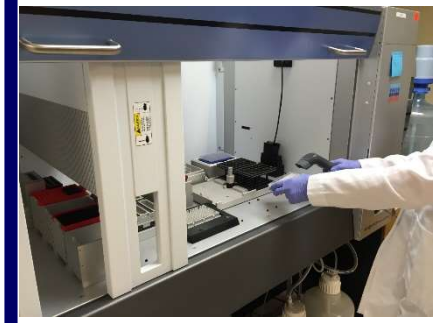
Blood and Saliva Hormone Testing- What to Choose?



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Body Fluids Commonly Used for Testing Steroid Hormones

- Blood
  - Serum (venipuncture)
  - Plasma (venipuncture)
  - Capillary Blood (finger/heel stick)
- Urine
- Saliva

Limitations of Hormone Testing in Different Body Fluids

Hormones Tested	Saliva	Blood	Urine
Estradiol	Yes	Yes	Yes
Progesterone	Yes	Yes	Yes
Testosterone	Yes	Yes	Yes
DHEA(S)	Yes	Yes	Yes
Cortisol	Yes	Yes	Yes
FSH	No	Yes	Yes
LH	No	Yes	Yes
fT3	No	Yes	Yes
fT4	No	Yes	No
TSH	No	Yes	No
IGF1	No	Yes	No
Insulin	No	Yes	No
Hs-CRP	No	Yes	No
Vitamin D3	No	Yes	No
PSA	No	Yes	No

A Guide to Steroid Hormone Testing in Different Body Fluids Following Different Routes of Hormone Administration

Type of Body Fluid	None Endogenous Steroids	Oral Steroids	Topical Steroids	Vaginal Steroids	Troche Steroids	Pellet/IM Steroids
Serum	Yes	Yes (1)	No (2)	No (2)	Yes	Yes
Saliva	Yes	Yes (3)	Yes (3)	Yes	No (4)	Yes
Urine	Yes	Yes (1)	No (2)	No (5)	No (2)	Yes (1)
DBS	Yes	Yes	Yes (6)	Yes	Yes	Yes

1) Overestimation: Metabolites interfere with immunoassays- LC-MS or GC-MS ok

2) Underestimation: Hormone levels not reflective of tissue uptake

3) Overestimation: Requires range adjustment

4) Overestimation: Direct contamination of oral mucosa/saliva

5) Overestimation: Direct contamination of urine

6) Overestimation: IF fingertips contaminated with topical hormones

HORMONE TESTING IN VENIPUNCTURE SERUM OR PLASMA

Serum/Plasma (Advantages)

- Wide range of hormone tests available
- Automated FDA-approved methodology
- Covered by most insurance carriers
- Familiarity with levels and ranges

Serum/Plasma (Disadvantages-1)

- Invasive-sharp needle in arm
- Stressful-inconvenient to patient-
  requires driving to blood draw station
- Phlebotomist required
- Processing of specimen-centrifugation

- Shipment-Biohazard labeling and requires cold packs

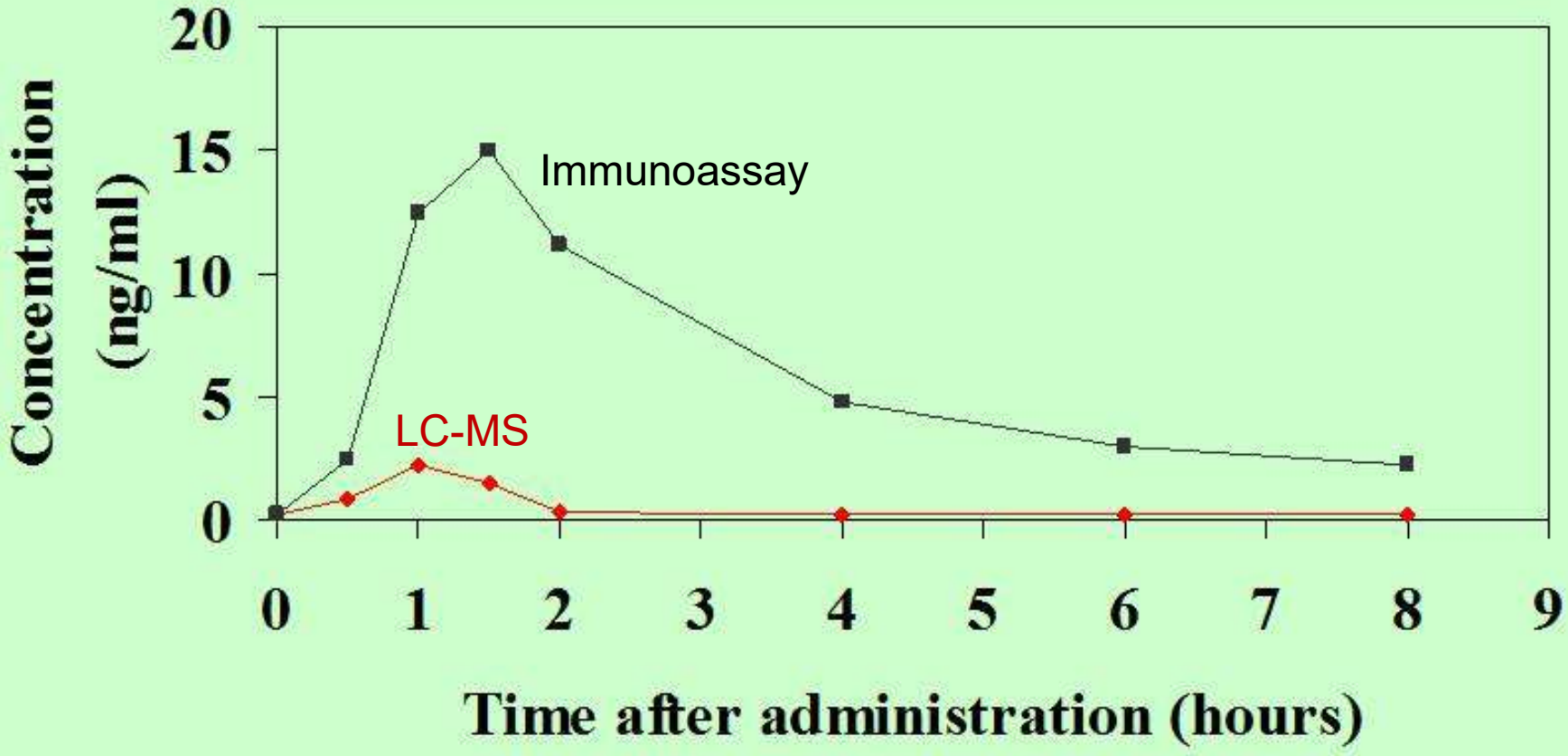
Serum/Plasma (Disadvantages-2)

- Normal ranges (+/- 2 SD) are too wide-more geared for disease than failing health
- Difficult to measure multiple times during day due to collection logistics
- Usually measures TOTAL and not BIOAVAILABLE fraction of hormone in bloodstream
- Not valid method for measuring hormones delivered *topically* -gross underestimation of hormone distribution to tissues

Serum Progesterone Following Oral Progesterone Delivery

Problem with high level of progesterone metabolites that are bioinert, but cross-react with antibodies used in immunoassays

Response to Oral Administration of 100 mg Progesterone



HORMONE TESTING
IN
SALIVA

Saliva (Advantages-1)

- Simple
- Stress-free
- Noninvasive (no needles)
- More convenient for health care provider and patient

Saliva (Advantages-2)

- Optimized for collection any time of day/month, any place
- No special processing (eg, centrifugation, ice-packs) prior to shipment
- Hormones stable in saliva for prolonged period of time
- Convenient shipment by regular US mail

Saliva Disadvantages

- Restricted to STEROID hormones-no thyroid or peptide (eg. FSH) hormones
- Technically more challenging: need 10-20x sensitivity-more problematic for hormones at very low concentration (i.e. estrogens)
- Interfering substances-food, beverages
- Sublingual use of hormones leads to spurious high test results (direct contamination of the oral mucosa)
- Problematic for those with poor saliva production

Salivary Sex Hormones (E2, Pg, T)

