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...
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 DEHA-S could be reliably measured in saliva
collected using two common saliva collection techniques:
passive drool and cotton salivette. In addition, we exam-
ined 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 Cam-
pus 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 sal-
iva samples, one using the Salivette® (Sarstedt, New-
ton, 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 plas-
cinsert removed (passive drool). Following informed
consent, participants were asked to think of their
avorite 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 pas-
ive 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., with-
out 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 free-
zer 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 immu-
osorbet 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 inde-
pendent variables, with levels of salivary DHEA-S col-
lected 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 com-
pared 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,
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 \[r(14) = +0.82; r(13) = +0.83; r(13) = +0.95; r(15) = +0.95, \text{respectively}; p's < 0.001\]. 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 \[1.42 \pm 0.10 \text{ vs } 2.45 \pm 0.11 \text{ respectively, } F(1,37) = 85.64, p < 0.05\]. However, saliva weights were not correlated with DHEA-S values for either passive drool or Salivette \[r(35) = -0.12, \text{n.s.}; r(37) = -0.12, \text{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 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 sulphate; EIA: enzyme immunoassay; ANOVA: analysis of variance; RMANOVA: Repeated-measures analysis of variance

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Authors’ contributions
LCX provided funding. CAW and LCX 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 contributions to the methodology section. LCK analyzed the data and drafted the results section. CAW and LCK contributed equally to the writing of the manuscript. We thank the Executive Editor of Psychoneuroendocrinology 2001, 26:165-173.

Competing interests
The authors declare that they have no competing interests.

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References
1. Seeman TE, Mayer EH, Seidman SN, et al. Health, aging, and the brain. J Am Geriatr Soc 2004, 52(2):S7-S16.
2. Seeman TE, Singer BH, Rowe JW, Horwitz RI, McEwen BS. Adaptation: Allostatic Load and Its Health Consequences. MacArthur studies of successful aging. J Clin Endocrinol Metab 1997, 82(6):1341-1357.
3. Almeida DM, Piazza JR, Dmitrieva ND, Klein LC. Frontiers in the use of biomarkers of health in research on stress and aging. J Clin Endocrinol Metab 1998, 83(10):3185-3192.
4. Di Luigi L, Guidetti C, Baldari MC, Gallotta P, Sgro P, Perroni F, Romanelli F, Castiello D. Salivary cortisol, salivary alpha-amylase, and their ratio in the assessment of daily stress burden in similar environmental conditions. J Clin Endocrinol Metab 2009, 94(3):1115-1122.
5. Huang YJ, Chen MT, Fang CL, Lee WC, Lin HC, Chou CH, Chen YJ. Elevated salivary dehydroepiandrosterone sulphate and cortisol concentrations in medicated depressed patients: preliminary findings. Psychopharmacology 2004, 178(2):117-122.
6. Taylor MK, Sausen KP, Potterer EG, Muscila-Parodi LR, Reis JP, Markham AE, Padilla GA, Taylor DL. Stressful military training: endocrine reactivity, performance, and psychological impact. Aviat Space Environ Med 2007, 78(12):1143-1149.
7. Kivilahti KT, Granger DA, Schwartz EB, Nelson V, Curran M, Shumaker SA. Assessing cortisol and dehydroepiandrosterone (DHEA) in saliva: effects of collection method. J Psychopharmacol 2006, 20(5):643-649.
8. Lampe PJ, Nolan A. Assessing cortisol and dehydroepiandrosterone (DHEA) in saliva: effects of collection method. J Psychopharmacol 2006, 20(5):643-649.
9. Lampe PJ, Nolan A. A recovery of human saliva using the Salivette system. Eur J Clin Chem Clin Biochem 1994, 32(9):727-728.
10. Grosch M, Suchard M. Use of salivary biomarkers in biobehavioral research: cotton-mouthed sample collection methods can interfere with salivary immunoassay results. Psychoneuroendocrinology 2001, 26:165-173.
11. Kivilahti KT, Granger DA, Schwartz EB, Nelson V, Curran M, Shumaker SA. Assessing cortisol and dehydroepiandrosterone (DHEA) in saliva: effects of collection method. J Psychopharmacol 2006, 20(5):643-649.
12. Grosch M, Kohler H, Topf HG, Rupprecht T, Rauh M. Evaluation of saliva collection devices for saliva collection. J Pharm Biomed Anal 2008, 47(3):478-486.
13. Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 1984, 59(3):551-555.
14. Hornby PJ. Biosynthesis of DHEAS by the human adrenal cortex and its age-related decline. Ann N Y Acad Sci 1995, 774:29-46.
15. Baulieu EE. Dehydroepiandrosterone (DHEA): a fountain of youth? Endocr Rev 1985, 6(4):223-246.
16. Grosch M, Kohler H, Topf HG, Rupprecht T, Rauh M. Evaluation of saliva collection devices for saliva collection. J Pharm Biomed Anal 2008, 47(3):478-486.
17. Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 1984, 59(3):551-555.
18. Hornby PJ. Biosynthesis of DHEAS by the human adrenal cortex and its age-related decline. Ann N Y Acad Sci 1995, 774:29-46.
19. Baulieu EE. Dehydroepiandrosterone (DHEA): a fountain of youth? J Clin Endocrinol Metab 1986, 61(3):3147-3151.
20. Kroboth PD, Salek FJ, Pittenger AL, Fabian TJ, Frye RF. DHEA and DHEA-S: a review. J Clin Pharmacol 1990, 30(4):327-340.
21. Padilla GA, Taylor DL. Cortisol, dehydroepiandrosterone sulphate (DHEA-S) and testosterone in women with chronic migraine. Neuroimmunomodulation 1998, 5(2):91-95.
22. Patacchioli FR, Monnazzi P, Simeoni S, De Filippis S, Salvatori E, Coloprisco G, Martelliti G. Salivary cortisol, dehydroepiandrosterone sulphate (DHEA-S) and testosterone in women with chronic migraine. J Headache Pain 2006, 7(2):90-94.
23. Zhao ZY, Xie Y, Fu YR, Li YY, Bogdan A, Touitou Y. Circadian rhythm characteristics of serum cortisol and dehydroepiandrosterone sulphate in healthy Chinese men aged 30-60 years. A cross-sectional study. Steroids 2003, 68(2):133-138.
24. Del Ponte A, Di Monte MG, Graziani D, Guagnano MT, Mondini P, Wistull F, Sens C. Changes in plasma DHEAS circadian rhythm in elderly men. Prog Clin Biol Res 1990, 341A:791-796.
25. Nicolau GY, Haus E, Lakatua DJ, Bogdan C, Sackett-Lundeen L, Popescu M, Berg H, Petrescu E, Robu E. Circadian rhythm and circannual variations of FSH, LH, testosterone and dehydroepiandrosterone-sulphate (DHEA-S) and testosterone in women with chronic migraine. J Headache Pain 2006, 7(2):90-94.
26. Harmon AG, Hibel LC, Rupprecht T, Rauh M. Evaluation of saliva collection devices for saliva collection. J Pharm Biomed Anal 2008, 47(3):478-486.
27. Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 1984, 59(3):551-555.
28. Hornby PJ. Biosynthesis of DHEAS by the human adrenal cortex and its age-related decline. Ann N Y Acad Sci 1995, 774:29-46.
29. Baulieu EE. Dehydroepiandrosterone (DHEA): a fountain of youth? J Clin Endocrinol Metab 1986, 61(3):3147-3151.
30. Kroboth PD, Salek FJ, Pittenger AL, Fabian TJ, Frye RF. DHEA and DHEA-S: a review. J Clin Pharmacol 1990, 30(4):327-340.
31. Padilla GA, Taylor DL. Cortisol, dehydroepiandrosterone sulphate (DHEA-S) and testosterone in women with chronic migraine. J Headache Pain 2006, 7(2):90-94.
32. Zhao ZY, Xie Y, Fu YR, Li YY, Bogdan A, Touitou Y. Circadian rhythm characteristics of serum cortisol and dehydroepiandrosterone sulphate in healthy Chinese men aged 30-60 years. A cross-sectional study. Steroids 2003, 68(2):133-138.
33. Del Ponte A, Di Monte MG, Graziani D, Guagnano MT, Mondini P, Wistull F, Sens C. Changes in plasma DHEAS circadian rhythm in elderly men. Prog Clin Biol Res 1990, 341A:791-796.
34. Nicolau GY, Haus E, Lakatua DJ, Bogdan C, Sackett-Lundeen L, Popescu M, Berg H, Petrescu E, Robu E. Circadian rhythm and circannual variations of FSH, LH, testosterone and dehydroepiandrosterone-sulphate (DHEA-S) and testosterone in women with chronic migraine. J Headache Pain 2006, 7(2):90-94.
28. Whetzel CA, Corwin EJ, Klein LC: Disruption in Th1:Th2 immune response in young adult smokers. Addict Behav 2007, 32(1):1-8.

