Dehydroepiandrosterone (DHEA)

Salivary DHEA Measurement in Wave I of the Social Life Health & Aging Project

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Rationale

The adrenal cortex is the primary source of circulating concentrations of DHEA and DHEA-S, (Williams and Wilson 1998) which are secreted in response to adrenal corticotropin-releasing hormone (ACTH) (Parker, Azziz et al. 1996). DHEA and DHEA-S act as the precursors (Regelson, Loria et al. 1994) to the approximately 50% of androgens in adult men, 75% of active estrogens in premenopausal women, and almost all of active estrogens after menopause (Labrie, Belanger et al. 1997).

DHEA production and concentration declines significantly and steadily during aging (Ravaglia, Forti et al. 1996; Speroff, Glass et al. 1999; Hackbert and Heiman 2002; Villareal and Holloszy

2004). Some have considered DHEA concentration, along with its sulfated counterpart, DHEA-S, to be possible markers of physiologic aging (Rudman, Shetty et al. 1990).

Inverse relationships between DHEA concentration and extent of cardiovascular disease (Alexandersen, Haarbo et al. 1996), progression of HIV infection (Christeff, Lortholary et al. 1996), symptomatic progression of systemic lupus erythematosus (SLE) (Spark 2002), presence of rheumatoid arthritis among postmenopausal women (Masi 1995), and bone resorption and skin pigmentation (Spark 2002) have been reported. Activities, such as smoking, among middle-aged men (Field, Colditz et al. 1994), and exercise (Kroboth, Salek et al. 1999), have been shown to influence DHEA concentration.

DHEA has been explored for its role in mood and well-being (Hackbert and Heiman 2002; Spark 2002), sexual physiology and treatment of sexual problems (Spark 2002).

Measurement

Sex hormone assays, particularly in the clinical setting, are typically performed on a serum specimen. Salivary measures have been developed and offer a relatively convenient and minimally-invasive approach for obtaining sex hormone data (Gallagher, Leitch et al. 2006). These measures are representative of active, unbound steroid concentrations in the blood. No serum binding globulin has been identified for DHEA (Gallagher, Leitch et al. 2006).

DHEA is rapidly cleared from the blood (Longcope 1996) Levels in saliva are significantly lower than levels found in circulating serum (Gallagher, Leitch et al. 2006; Salimetrics 2006). Previous studies have indicated a strong positive correlation between salivary and serum DHEA levels (Shirtcliff, Granger et al. 2001; Gallagher, Leitch et al. 2006), though as expected, absolute concentration of DHEA is higher in serum. In a recent study done by Shirtcliff et al. (2001), the relationship between salivary DHEA levels and serum DHEA was only maintained when samples were collected using non-cotton based methods.

Scoring

Salivary values are reported in picograms per milliliter (pg/mL). The assay range was ≥5 pg/mL. (Serum values are typically reported in nanograms/mL.)

Population Norms

Note: 1 nanogram (ng) = 1,000 picogram (pg)

Table 1.

*Salivary DHEA Expected Ranges:

Group	N	Mean (pg/mL)	Standard Deviation (pg/mL)
Females	19	165.6	71.6
Males	20	153.5	68.8

Reproduced with permission from DHEA Quantitative Immunoassay Kit, 1-1502/1-1512, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

Note: These values were taken from young adults, aged 18-30.

DHEA concentrations have generally been reported to be higher in women than in men (Sulcova, Hill et al. 1997), though some have reported equal concentrations (Labrie, Belanger et al. 1997). The following are population-based, serum DHEA data, and non-population-based salivary data:

- Massachusetts Male Aging Study (Feldman, Longcope et al. 2002):
  - *Serum* samples were taken from 1,709 men 40 to 70 years of age (mean = 55.2) at baseline (1987-1989). Average DHEA = 2.3 ng/ml, +/- 1.6 (standard deviation). At follow-up (1995-1997), 1,156 samples were taken (mean age = 62.7). Average DHEA = 1.9 +/- 1.0 ng/ml.
- Massachusetts Women's Health Study (Johannes, Stellato et al. 1999):
  - *Serum* samples were taken from 224 women, 50 to 60 years of age (mean = 52.7). Mean DHEA level was 2.19 ng/ml.

Table 2. Salivary Concentrations

Group	N	Age	Mean Concentration +/- Dispersion Value*	Units	Source
Saliva					
Women	16	30-34	374.0 SEM = +/- 35.4	pg/ml	(Granger, Schwartz et al. 1999)
Women	16	35-39	299.4 SEM = +/- 30.9	pg/ml	(Granger, Schwartz et al. 1999)
Women	16	40-45	207.6 SEM = +/- 32.7	pg/ml	(Granger, Schwartz et al. 1999)

* Averaged AM and PM values.

Specimen Collection

All NSHAP respondents were asked to provide a salivary specimen. 90.8% (N=2,721) agreed. 2,640 respondents were able to provide a salivary specimen. This involved production of approximately 2 milliliters of saliva (unstimulated passive drool) into a small, code-labeled polystyrene collection vial via a 5-centimeter section of a household plastic straw, following procedures recommended by Salimetrics, LLC. The procedure required approximately 5 minutes. The time of last food or water consumption prior to saliva collection was recorded.

Shipping and Storage

The salivary specimens were transported from the interview to a freezer using cold packs. Salivary specimens were stored in a freezer until they were shipped. The salivary samples were shipped to the lab on dry ice according to instructions. Upon receipt at Salimetrics, specimens were stored at -80°C in lab grade freezers.