=

1-3% Serum Hormones

e.g. Estradiol: Reported 1.5-2%

Average Premeno Serum = 100 pg/ml

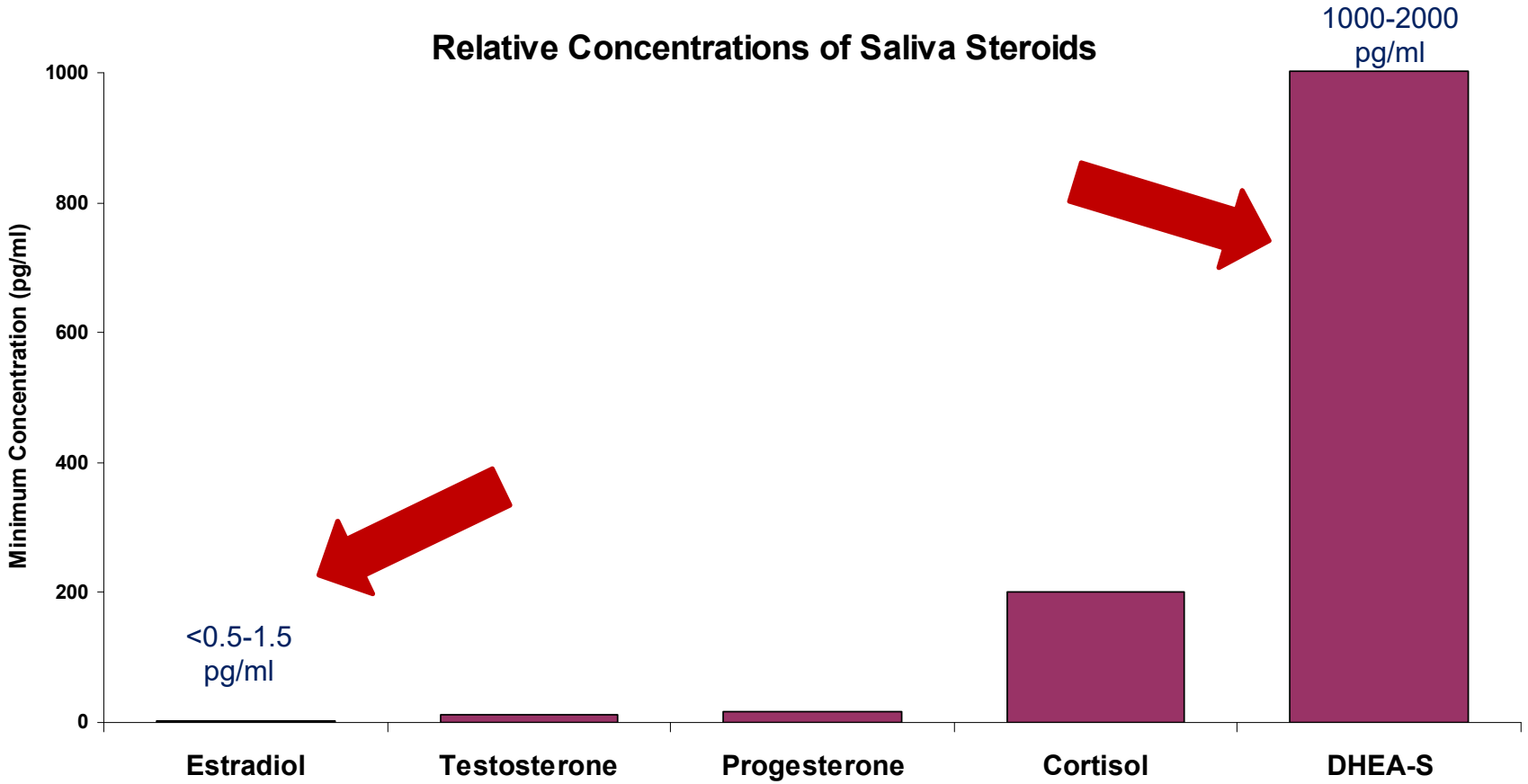
Average Premeno Saliva = 2.0 pg/ml @ 2%

Actual Serum E2 Range: 43-180 pg/ml

Calculated Saliva Range @ 2%: 0.9-3.6 pg/ml

Actual Saliva Range: 1.3-3.3 pg/ml

Assay Challenge: Relative Lowest Expected Concentrations of Steroids in Saliva



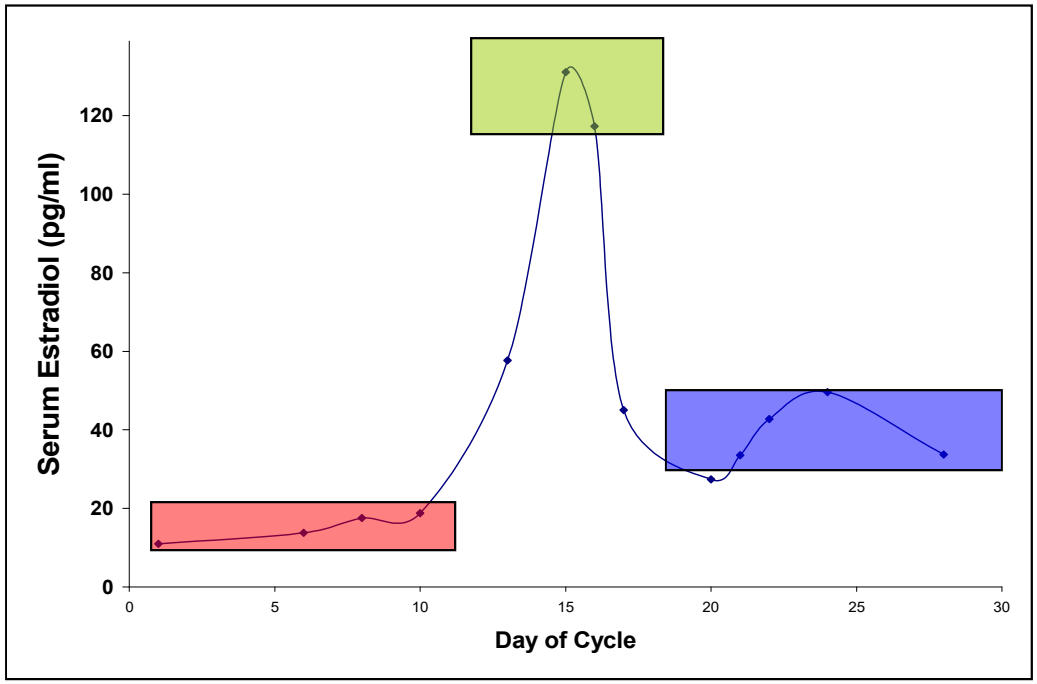
Clinical Utility of Salivary Hormone Testing

- Do salivary hormone levels show expected relationships with:
  - Menstrual Cycle (Estradiol and Progesterone)
  - Age: (e.g. Premenopausal vs Postmenopausal)
  - Diurnal Patterns-High Morning/Low Night
  - Symptoms of Hormone Imbalance-Deficiency and Excess
  - Hormone Supplementation

