

SHORT REPORT

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Measuring DHEA-S in saliva: time of day differences and positive correlations between two different types of collection methods

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Abstract

Background: The anabolic steroid, dehydroepiandrosterone sulfate (DHEA-S), is secreted from the adrenal cortex. It plays a significant role in the body as a precursor to sex steroids as well as a lesser known role in the hypothalamic pituitary adrenal axis (HPA) response to stress. DHEA-S can be measured reliably in saliva, making saliva collection a valuable tool for health research because it minimizes the need for invasive sampling procedures (e.g., blood draws). Typical saliva collection methods include the use of plain cotton swab collection devices (e.g., Salivette®) or passive drool. There has been some speculation that the plain saliva cotton collection device may interfere with determination of DHEA-S by enzyme immunoassay (EIA) bringing this saliva collection method into question. Because of the increasing popularity of salivary biomarker research, we sought to determine whether the cotton swab interferes with DHEA-S determination through EIA techniques.

Findings: Fifty-six healthy young adult men and women aged 18-30 years came to the lab in the morning (0800 hrs; 14 men, 14 women) or late afternoon (1600 hrs; 14 men, 14 women) and provided saliva samples via cotton Salivette and passive drool. Passive drool collection was taken first to minimize particle cross contamination from the cotton swab. Samples were assayed for DHEA-S in duplicate using a commercially available kit (DSL, Inc., Webster, TX). DHEA-S levels collected via Salivette and passive drool were positively correlated ($r = + 0.83$, $p < 0.05$). Mean DHEA-S levels were not significantly different between collection methods. Salivary DHEA-S levels were significantly higher in males than in females, regardless of saliva collection method ($p < 0.05$), and morning DHEA-S values were higher than evening levels ($p < 0.05$).

Conclusions: Results suggest that DHEA-S can be measured accurately using passive drool or cotton Salivette collection methods. Results also suggest that DHEA-S levels change across the day and that future studies need to take this time of day difference into account when measuring DHEA-S.

Background

Dehydroepiandrosterone-sulfate (DHEA-S), the sulfated form of dehydroepiandrosterone (DHEA), is an important health biomarker used consistently in the measurement of allostatic load [1,2]. As a steroid, there has been interest in measuring DHEA-S in youth and aging studies [3-7]. Despite research interest in this biomarker, there is ambiguity regarding the best method to measure DHEA-S in saliva. For example, recent studies have incorporated salivary DHEA-S as an additional biomarker of hypothalamic-pituitary-adrenal axis (HPA)

function [8-11], however their collection methods vary between passive drool and cotton salivette. This collection method difference is important because hormone recovery from saliva can vary by collection device [12-14]. Specifically, hormones such as sex steroids (e.g., testosterone and estrogen) and DHEA that are detectable in saliva must be collected through the passive drool method because they bind to the collection device and provide either falsely inflated or deflated values [15-17]. It is not known how these collection methods alter DHEA-S determination in saliva.

Not only is it important to validate DHEA-S in different types of saliva collection methods, but it also is important to understand how DHEA-S levels differ

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across sex and across the day. DHEA-S levels show a life-time rhythm with values high at birth, a sharp decline during the first year, a peak again in the mid 20-30s, and then a decline throughout the life span. In addition, males have higher levels of DHEA-S than do females [18]. Literature suggests that serum DHEA-S values are stable across the day due to a 10-12 hour renal clearance rate [19-21]. However, studies also suggest that levels of DHEA-S in saliva or serum do change across the day, with some reporting higher morning levels compared to evening and other reporting the opposite pattern [22-25]. The discrepancy between these studies could be the result of improved assay techniques, different sample populations or different sampling methods. Thus, it remains unclear whether DHEA-S values do change across this day. Therefore, we sought to determine whether DHEA-S could be reliably measured in saliva collected using two common saliva collection techniques: passive drool and cotton salivette. In addition, we examined salivary DHEA-S values in the morning and in the evening to determine DHEA-S changes across the day in a normal, health population of young adult men and women.

Methods

Participants

Fifty-six (28 females and 28 males) participants between the ages of 18-30 years (mean 21.77 ± 0.35 years) were recruited from The Pennsylvania State University Campus to take part in a 10-minute lab session.

Experimental Procedure

Lab sessions were scheduled either between 0800-0900 hrs or 1600-1700 hrs. Each participant provided 2 saliva samples, one using the Salivette[®] (Sarstedt, Newton, NC) with the cotton swab device in place to collect saliva (Salivette) and one using a piece of straw to spit into a Salivette[®] that had the cotton and plastic insert removed (passive drool). Following informed consent, participants were asked to think of their favorite food and, using a straw, spit into a collection tube for 2 minutes. After the passive drool collection, participants filled out a brief demographic survey that took approximately 5 minutes to complete. This brief time interlude allowed a wash out period between passive drool and Salivette saliva collection. Passive drool collection was taken first for all participants in order to minimize particle cross contamination from the cotton Salivette. Following the demographic survey, participants placed a cotton swab from a Salivette in their mouths without touching the cotton and rolled the swab across their tongue for 2 minutes (i.e., without chewing on the swab; unstimulated saliva collection).