Shipping Address	Salimetrics, LLC Attn: Receiving Dept. 101 Innovation Blvd., Suite #302 State College, PA 16803 800-790-2258

Assay

(see Salimetrics Salivary DHEA Enzyme Immunoassay Kit package insert for details (http://salimetrics.com/pdf/DHEA%20Kit%20Insert.pdf) (Salimetrics 2006))

On day of assay, the specimens were thawed completely, vortexed, and centrifuged at 1500 x g (@3000 rpm) for 15 minutes. Clear samples were pipetted into wells. The enzyme immunoassay was conducted at Salimetrics, LLC. The assay range was > 5 pg/ml. Assays were conducted in the following priority order: 1) estrogen, 2) progesterone, 3) DHEA, 4) testosterone, 5) cotinine and underwent 2 to 3 freeze-thaw cycles:

thaw #1: sex hormone assays
thaw #2: a subset underwent repeat sex hormone testing based on quality indicators
thaw #3: cotinine assay

Table 3. NSHAP Salivary Testing Performed at Salimetrics

Test	Units	Highest Calibrator*	Lowest Calibrator*	Lower Limit of Sensitivity	None detected (ND) reported if value:	Interference likely if value:
Estradiol	pg/mL	64	2	1 pg/mL	<0.5 pg/mL	>320 pg/mL
Progesterone	pg/mL	2430	10	5 pg/mL	<2 pg/mL	>5x highest calibrator
DHEA	pg/mL	1000	10.2	5 pg/mL	≤2 pg/mL	>5x highest calibrator
Testosterone	pg/mL	600	6.1	1 pg/mL	≤0.5 pg/mL	>5x highest calibrator
Cotinine	ng/mL	200	0.8	0.05 ng/mL	unable to get a number value because result is too low	dilute sample x20; report >3000 if value is still high

* Calibrator values are used to adjust instrumentation by establishing the relationship (under specified conditions) between known, standard values and the values indicated by a particular measuring instrument. See package insert for calibration curve.

Scoring

Values reported in picograms per milliliter (pg/mL). Assay range ≥ 5 pg/mL.

Performance Characteristics

A. Precision

Intra-assay precision was determined from the mean of 12 replicates at high (618.6 pg/mL) and low (44.6 pg/mL) DHEA levels. The average intra-assay coefficient of variation was 5.6%.

Inter-assay precision was determined from the mean of averaged duplicates for 12 separate runs. The average inter-assay coefficient of variation was 7.9% for high (579.5 pg/mL) and 8.5% for low (34.8 pg/mL) DHEA levels.

B. Sensitivity

The lower limit of sensitivity was determined by interpolating the mean minus 2 SD for 10 sets of duplicates for the 0 pg/mL standard. The minimal concentration of DHEA that can be distinguished from 0 is 5 pg/mL.

C. Expected Ranges

Table 4.

*Salivary DHEA Expected Ranges:

Group	N	Mean (pg/mL)	Standard Deviation (pg/mL)
Females	19	165.6	71.6
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Note: These values were taken from young adults, aged 18-30.

D. Correlation with Serum

The DHEA serum-saliva correlation, using a log 10 transformation for the total (n = 39), combined males and females, is 0.857, $p < 0.0001$ (Salimetrics 2006). The conversion equation from salivary concentration to serum concentration for this particular DHEA assay is:

Serum DHEA (ng/mL) = 0.0576*saliva DHEA (pg/mL) + 2.3074.

Quality Control (see Table 3)

Run on each EIA test plate were six (6) standard calibrators ranging from 10.2 pg/mL to 1000 pg/mL and two sets of high and low controls with established ranges. A sufficient number of assay kits and controls were sequestered for the project to minimize any lot-to-lot variations over the course of the study.

Subjects' saliva samples were run in duplicate (saliva pipetted into side-by-side wells) on a single EIA plate. Assay results for each subject were acceptable when the coefficient of variation (%CV) between the duplicate results (result 1 and result 2) was <15%. In instances where the %CV between duplicates was >15%, results were accepted if the absolute value between result 1 and result 2 was <8 pg/mL. Values greater than the upper assay limit of 1000 pg/mL were run on dilution to bring the OD readings within accepted range (10.2 pg/mL - 1000 pg/mL). In instances when a sample returned an extremely high result (5 times the highest calibrator), dilutions were made up to 5,000 pg/mL and a flag (**) and comment "interference likely" were added to the report. Samples with results < 5 pg/mL were also repeated. Values falling between 2.1 pg/mL and 5 pg/mL were reported and flagged (*) with the comment "below lower limit of assay." DHEA values less than or equal to 2 pg/mL were reported as "none detected". In rare instances, repeat results where lower than initial values and did not fall within the accepted criteria (CV% <15% or difference <8 pg/mL between values 1 & 2). These data were reported with the comment "repeat affected by freeze/thaw." If not enough sample remained to run a duplicate assay, "quantity not sufficient (qns)" was reported as the result.

DHEA data were compiled in Excel by the testing manager and checked for accuracy by the technical supervisor before final reports were delivered. Data was supplied with corresponding assay plate number to facilitate the calculation of intra-assay and inter-assay control values.

Availability

Product Name	Salivary DHEA Enzyme Immunoassay Kit
Manufacturer	Salimetrics, LLC
Location of Manufacturer	101 Innovation Blvd., Suite 302 State College, PA 16803 USA 800-790-2258 (USA & Canada only)
Catalog No.	1-1202/1-1212, 96-Well Kit

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