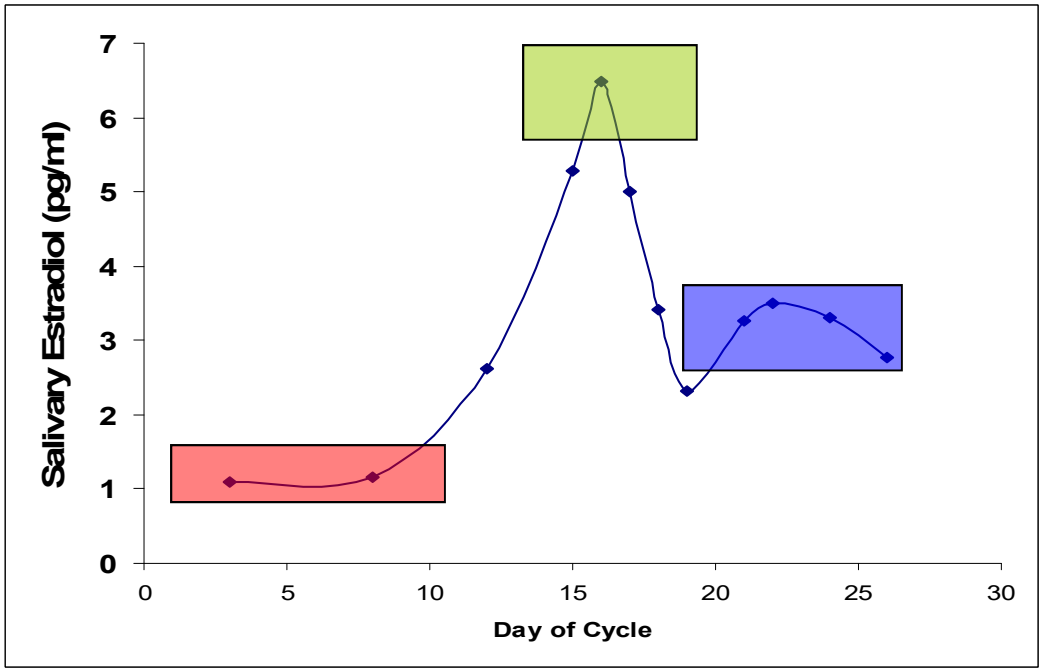
Menstrual Cycle Variation

Monthly Cycle

Serum Estradiol



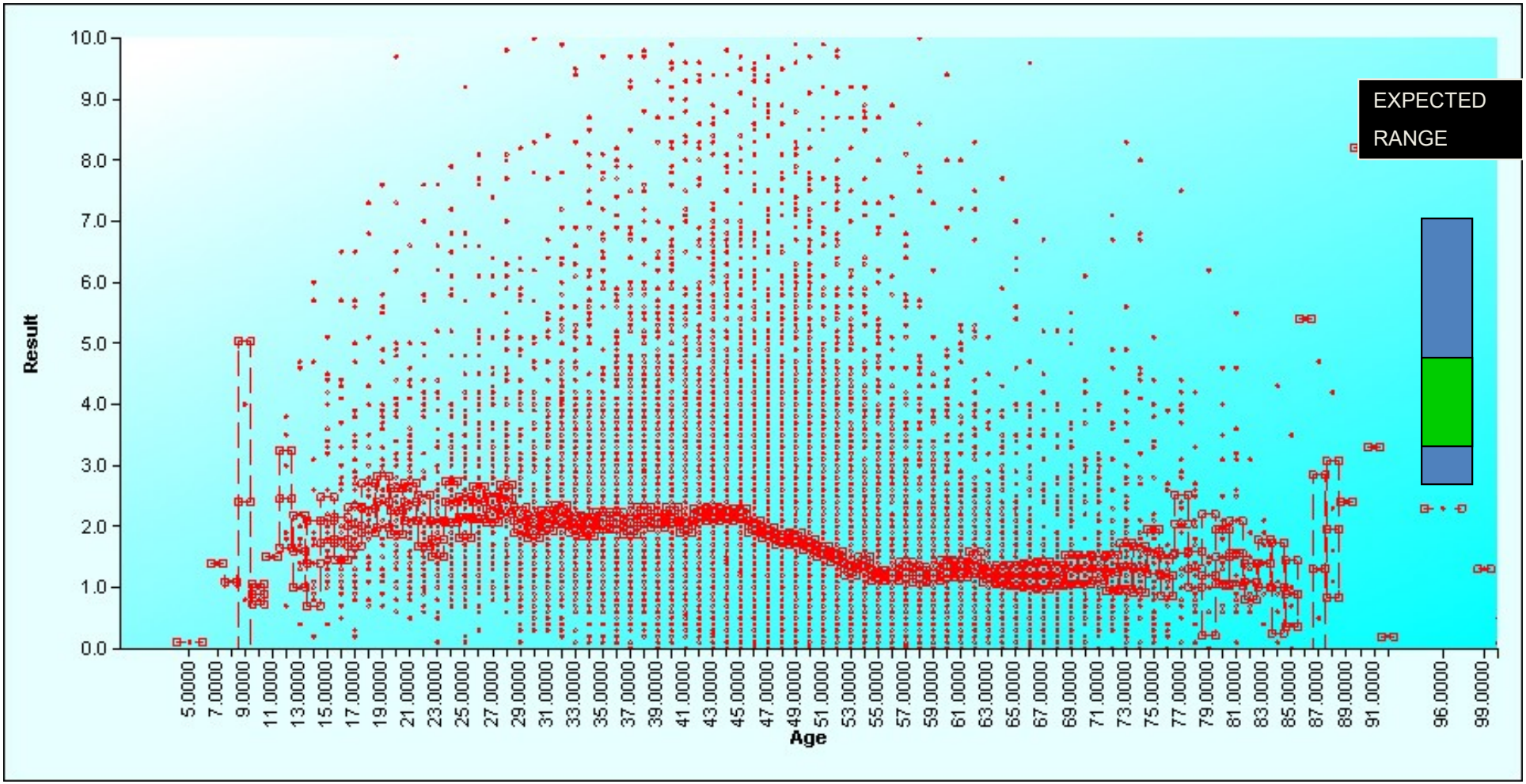
Salivary Estradiol



Age
Variation

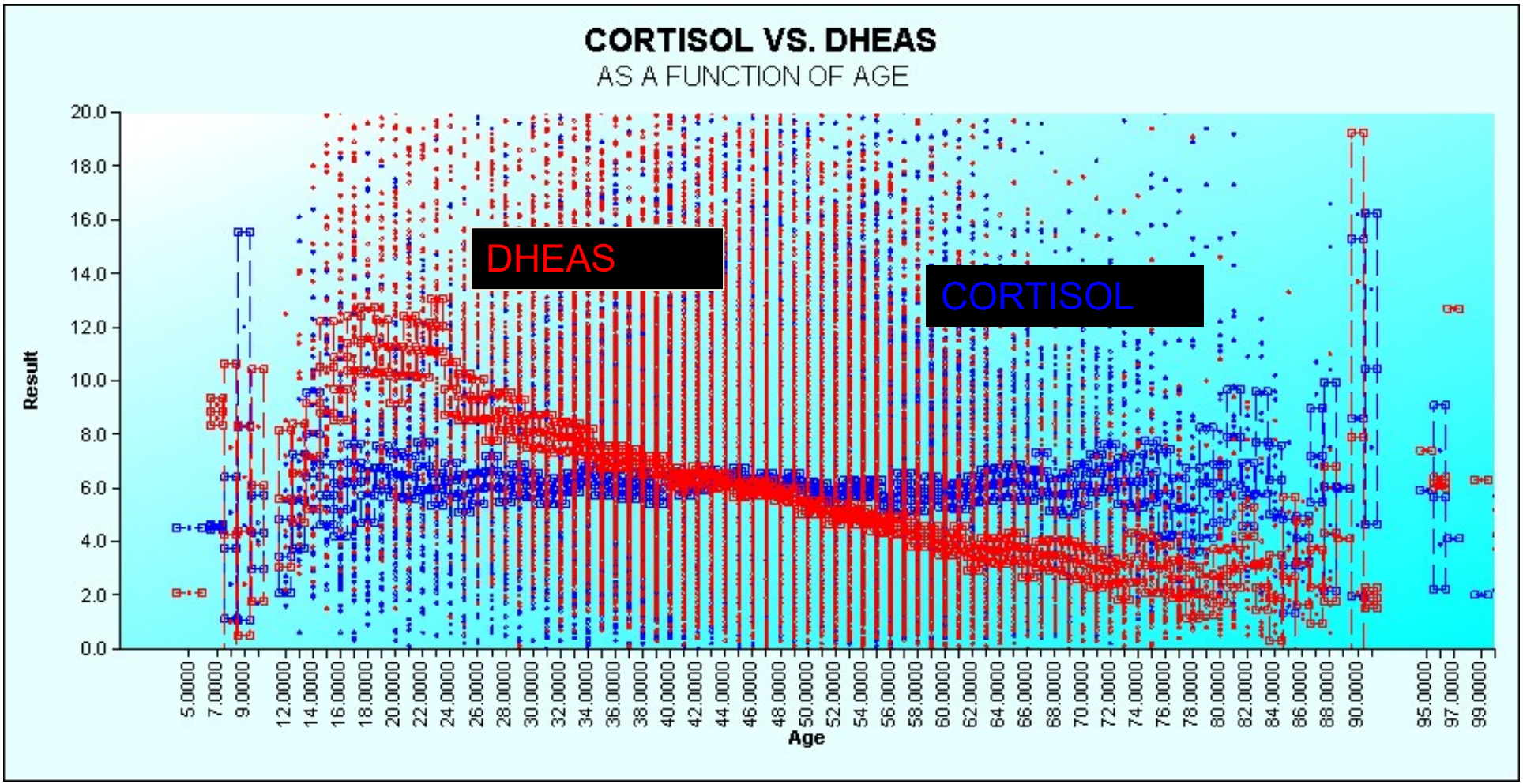
Estradiol levels decline dramatically at menopause

(N =17,349)



Androgen (DHEAS) levels decline progressively and steadily with age

(N = 19, 270)



Diurnal Pattern

Diurnal Patterns of Cortisol and Testosterone in Saliva by LC/MS/MS

Endocr. J./ F. MATSUI *et al.*: SALIVARY TESTOSTERONE AND CORTISOL
doi: 10.1507/endocrj.K09E-186

