress-related systems.

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Table 1

Means and Standard Deviations of the Central Study Variables as well as their Intercorrelations (N = 185)

	M (SD)	2	3	4	5	6	7	8	9
1 Age (years)	48.55 (19.19)	-.03	.144*	.31**	.24**	-.02	.19**	-.17**	-.02
2 Sex (1 = woman)	51% female		-.08	-.06	-.03	.01	-.04	.02	-.13
3 BMI	25.83 (4.61)			-.08	.03	.04	.13	-.06	.01
4 AUC (Cortisol)	90.27 nmol/l (23.83 nmol/l)				.23**	.05	.06	.04	.01
5 Slope (Cortisol)	-.13 (.04)					.07	.01	-.07	-.02
6 Waking response (Cortisol)	.67 (1.51)						.03	.01	.29**
7 AUC (Amylase)	1653.46 U/ml (865.63 U/ml)							-.14	-.02
8 Slope (Amylase)	.04 (.04)								.44**
9 Waking response (Amylase)	-.52 (1.32)								

Note.

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p<.01.

Table 2

Two-Level Models Predicting Salivary Cortisol and Salivary Alpha-Amylase from Gender, Age, and Body Mass using Restricted Maximum Likelihood Estimation (N = 185)

	Salivary Cortisol		Salivary Alpha-Amylase			
	Model 1: Area under the curve	Model 2: Wake-evening slope	Model 3: Awakening response	Model 4: Area under the curve	Model 5: Wake-evening slope	Model 6: Awakening response
Fixed effects	Coefficients (SE)	Coefficients (SE)	Coefficients (SE)	Coefficients (SE)	Coefficients (SE)	Coefficients (SE)
Intercept	91.62 ^{**} (2.51)	-0.13 ^{**} (0.03)	2.63 ^{**} (0.04)	1675.82 ^{**} (88.06)	3.39 ^{**} (0.49)	4.16 ^{**} (0.08)
Gender	-2.92 (3.36)	0.00 (0.01)	-0.00 (0.05)	-41.04 (124.00)	-0.62 (0.64)	-0.03 (0.11)
Age	0.39 ^{**} (0.09)	0.00 (0.00) ^{**}	-0.00 (0.00)	8.06 [*] (3.18)	-0.02 (0.02)	0.01 ^{**} (0.00)
Body mass index	-0.06 (0.39)	-0.00 (0.00)	-0.01 [*] (0.01)	18.30 (12.83)	-0.05 (0.07)	0.02 (0.01)
Average daily cigarettes	0.18 (0.79)	0.00 (0.00)	-0.00 (0.02)	23.40 (35.16)	-0.08 (0.07)	0.03 (0.02)
Average daily coffees	1.77 (1.61)	0.00 (0.00)	0.01 (0.02)	-20.25 (50.77)	-0.03 (0.25)	-0.07 (0.05)
Time since waking			0.0001 ^{**} (0.00)			-0.00 ^{**} (0.00)
Time since waking X Age			0.0000 [*] (0.00)			0.00 (0.00)
Random effects						
Residual	712.15	0.00	0.17	297997.58	28.75	0.27
Intercept lv 1	432.44 ^{**}	0.00 ^{**}	0.12	690717.52 ^{**}	14.89 ^{**}	0.64 ^{**}

Note.

* p<.05;

** p<.01