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Diurnal patterns and associations among salivary cortisol, DHEA and alpha-amylase in older adults ☆

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HIGHLIGHTS

- We studied diurnal patterns and associations among cortisol, DHEA and α-amylase (sAA).
- Diurnal patterns, based on medians, were similar to some past studies but not others.
- The nature of the associations depends on the time of day.
- The association between awakening cortisol and sAA depends on the level of DHEA.
- After awakening, no association between cortisol, DHEA versus sAA is found.

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ABSTRACT

Background: Cortisol and dehydroepiandrosterone (DHEA) are considered to be valuable markers of the hypothalamus–pituitary–adrenal (HPA) axis, while salivary alpha-amylase (sAA) reflects the autonomic nervous system. Past studies have found certain diurnal patterns among these biomarkers, with some studies reporting results that differ from others. Also, some past studies have found an association among these three biomarkers while other studies have not. This study investigates these patterns and associations in older adults by taking advantage of modern statistical methods for dealing with non-normality, outliers and curvature. Basic characteristics of the data are reported as well, which are relevant to understanding the nature of any patterns and associations.
Methods: Boxplots were used to check on the skewness and presence of outliers, including the impact of using simple transformations for dealing with non-normality. Diurnal patterns were investigated using recent advances aimed at comparing medians. When studying associations, the initial step was to check for curvature using a non-parametric regression estimator. Based on the resulting fit, a robust regression estimator was used that is designed to deal with skewed distributions and outliers.
Results: Boxplots indicated highly skewed distributions with outliers. Simple transformations (such as taking logs) did not deal with this issue in an effective manner. Consequently, diurnal patterns were investigated using medians and found to be consistent with some previous studies but not others. A positive association between awakening cortisol levels and DHEA was found when DHEA is relatively low; otherwise no association was found. The nature of the association between cortisol and DHEA was found to change during the course of the day. Upon awakening, cortisol was found to have no association with sAA when DHEA levels are relatively low, but otherwise there is a negative association. DHEA was found to have a positive association with sAA upon awakening. Shortly after awakening and for the remainder of the day, no association was found between DHEA and sAA ignoring cortisol. For DHEA and cortisol (taken as the independent variables) versus sAA (the dependent variable), again an association is found only upon awakening.

1. Introduction

The hypothalamus–pituitary–adrenal (HPA) axis and the sympathetic–adrenomedullary system (SAM) are two major biological systems involved in homeostatic and allostatic adaptations to environmental and internal stimuli [37,23,42,12,43]. Dysfunction in the HPA axis is implicated in the development of a variety of sub-clinical and

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clinical conditions [43,44,3]. Cortisol, the primary hormone product secreted by the HPA axis, is considered to be a biomarker of HPA axis activity (e.g., [22]). Dehydroepiandrosterone (DHEA) is also secreted by the adrenal cortex and plays a pivotal role in the regulation of HPA activity, with effects that are opposite to cortisol at both peripheral and central levels [40,14,17].

Past studies have found that mean cortisol levels exhibit an initial rise after awakening, referred to as the cortisol awakening response (CAR), followed by a decline in cortisol during the remainder of the day. Pruessner et al. [53] were the first to propose that the repeated assessment of salivary cortisol increase after awakening might represent a useful and easy measure index of cortisol regulation. In most studies, an increase in salivary cortisol levels of about 50–75% within 30–45 min after awakening has been found. The CAR is increasingly used in psychoneuroendocrinology as an indicator of HPA axis activity. For reviews of the literature, see Clow et al. [11], Chida and Steptoe [8] and Fries et al. [20]. The CAR is considered to be a marker of the integrity of the HPA axis [29]. Exhibiting an absence or an exacerbation of this increase is associated with several adverse psychological and physiological outcomes (e.g., [51,50]). Both enhanced and reduced CARs are associated with various psychosocial factors [36,8], including depression and anxiety disorders (e.g., [52,64,4,65,66]).

Currently, only a few studies have reported results about the daily fluctuations of DHEA and sAA. Regarding DHEA, a circadian variation (with a trough concentration later in the day) has been reported in adults [72,22] but not in the elderly [13].

The CAR is considered a reliable parameter for detecting participants who are non-adherent to a study protocol [63,61,46]. Consequently, in a study focusing on young adults, Ghiciuc et al. [22] examine diurnal patterns for participants who exhibited cortisol increases by at least 50% after awakening and found that mean DHEA levels decreased significantly 15 min after awakening. A non-significant increase was observed at 30 and 45 min and a significant increase was reported about 13 h after awakening. The rationale for focusing only on participants with a CAR of 50% was that failure to comply with the strict timing of saliva sampling can influence hormonal measurements and compromise the accuracy and reliability of the results [27,38].