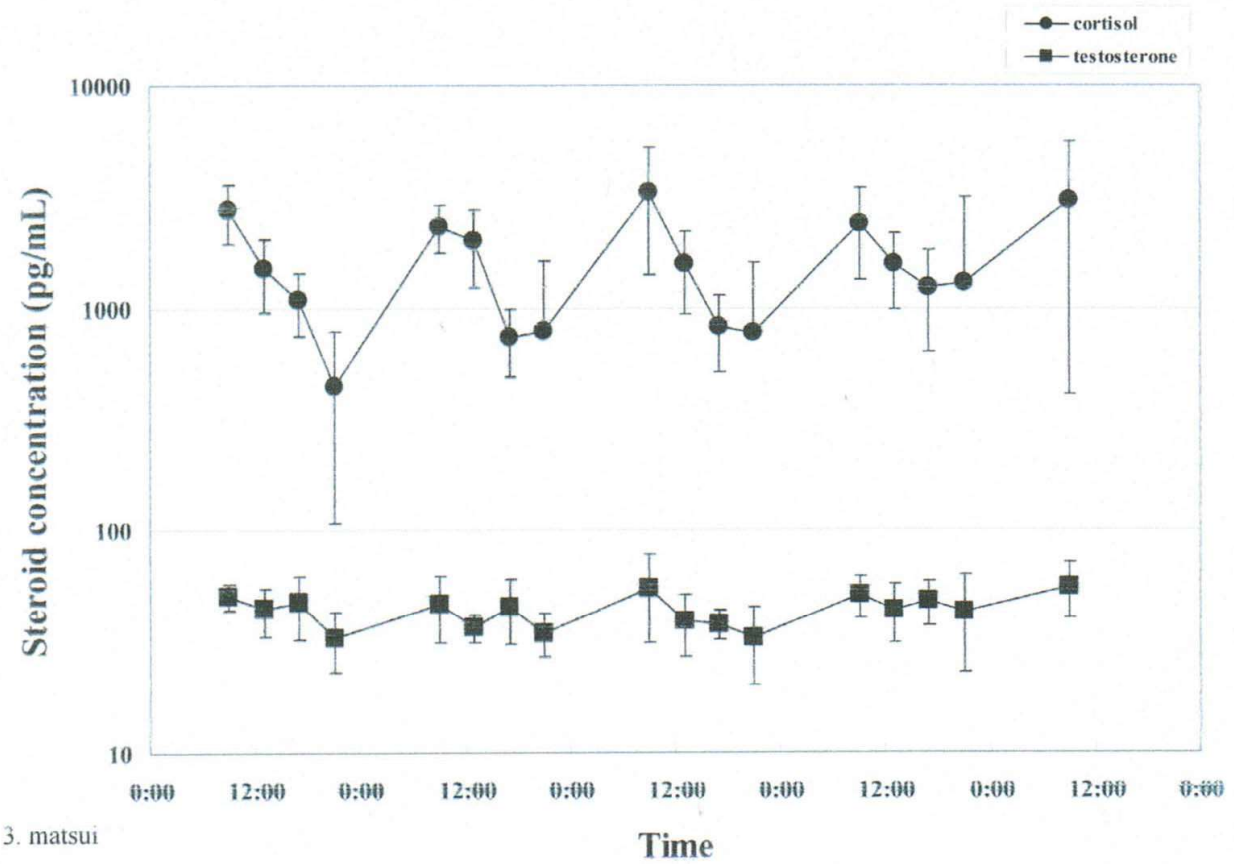
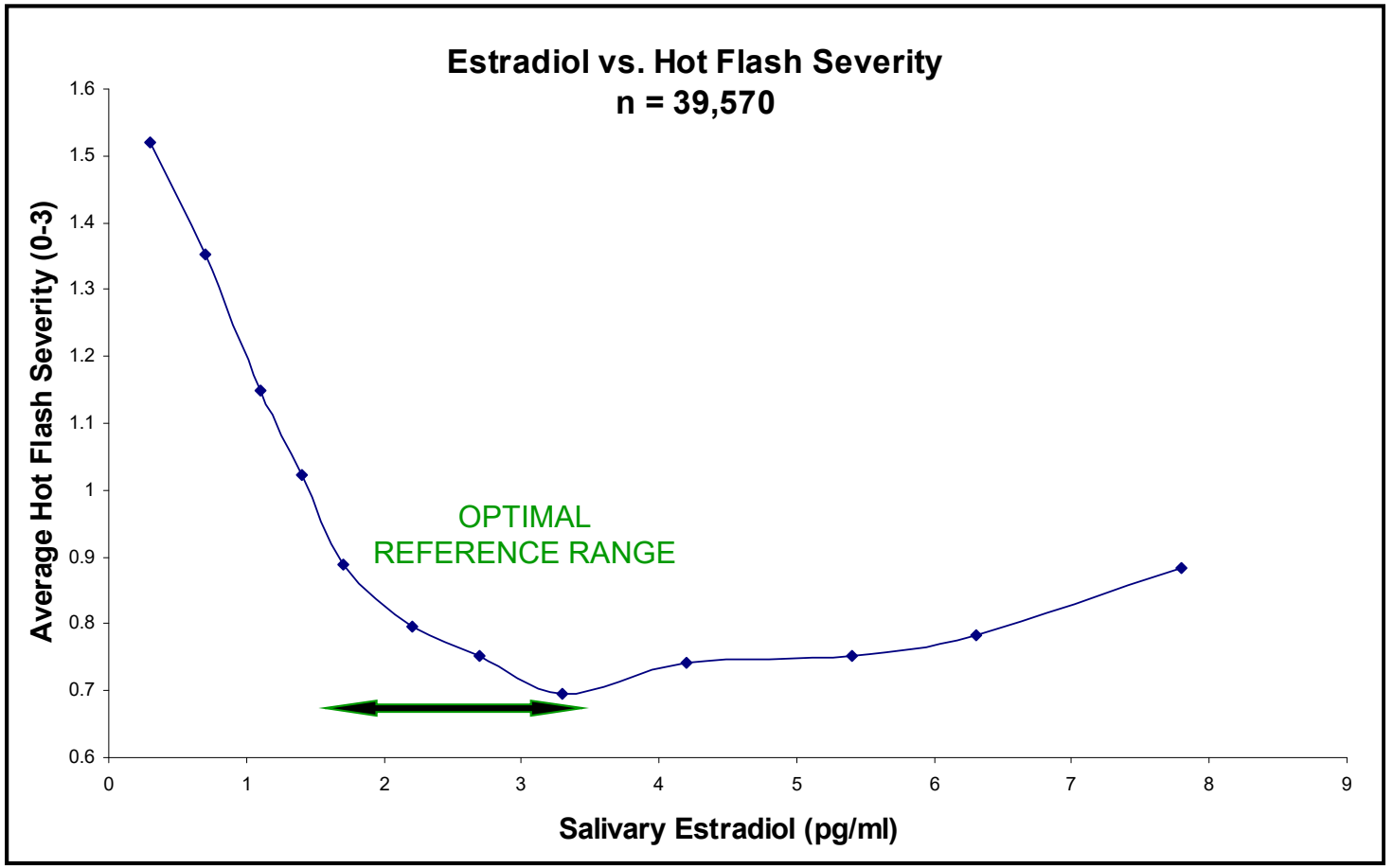


Fig. 3. matsui

Fig. 3. The diurnal change of the average value of salivary testosterone and cortisol value over 4 days (n=5). Bars indicate SD (standard deviation) in each point.

