Estradiol

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

Authors:

Karl Mendoza, BS, University of Chicago, Department of Ob/Gyn
Mary Curran, Salimetrics LLC
Stacy Tessler Lindau, MD, MAPP, University of Chicago, Departments of Ob/Gyn and Medicine – Geriatrics*

* Corresponding author. Fax: +1 773 834 5664.
E-mail address: slindau@uchicago.edu (S.T. Lindau).

Suggested Citation: Mendoza, K., M. Curran, and S.T. Lindau. (2007). Salivary Estradiol Measurement in Wave I of the National Social Life, Health & Aging Project (NSHAP). Chicago Core on Biomarkers in Population-Based Aging Research
http://biomarkers.uchicago.edu/pdfs/TR-Estradiol.pdf

Date: December 17, 2007

Contents

Rationale

Estradiol (17-beta-estradiol or E_2), a steroid hormone derived from cholesterol, targets a variety of tissues, located in the female and male reproductive tracts, mammary gland, and skeletal and cardiovascular systems (Hall, Couse et al. 2001). Among women, it is primarily synthesized from testosterone in the ovarian follicles, whereas among men, it is produced by the testes and extraglandular conversion of androgens (Tivis, Richardson et al. 2005; Salimetrics 2006).

In women, estradiol synthesis normally declines after menopause (Manly, Merchant et al. 2000; Meston and Frohlich 2000). This causes decreased vaginal lubrication and atrophy of the vaginal epithelium, due to diminished genital vasocongestion (Meston and Frohlich 2000). Though the

role of estrogens in female sexual desire is not fully understood, they may influence sexual desire (Dennerstein, Gotts et al. 1994; Meston and Frohlich 2000); estrogen replacement therapy may indirectly enhance female sexuality, by restoring vaginal lubrication (Meston and Frohlich 2000) or promoting positive body image and overall sense of well-being.

A number of studies have linked higher levels of serum estradiol to increased risk for developing breast cancer (Clemons and Goss 2001; Chlebowski, Hendrix et al. 2003; Tivis, Richardson et al. 2005) and coronary problems (Manson, Hsia et al. 2003; Tivis, Richardson et al. 2005). For women, higher estradiol levels have also been associated with improved cognition (Maki and Resnick 2000; Carlson, Zandi et al. 2001; Tivis, Richardson et al. 2005), mood, and memory (Tivis, Richardson et al. 2005). Estradiol's effects on women's skeletal health, however, are inconclusive.

For elderly men, estradiol may have a role, in combination with testosterone and other factors, in preservation of memory and cognitive function (Barrett-Connor, Goodman-Gruen et al. 1999; Carlson and Sherwin 2000).

Measurement

Sex hormone assays, particularly in the clinical setting, are typically performed on a serum specimen (Kaufman and Lamster 2002). Salivary measures have been developed and offer a relatively convenient and minimally-invasive approach for obtaining sex hormone data (Worthman, Stallings et al. 1990; Kaufman and Lamster 2002; Granger, Shirtcliff et al. 2004). These measures are representative of active, unbound steroid concentrations in the blood (Worthman, Stallings et al. 1990; Lu, Bentley et al. 1999).

In the case of estradiol, good correlation between salivary and serum levels have been reported (Worthman, Stallings et al. 1990), including one study of people ages 18 to 28 (Shirtcliff, Granger et al. 2000) ($n = 31$) finding a correlation of $r = 0.68$ ($p < 0.001$), where a stronger, significant association was detected among women ($n = 16$, $r = 0.60$, $p < 0.013$) than men ($n = 15$, $r = 0.60$, $p>0.05$). Reported salivary concentrations, relative to free serum concentrations, have varied (Worthman, Stallings et al. 1990) from 0.2% to 7.90% (Lu, Bentley et al. 1999). In one study, salivary estradiol concentrations accounted for, on average, 1.65% of serum concentrations among men, and 3.33% among women (Shirtcliff, Granger et al. 2000). Among premenopausal women, salivary estradiol levels vary significantly across the menstrual cycle (Shirtcliff, Granger et al. 2000), with the lowest levels occurring during menstruation (Shirtcliff, Granger et al. 2000; Salimetrics 2006).

Population Norms

To our knowledge, population-based, published literature on *salivary* estradiol levels, particularly for postmenopausal women and older men, are limited.