29. Goodyer IM, Herbert J, Altham PM, Pearson J, Secher SM, Shiers HM: Adrenal secretion during major depression in 8- to 16-year-olds, I. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. Psychol Med 1996, 26(2):245-256.

30. Hucklebridge F, Hussain T, Evans P, Clow A: The diurnal patterns of the adrenal steroids cortisol and dehydroepiandrosterone (DHEA) in relation to awakening. Psychoneuroendocrinology 2005, 30(1):51-57.

31. Morgan CA, Rasmussen A, Pietrzak RH, Conic V, Southwick SM: Relationships among plasma dehydroepiandrosterone and dehydroepiandrosterone sulfate, cortisol, symptoms of dissociation, and objective performance in humans exposed to underwater navigation stress. Biol Psychiatry 2009, 66(4):334-340.

32. Carlson LE, Speca M, Patel KD, Goodey E: Mindfulness-based stress reduction in relation to quality of life, mood, symptoms of stress and levels of cortisol, dehydroepiandrosterone sulfate (DHEAS) and melatonin in breast and prostate cancer outpatients. Psychoneuroendocrinology 2004, 29(4):448-474.

33. de Bruin VM, Vieira MC, Rocha MN, Viana GS: Cortisol and dehydroepiandrosterone sulfate plasma levels and their relationship to aging, cognitive function, and dementia. Brain Cogn 2002, 50(2):316-323.

34. Cruess DG, Antoni MH, Kumar M, Ironson G, McCabe P, Fernandez JB, Fletcher M, Schneiderman N: Cognitive-behavioral stress management buffers decreases in dehydroepiandrosterone sulfate (DHEA-S) and increases in the cortisol/DHEA-S ratio and reduces mood disturbance and perceived stress among HIV-seropositive men. Psychoneuroendocrinology 1999, 24(5):537-549.

35. Strazdins L, Meyerkort S, Brent V, D’Souza RN, Broom DH, Kyd JM: Impact of saliva collection methods on sIgA and cortisol assays and acceptability to participants. J Immunol Methods 2005, 307(1-2):167-171.

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