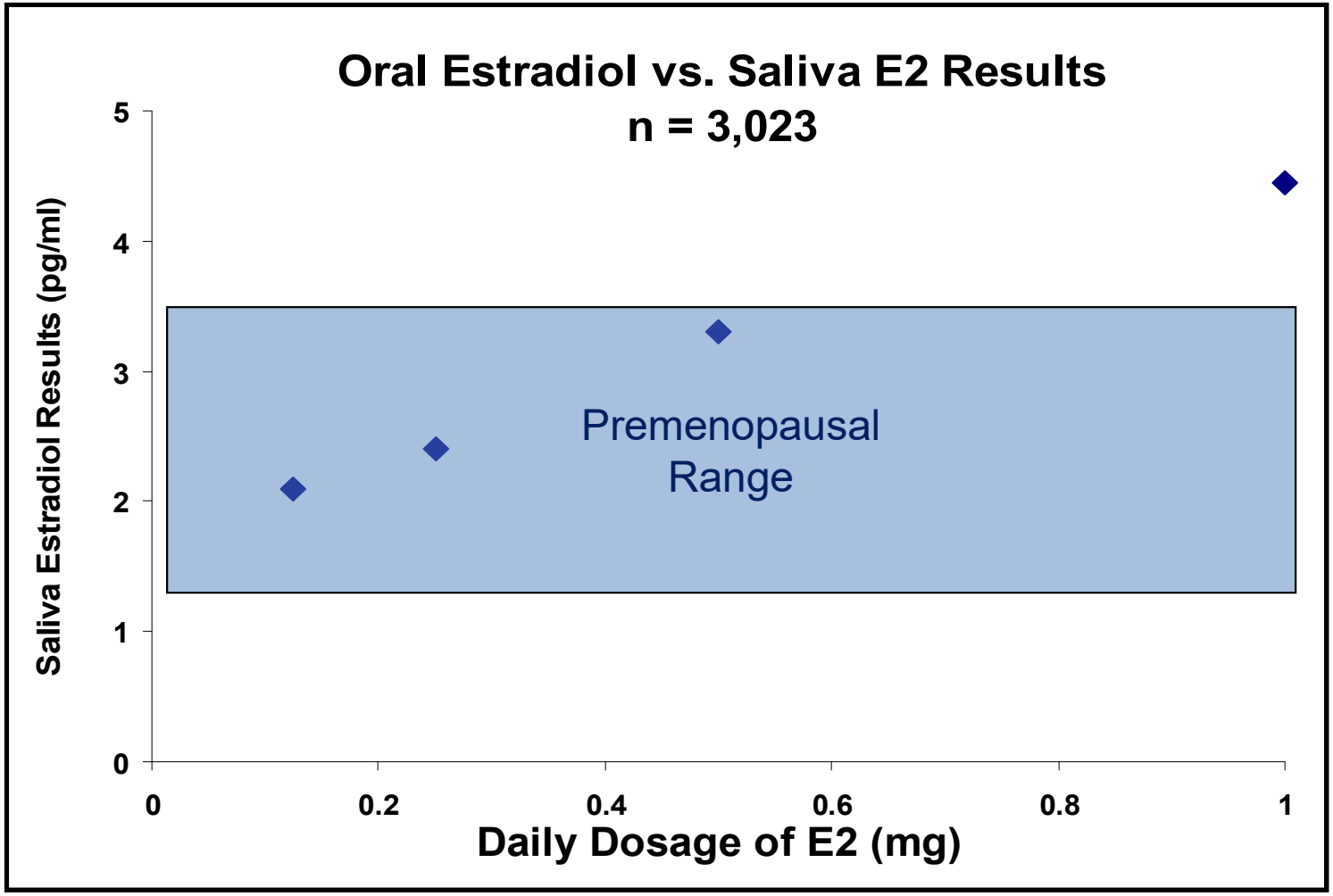
Symptom Association
with
Salivary Hormone Levels

Salivary Estradiol & Hot Flashes

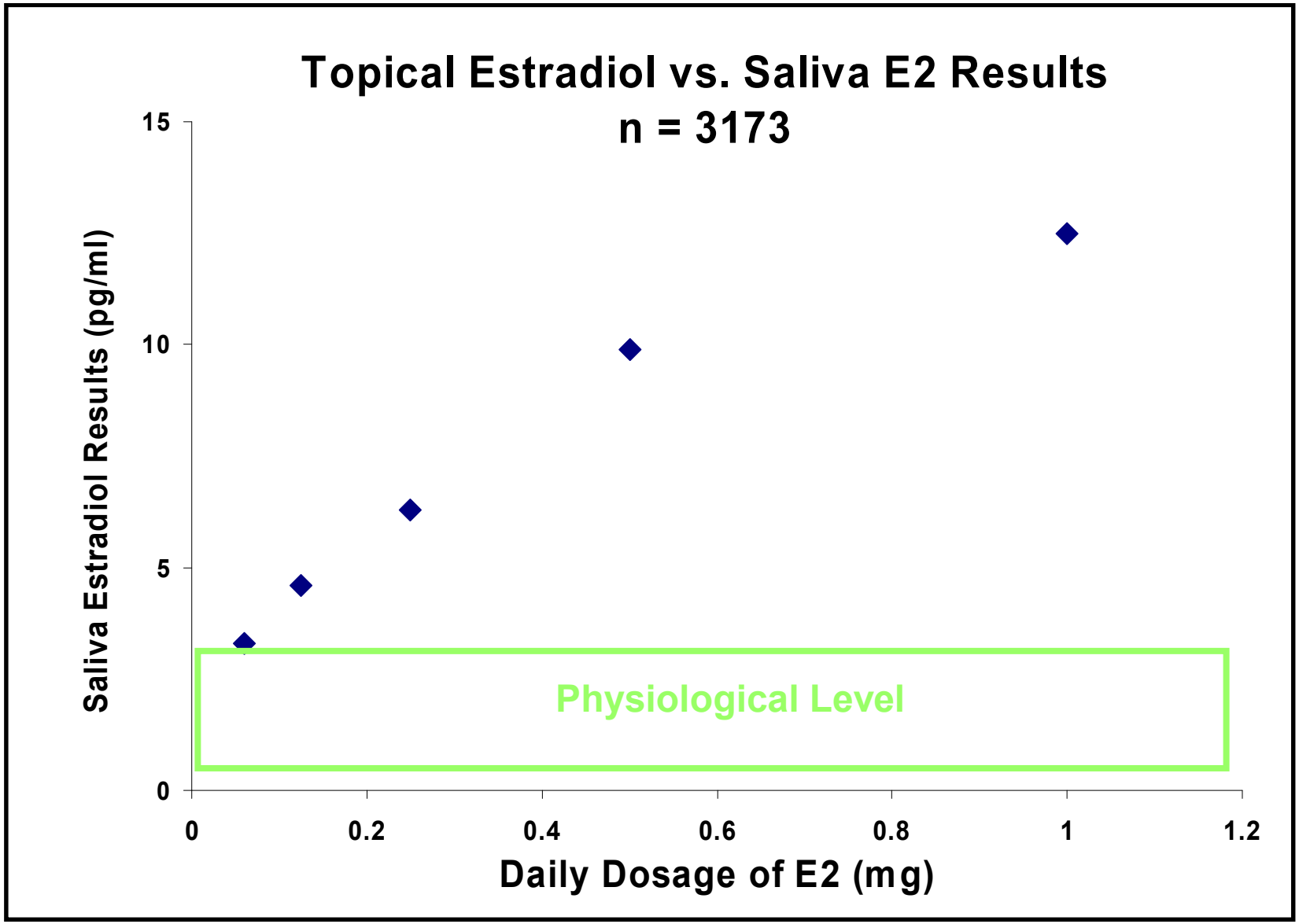


Dose Response to Exogenous Hormone Therapy

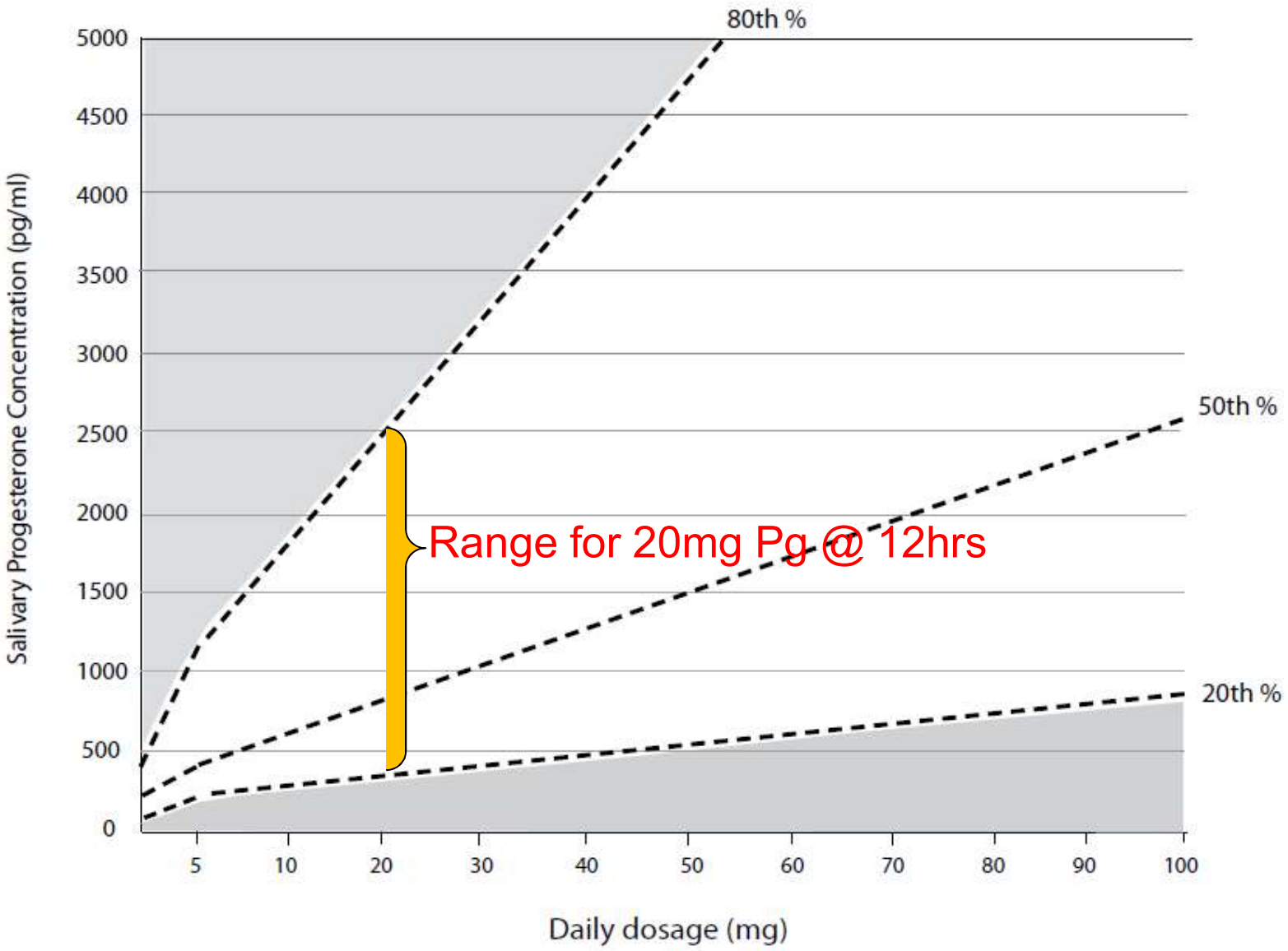
Salivary Estradiol: Monitoring Supplementation