Secretion of sAA has been proposed as an indicator of plasma catecholamine modifications under a variety of conditions [7,67,62,39,70]. Significant diurnal fluctuations in sAA have been reported, with low values reached within 60 min after awakening [45] and much higher values reached later in the day [47,2,71,45,22].

Very little is known about the associations among cortisol, DHEA and sAA, particularly for older adults. The aim of our study is to help fill this gap by reporting results on the associations among cortisol, DHEA and sAA in the well elderly. A unique feature of the present study is the use of modern statistical methods that provide improved techniques aimed at dealing with outliers, non-normality and curvature. Another goal was to provide new information and perspectives regarding the diurnal patterns of cortisol, DHEA and sAA.

2. Material and methods

2.1. Participants and study design

The data stem from the Well Elderly II study [9,34]. The participants were 460 men and women aged 60 to 95 years (mean age 74.9). All participants were residents of, users of, or visitors to the study recruitment sites, demonstrated no overt signs of psychosis or dementia (based on a cursory screening procedure), and were able to complete the study assessment battery (with assistance, if necessary). All prospective participants completed the informed consent process prior to study entry. Participants were recruited from 21 sites in the greater Los Angeles area, including nine senior activity centers, eleven senior housing residences, and one graduated care retirement community. Recruitment strategies included providing sign-up booths, giving presentations at meetings and social events, and distributing flyers and posters. Recruitment was undertaken in two successive cohorts to reduce temporal influences on study outcomes, overcome logistical difficulties, minimize interactions among participants, and allow adjustments in ethnic stratification. Individuals in cohort 1 ($n = 205$) began participation between November, 2004 and June, 2005, whereas those in cohort 2 ($n = 255$) began participation between March and August, 2006. Here, the two cohorts are combined in all analyses.

2.2. Assessment

Saliva samples were taken at four times: upon awakening, 30–45 min later, 5 h later, and 5 h later. Samples were assayed for sAA using a commercially available kinetic reaction assay (Salimetrics, State College, PA). The assay employs a chromogenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. The enzymatic action of sAA on this substrate yields 2-chloro-pnitrophenol, which can be spectrophotometrically measured at 405 nm using a standard laboratory plate reader. The amount of activity present in the sample is directly proportional to the increase (over a 2 min period) in absorbance at 405 nm. Results are computed in units per milliliter of sAA using the formula: [Absorbance difference per minute × total assay volume (328 ml) × dilution factor (200)] / [millimolar absorptivity of 2-chloro-p-nitrophenol (12.9) × sample volume (.008 ml) × light path (.97)].

Samples were assayed for cortisol using a highly sensitive enzyme immunoassay. The test uses 25 μl/dl, ranges in sensitivity from .007 to 1.2 μl/dl, and has average intra- and inter-assay coefficients of variation of 4.13% and 8.89%, respectively. Samples were assayed for salivary DHEA using a double antibody radioimmunoassay developed at Penn State Behavioral Endocrinology Laboratory (Granger et al., 1999). The test uses 100 μl of saliva, has a minimum detection limit of 4 pg/ml and average intra- and inter-assay coefficients of variation less than 4.05% and 12.57%, respectively.

2.3. Data analysis and statistics

All analyses were performed with the software R [54]. The median levels of the biomarkers were compared using a percentile bootstrap method that deals effectively with tied (duplicated) values (e.g., [68], Section 5.9.11). As will be seen, the data in the present study are skewed with outliers suggesting that conventional methods for comparing means can have relatively low power and poor control over the Type I error probability (e.g., [58,41,30,32,68,26]). Indeed, even a single outlier might result in poor power. Nonparametric (rank-based) methods are sometimes suggested for comparing medians. There are exceptions, but under general conditions, nonparametric methods do not compare medians (e.g., [21,31,6]). The simple strategy of transforming the data is relatively ineffective by modern standards in terms of dealing with outliers and skewed distributions. For example, taking logs, typically outliers remain and distributions are still skewed, which proved to be the case for the data at hand.

When using least squares regression, again outliers can wreak havoc on power and they can result in a highly misleading summary of the association among the bulk of the points. Here, outliers among the dependent variable were addressed using the regression estimator derived by Theil [60] and Sen [59], which is designed to estimate the median of a dependent variable rather than the mean. As is the case when dealing with means, simply discarding outliers among the dependent variable and applying least squares regression to the remaining data generally yields poor control over the probability of a Type I error. As for the independent variable, theory allows one to remove outliers. This was done here using an outlier detection method that has been studied extensively in the statistics literature (e.g., [56,69]). These books also explain why outlier detection techniques, based on the mean and variance, are highly unsatisfactory. Multivariate outliers were detected using the method in Wilcox ([69], Section 6.4.7).