Table 1. Mean Serum and Salivary Estradiol Levels

	Age Range	N	Mean SERUM levels (pg/ml)	SD	Range	Mean SALIVARY levels (pg/ml)	SD	Range	Source
Male	*8-9*	17				0.83		< 0.25 - 2.46	*[1]*
	18-27	15	32.37	7.53		0.53	0.19		*[1]*
	32-49	19				1.02		< 0.25 - 3.93	*[1]*
Female	*11-12 (noncycling)*	18				0.82		< 0.25 - 2.45	*[1]*
	18-27	16	70.39	45.42		0.98	0.56		*[1]*
	36-51 (cycling)	54				2.01		< 0.25 - 6.13	*[1]*
	48-65 (w/ estrogen therapy)	28	136.17*	17.76	55.1 - 496.3	1.61	0.23	0.36 - 6.17	*[2]*
	50-66 (postmenopausal)	12	15.48	2.29	4.0 - 28.3	1.39	0.33	0.54 - 4.78	*[2]*
	Perimenopausal women, mean age: 46.25 (SD = 2.63)	3,029	77.3	81.1					*[3]*
	42-52 (perimenopausal women)	2,930	76.0 (Median: 54.0)	77.0					*[4]*

*Women who were undergoing estrogen therapy had a significantly higher serum estradiol level ($p < 0.0001$)

[1] Shirtcliff et al. 2000
[2] Tivis et al. 2005
[3] Lasley et al. 2002
[4] Randolph et al. 2003

Specimen Collection

All 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 polypropylene 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.

Storage and Shipping

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 Estradiol Enzyme Immunoassay Kit package insert for details http://salimetrics.com/pdf/HS%20Estradiol%20E2%20Kit%20Insert.pdf)

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 > 1 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 2. 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 ≥ 1 pg/mL.

Performance Characteristics

A. Precision

Table 3.

The intra-assay precision was determined from the mean of 14 replicates each.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (%)
High	14	20.26	1.42	7.0
Mid	14	7.24	0.45	6.3
Low	14	3.81	0.31	8.1

The inter-assay precision was determined from the mean of average duplicates for 10 separate runs.

Sample	N	Mean (pg/ml)	Standard Deviation (pg/ml)	Coefficient of Variation (pg/ml)
High	10	24.62	1.47	6.0
Low	10	4.76	0.42	8.9

Reproduced with permission from Estradiol Quantitative Immunoassay Kit, 1-3702/1-3712, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

B. Sensitivity

The lower limit of sensitivity was determined by interpolating the mean minus 2 SD's of the optical densities of 10 sets of duplicates at the 0 pg/mL standard. The minimal concentration of estradiol that can be distinguished from 0 is less than 1.0 pg/mL.

C. Correlation with Serum

The correlation between saliva and serum estradiol in females was determined by assaying 11 matched samples. Samples were screened for pH and blood contamination. The magnitude of the saliva-serum correlation, r= 0.80, p <0.001, is consistent with the literature, in that the saliva-serum correlation is stronger among women, than men (Shirtcliff, Granger et al. 2000), and intra-subject correlation is higher, compared to between-subjects (Ellison and Lipson 1999). The conversion equation from salivary concentration to serum concentration for this particular estradiol assay is:

Serum E2 (pg/mL) = 2.171*salivary E2 (pg/mL) +7.1846.

D. Specificity of Antiserum

Table 4.

Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Estradiol EIA
Estriol	10	0.234
Estrone	1	1.276
Progesterone	100	ND
17 α-Hydroxyprogesterone	1000	ND
Testosterone	1000	ND
Cortisol	1000	ND
DHEA	1000	ND
Androstenedione	1000	ND
Aldosterone	1000	ND
Cortisone	1000	ND
11-Deoxycortisol	1000	ND
21-Deoxycortisol	1000	ND
Dexamethasone	1000	ND
Triamincinolone	1000	ND
Corticosterone	1000	ND
Prednisolone	1000	ND
Prednisone	100	0.016
Transferrin	1000	ND

ND = None detected (<0.004)

Reproduced with permission from Estradiol Quantitative Immunoassay Kit, 1-3702/1-3712, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

E. Recovery

Table 5.

Five saliva samples was spiked with different levels of estradiol and assayed.

Sample	Endogenous (pg/ml)	Added (pg/ml)	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
I	2.92	20.48	23.40	23.84	101.9
II	4.68	13.65	18.33	17.91	97.7
III	3.80	3.20	7.00	6.78	96.9
IV	5.41	20.48	25.89	28.2	108.9
V	3.69	3.20	7.16	8.26	115.4

Reproduced with permission from Estradiol Quantitative Immunoassay Kit, 1-3702/1-3712, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

F. Linearity of Dilution

Table 6.

Four saliva samples were diluted with PBS and assayed.

Sample	Dilution	Expected (pg/ml)	Observed (pg/ml)	Recovery (%)
I			28.98	
	1:2	14.49	13.57	93.7
	1:4	7.25	7.24	99.9
	1:8	3.62	3.73	103.0
II			23.84	
	1:2	11.92	12.03	100.9
	1:4	5.96	5.56	93.3
	1:8	2.98	3.60	120.8
III			6.78	
	1:2	3.39	3.07	90.6
	1:4	1.70	1.70	100.0
IV			8.54	
	1:2	4.27	4.55	106.6
	1:4	2.14	1.93	90.2

Reproduced with permission from Estradiol Quantitative Immunoassay Kit, 1-3702/1-3712, 96-Well Kit, April 10, 2006 *(Salimetrics 2006)*

Quality Control (see Table 2)