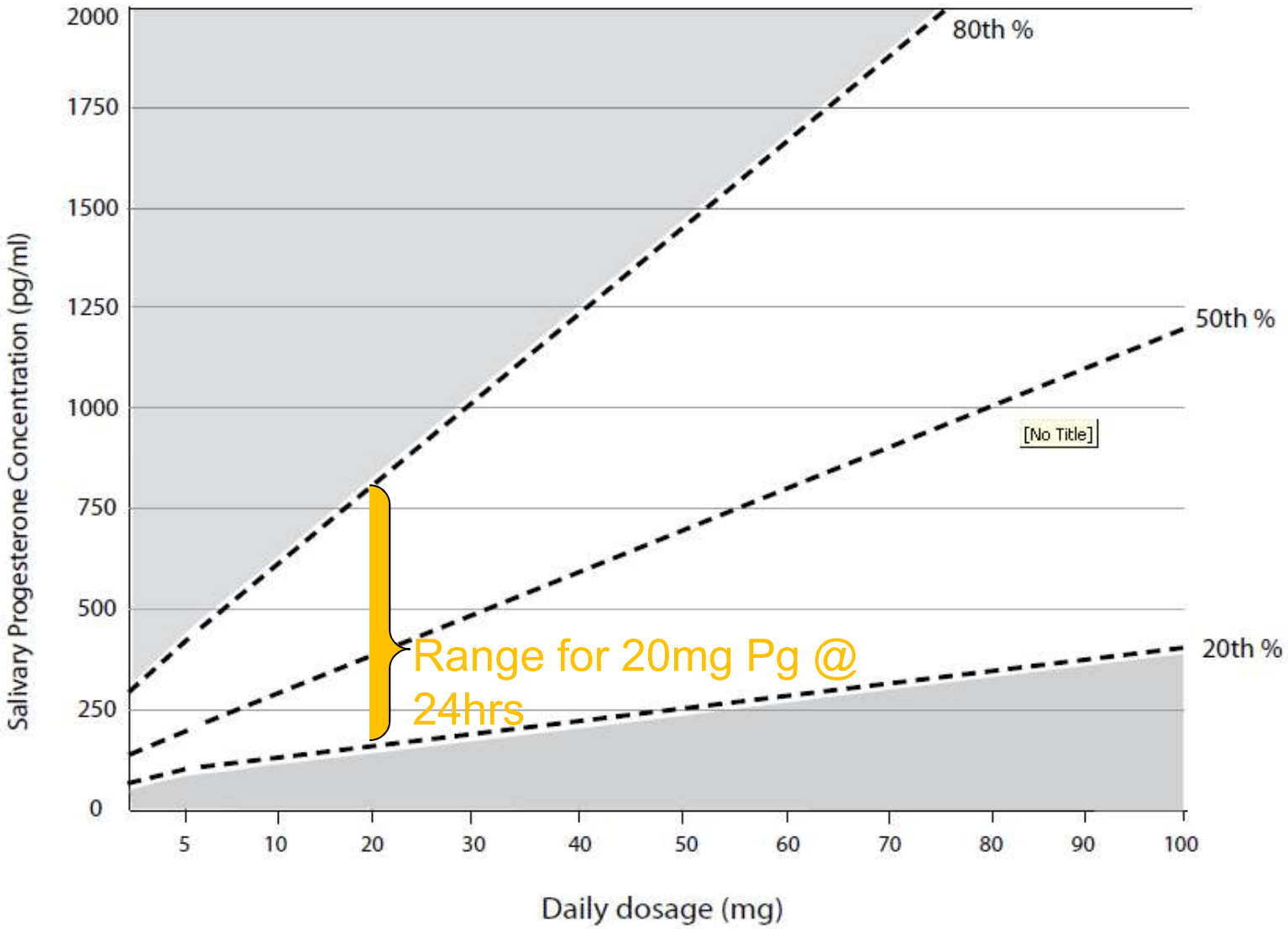
Salivary Estradiol Increases Linearly with Increasing Topical Dosage



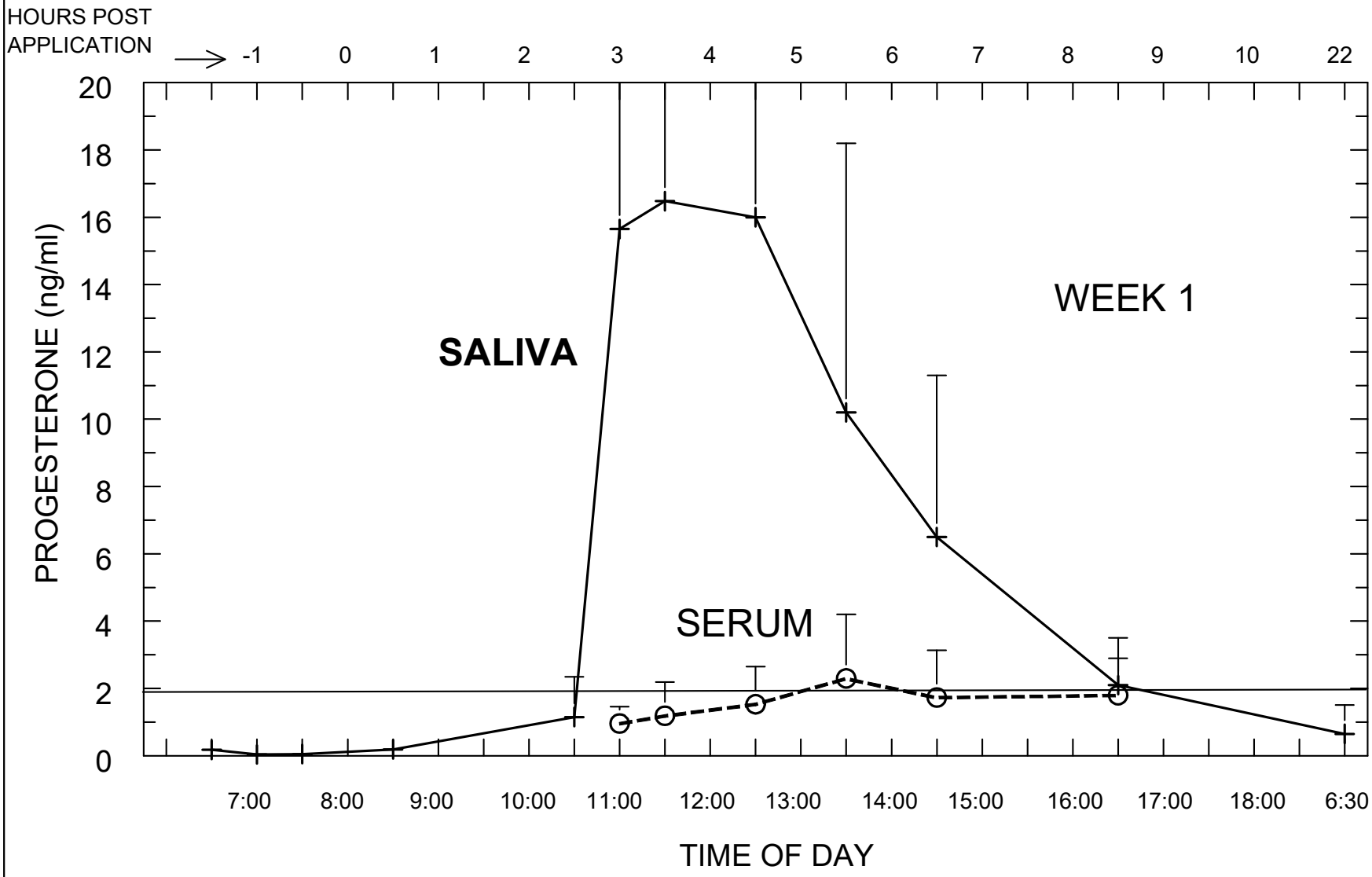
Salivary Progesterone -
12 hours After Topical Supplementation