All tubes were weighed before and after saliva collection to account for saliva volume (as per Harmon and colleagues) [26]. After both saliva samples were taken, collection tubes were placed in a -20 degree freezer and transferred to a -80 degree freezer within 24 hours for later assay. Participants were compensated \$5.00 for their time. Study procedures were reviewed and approved by The Pennsylvania State University Institutional Review Board.

Salivary DHEA-S Assessment

On the day of assay, samples were brought to room temperature and centrifuged for 5 minutes at 1500 x g to separate mucin from clear saliva. Salivary DHEA-S levels were evaluated in duplicate with sensitivity at 0.08 ng/mL by commercially available enzyme linked immunosorbent assay (EIA) kits (DSL, Webster, TX) in the core laboratory of The Pennsylvania State University General Clinical Research Center. Samples were balanced across assay plates so that each plate had the same number of morning and evening participants as well as male and female participants. Samples within each participant were kept together on a single assay plate.

Statistical Analyses

Repeated-measures analysis of variance (RMANOVA) was used to determine differences in "type" (passive drool vs. Salivette) of saliva collection. Time of day (i.e., morning vs. evening participants) and sex were the independent variables, with levels of salivary DHEA-S collected by passive drool (time 1) and Salivette (time 2) as the dependent variables. Further analyses then were run to examine differences within each saliva collection type by performing separate 2-way ANOVAs (with time of day and sex as independent variables) on the passive drool collection and on the Salivette collection methods separately. Natural logarithmic transformations were applied to the data because they were skewed [27,28]; this transformation resulted in normal distribution of the data. Transformed data were used for analyses. However, raw data are reported in Figure 1 for clarity [27,28]. All tests were two-tailed and significance was determined at the $\alpha = 0.05$ value.

Results

DHEA-S levels were similar between passive drool and Salivette collection methods. Consistent with prior research, DHEA-S values among men were higher in both passive drool and Salivette saliva samples compared to women, regardless of time of day [$F(1,50) = 10.56$, $p < 0.05$] (see Figure 1) [18]. Further, DHEA-S values were higher for both collection techniques in the morning compared to the afternoon [$F(1,50) = 9.27$,

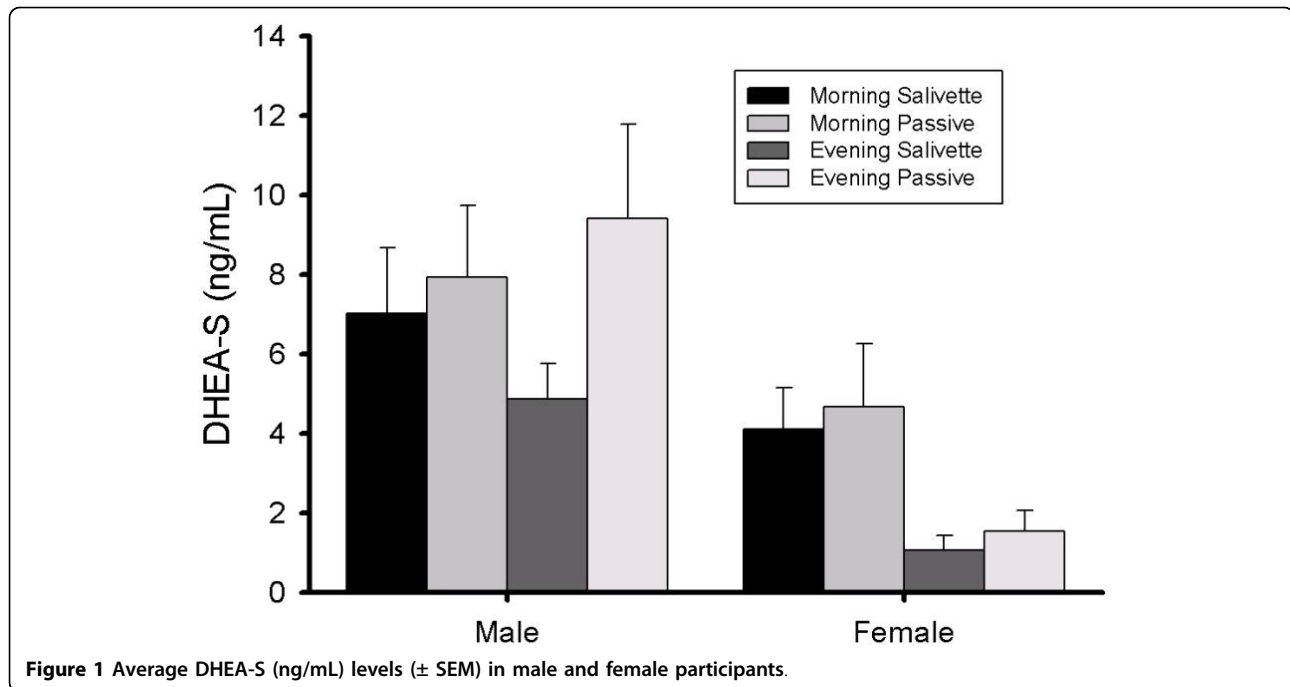


Figure 1 Average DHEA-S (ng/mL) levels (\pm SEM) in male and female participants.