Run on each EIA test plate were six (6) standard calibrators ranging from 2 pg/mL to 64 pg/mL and two sets of high and low controls. The control ranges were established using a minimum of 10 sets of data points across 10 plates. The mean and standard deviations were calculated and a range was established (mean +/- 2 S.D.). 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 values: 1.) fell within the range of the calibrators (2 pg/mL - 64 pg/mL) , 2.) were >1 pg/mL, and 3.) 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 <2 pg/mL. Values greater than the upper

assay limit of 64 pg/mL were run on dilution to bring the OD readings within acceptable range (2 pg/mL - 64 pg/mL). In instances when a sample returned an extremely high result, dilutions were made up to 320 pg/mL and a flag (**) and comment “interference likely” were added to the report. Samples with results < 1pg/mL were also repeated. Values falling between 0.5 pg/mL and 1 pg/mL were reported and flagged (*) with the comment “below lower limit of assay”. Estradiol values less than 0.5 pg/mL were reported as “none detected”.

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

Availability

Product Name	High Sensitivity Salivary Estradiol 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.	1-3702/1-3712, 96-Well Kit

References

Barrett-Connor, E., D. Goodman-Gruen, et al. (1999). "Endogenous sex hormones and cognitive function in older men." J Clin Endocrinol Metab **84**(10): 3681-5.

Carlson, L. E. and B. B. Sherwin (2000). "Higher levels of plasma estradiol and testosterone in healthy elderly men compared with age-matched women may protect aspects of explicit memory." Menopause **7**(3): 168-77.

Carlson, M. C., P. P. Zandi, et al. (2001). "Hormone replacement therapy and reduced cognitive decline in older women: the Cache County Study." Neurology **57**(12): 2210-6.

Chlebowski, R. T., S. L. Hendrix, et al. (2003). "Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial." Jama **289**(24): 3243-53.

Clemons, M. and P. Goss (2001). "Estrogen and the risk of breast cancer." N Engl J Med **344**(4): 276-85.

Dennerstein, L., G. Gotts, et al. (1994). "The relationship between the menstrual cycle and female sexual interest in women with PMS complaints and volunteers." Psychoneuroendocrinology **19**(3): 293-304.

Ellison, P. T. and S. F. Lipson (1999). "Salivary estradiol--a viable alternative?" Fertil Steril **72**(5): 951-2.

Granger, D. A., E. A. Shirtcliff, et al. (2004). "The "trouble" with salivary testosterone." Psychoneuroendocrinology **29**(10): 1229-40.

Hall, J. M., J. F. Couse, et al. (2001). "The multifaceted mechanisms of estradiol and estrogen receptor signaling." J Biol Chem **276**(40): 36869-72.

Kaufman, E. and I. B. Lamster (2002). "The diagnostic applications of saliva--a review." Crit Rev Oral Biol Med **13**(2): 197-212.

Lasley BL, Santoro N, Randolf JF, Gold EB, Crawford S, Weiss G, et al. The relationship of circulating dehydroepiandrosterone, testosterone, and estradiol to stages of the menopausal transition and ethnicity. Journal of Clinical Endocrinology & Metabolism 2002;87(8):3760-7.

Lu, Y., G. R. Bentley, et al. (1999). "Salivary estradiol and progesterone levels in conception and nonconception cycles in women: evaluation of a new assay for salivary estradiol." Fertil Steril **71**(5): 863-8.

Maki, P. M. and S. M. Resnick (2000). "Longitudinal effects of estrogen replacement therapy on PET cerebral blood flow and cognition." Neurobiol Aging **21**(2): 373-83.

Manly, J. J., C. A. Merchant, et al. (2000). "Endogenous estrogen levels and Alzheimer's disease among postmenopausal women." Neurology **54**(4): 833-7.

Manson, J. E., J. Hsia, et al. (2003). "Estrogen plus progestin and the risk of coronary heart disease." N Engl J Med **349**(6): 523-34.

Meston, C. M. and P. F. Frohlich (2000). "The neurobiology of sexual function." Arch Gen Psychiatry **57**(11): 1012-30.

Randolph, J. F., Jr., M. Sowers, et al. (2003). "Reproductive hormones in the early menopausal transition: relationship to ethnicity, body size, and menopausal status." Journal of Clinical Endocrinology & Metabolism **88**(4): 1516-22.

Salimetrics (2006). High Sensitivity Salivary Estradiol Enzyme Immunoassay Kit. State College, PA, Salimetrics LLC**:** 3.

Salimetrics (2006). Salivary Testosterone Enzyme Immunoassay Kit.

Shirtcliff, E. A., D. A. Granger, et al. (2000). "Assessing estradiol in biobehavioral studies using saliva and blood spots: simple radioimmunoassay protocols, reliability, and comparative validity." Horm Behav **38**(2): 137-47.

Tivis, L. J., M. D. Richardson, et al. (2005). "Saliva versus serum estradiol: implications for research studies using postmenopausal women." Prog Neuropsychopharmacol Biol Psychiatry **29**(5): 727-32.

Worthman, C. M., J. F. Stallings, et al. (1990). "Sensitive salivary estradiol assay for monitoring ovarian function." Clin Chem **36**(10): 1769-73.