Salivary Progesterone -
24 hours After Topical Supplementation



SALIVA AND SERUM PROGESTERONE LEVELS
FOLLOWING 30 MG TOPICAL PROGESTERONE



Serum vs. Saliva Steroid Hormone Levels

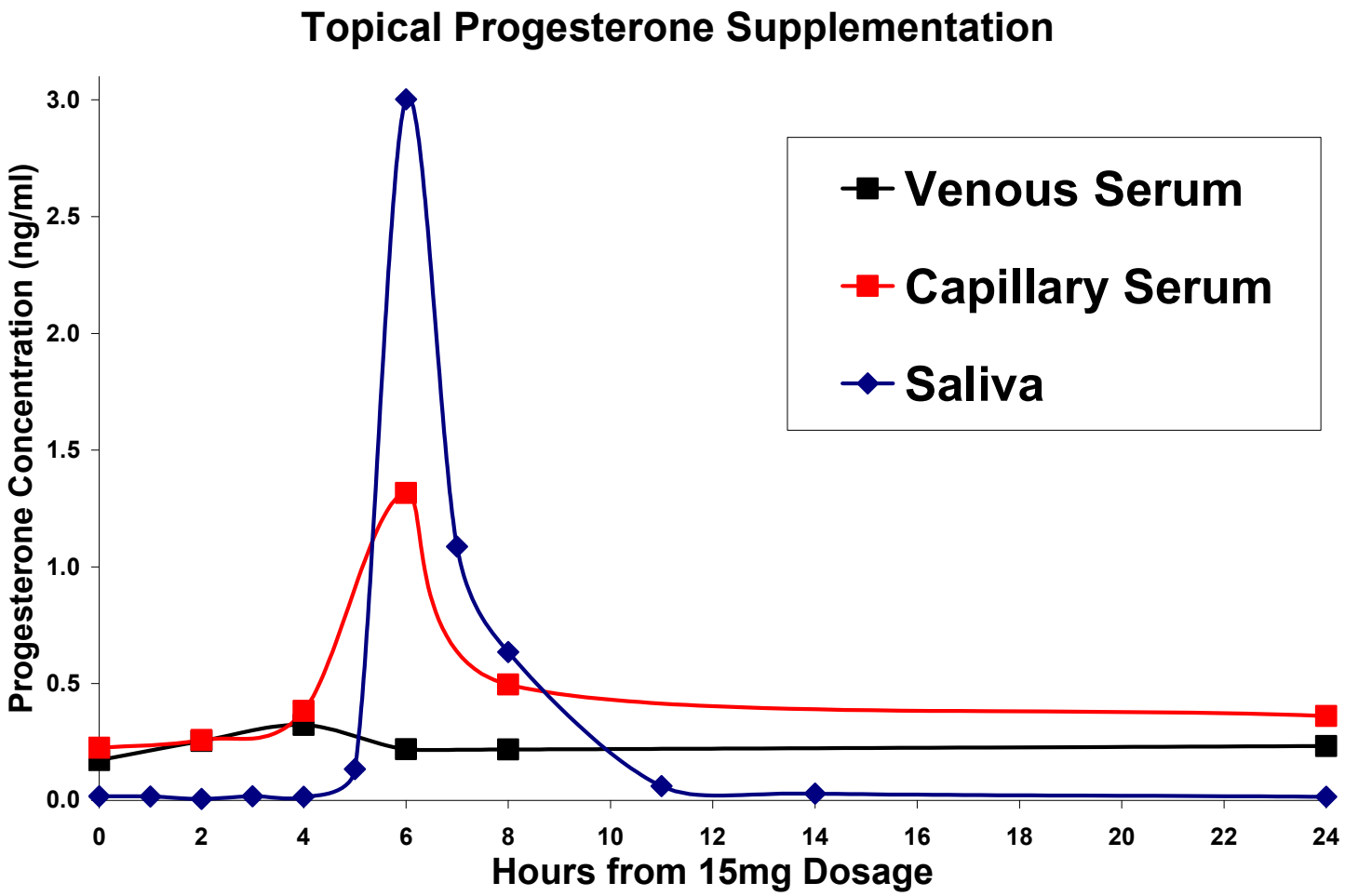
- Salivary/serum correlations are very good when hormones produced endogenously

HOWEVER

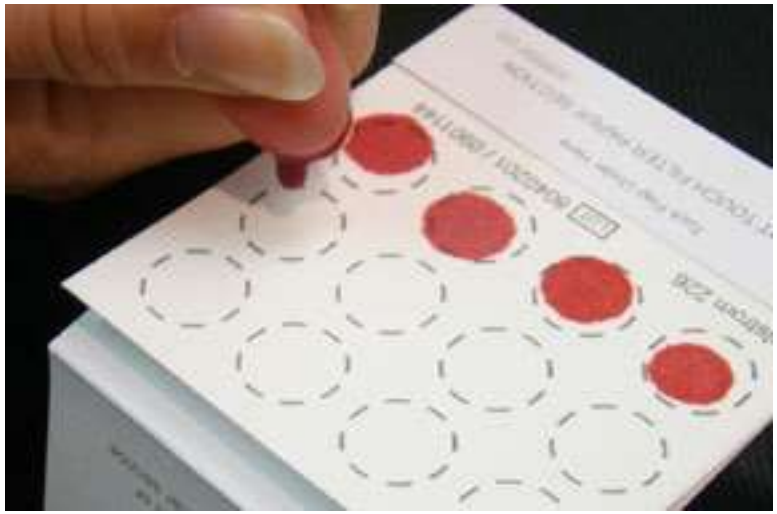
- Salivary/serum correlations become problematic with exogenous topical hormone replacement

TOPICAL application of ALL steroid hormones (Estradiol, Estriol, Progesterone, Testosterone, DHEA, Cortisol, etc.) results in a marked increase in saliva hormone levels but little change in serum hormone levels

Distribution of Progesterone in Different Body Fluids Following 15 mg Topical Pg



HORMONE TESTING
IN
DRIED BLOOD SPOTS

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DO NOT TOUCH FILTER PAPER SECTION