$p < 0.05$] (see Figure 1). There were no statistically significant two-way interactions between saliva collection type and sex, saliva collection type and time of day, and no 3-way interactions among saliva collection type, sex or time of day collected. Pearson product-moment correlation confirmed a significant positive correlation between DHEA-S levels measured in passive drool and levels determined in saliva from the Salivette among all participants [$r(55) = + 0.83$, $p < 0.001$]. Split by sex and time all four groups (i.e., morning male, evening male, morning female and evening female) passive drool and Salivette collected DHEA-S levels were also correlated within each group [$r(14) = + 0.82$; $r(13) = + 0.83$; $r(13) = + 0.95$; $r(15) = + 0.95$, respectively; p 's < 0.001]. Saliva weights were measured on a subset of participants, resulting in the passive drool collection method yielding less saliva than did the Salivette collection device [$1.42 \text{ g} \pm .10$ vs $2.45 \text{ g} \pm .11$ respectively, $F(1,37) = 85.64$, $p < 0.05$]. However, saliva weights were not correlated with DHEA-S values for either passive drool or Salivette [$r(35) = -0.12$, n.s.; $r(37) = -0.12$, respectively], and were not significant predictors of DHEA-S levels.

Conclusions

Results suggest that both Salivette and passive drool collection result in similar DHEA-S levels in the morning and late afternoon and among men and women. These results are consistent with Shirtcliff and colleagues' [12] report that DHEA-S can be reliably measured in passive drool and cotton swab using radioimmunoassay (RIA) methods. Our study advances these findings in two

important ways. First, we demonstrated reliable DHEA-S assessment in passive drool and cotton swab using enzyme immunolinked assay (ELISA) methods. Second, we used the standard cotton swab saliva collection procedure of rolling the swab over the tongue whereas Shirtcliff et al. [12] passed passive drool through a cotton swab. Together, our study and that of Shirtcliff et al. [12] suggest that DHEA-S can be reliably measured in saliva across these two collections and assay determination techniques.

Controversy still surrounds the type of collection device in regards to measuring hormones in saliva. Specifically, there are two issues: 1) low saliva volume may result in falsely low levels of hormones [26] and 2) blood contamination due to mucosal fissures of saliva samples could falsely inflate hormone values [12-14]. This study was designed to test the validity of DHEA-S in the Salivette compared to passive drool, however we also measured saliva volumes in order to record any differences in DHEA-S levels in lieu of the amount of saliva provided. Though blood contamination was not measured specifically in the current studies, unpublished studies in our lab have detected low levels of blood contamination in saliva samples collected in the field and that they do not appear to impact DHEA-S values.

Interestingly, results of this study also support data that report DHEA-S values may not be stable across the day as otherwise assumed in the literature (rhythm, [22,24,25]; no rhythm, [29-31]). Despite convincing literature reporting DHEA-S levels vary across the day, many studies have only measured DHEA-S at one time point [11,32-34].

Based on the limited prior studies and the present results, future studies need to take into consideration that DHEA-S levels may change across the day.

Salivette collection is a clean and easy method for collecting saliva (i.e., reduces pipette error due to mucin secretion), especially in field collection studies commonly designed for cortisol assessment [35]. If one is interested in measuring salivary sex steroids (i.e., estradiol, progesterone, testosterone), the Salivette is not recommended. However, several analytes can be measured reliably in saliva collected via Salivette such as salivary alpha-amylase, cortisol, DHEA-S and cotinine, the primary metabolite of nicotine found in cigarette smokers. The Salivette collection technique appears to be a clean and adequate collection method for determining DHEA-S.

Abbreviations

HPA: Hypothalamic-pituitary-adrenal axis; DHEA-S: Dehydroepiandrosterone sulfate; EIA: enzyme immunoassay; ANOVA: analysis of variance; RMANOVA: Repeated-measures analysis of variance

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Authors' contributions

LCK provided funding. CAW and LCK designed the experiment. CAW conducted the experiment and drafted the introduction and methods sections. CAW analyzed the data and drafted the results section. LCK made intellectual revisions to the entire manuscript. Both authors edited all sections of the manuscript and approved this version of the submitted manuscript.

Competing interests

The authors declare that they have no competing interests.

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