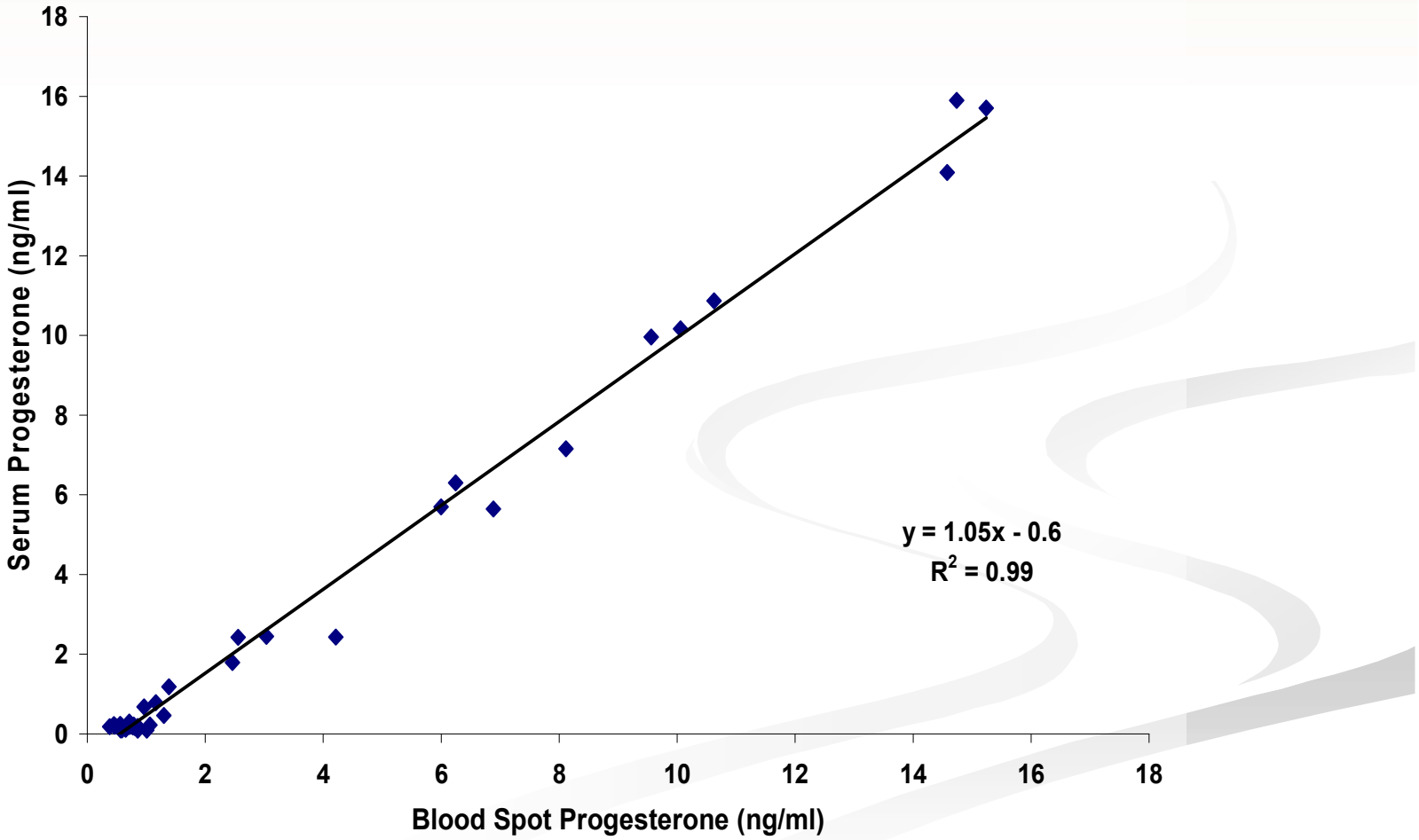
Capillary Dried Blood Spot-Advantages-1

- Convenient-Simple collection procedure
- More latitude in collection timing than serum-i.e. night time sampling for cortisol & insulin
- Dried serum analytes very stable for at least a month at ambient temperature-shipping simplified-allows for international shipment without need for Biohazard Label
- Wider range of analytes can be tested-similar to serum/plasma
- Results & ranges equivalent to serum/plasma for endogenously produced hormones
- More accurately reflects tissue uptake of exogenously used hormones than serum/plasma (gross underestimation)

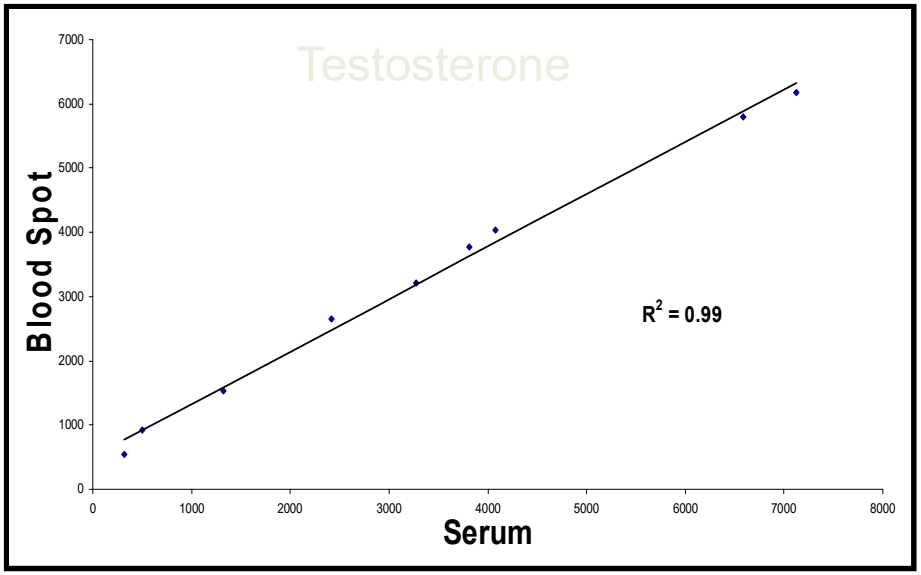
Why Blood Spot and Not Saliva?

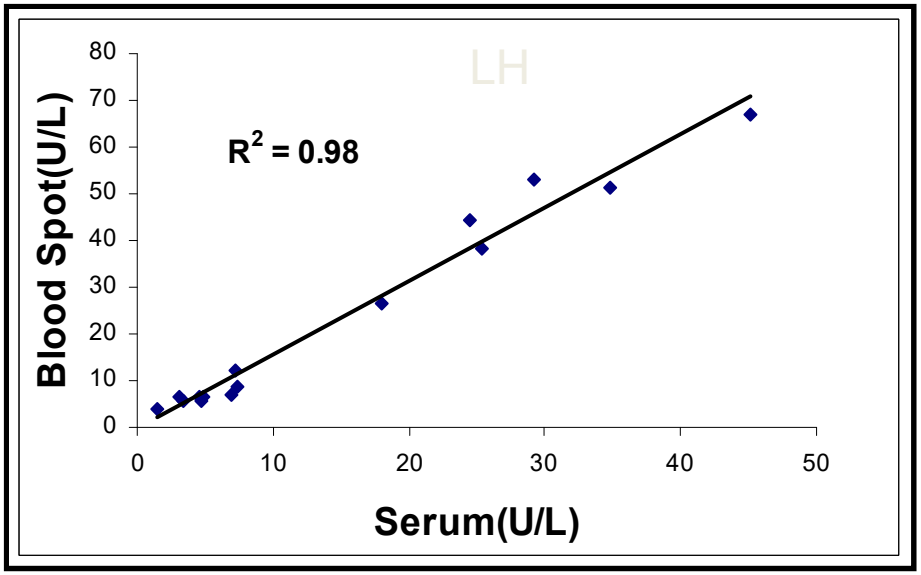
- Able to test peptide hormones (e.g. TSH, insulin) not present in saliva *and urine*
- Most accurate test for measuring steroid hormone levels in individuals supplementing with a sublingual or troche steroid hormone-gross overestimation with saliva testing due to local saturation of oral mucosa
- Allows for measurement of bioavailable fraction of steroid in blood when combined with measurement of blood binding protein (e.g. ABI = T/SHBG)

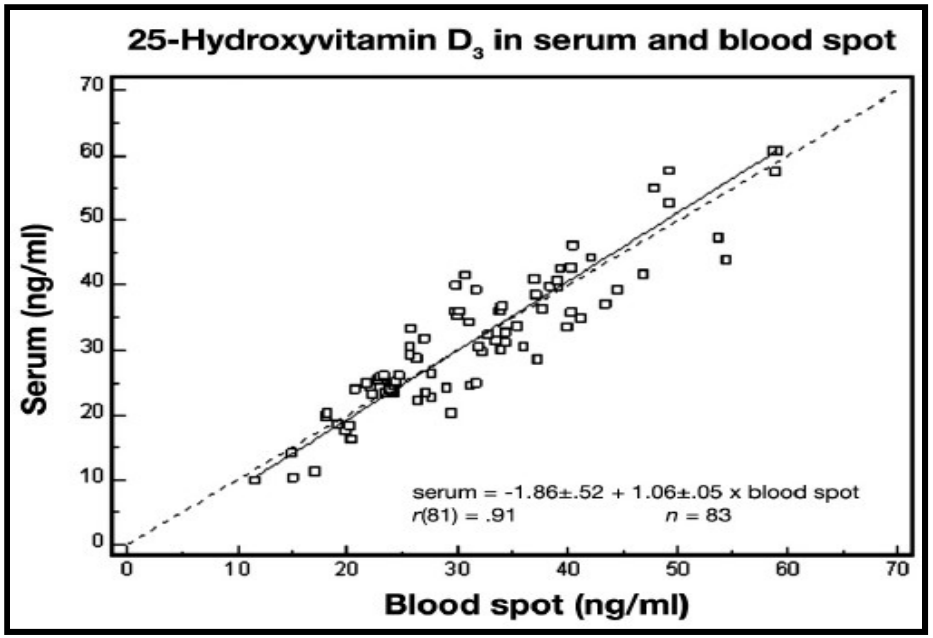
Progesterone
Blood Spot/Serum Correlation

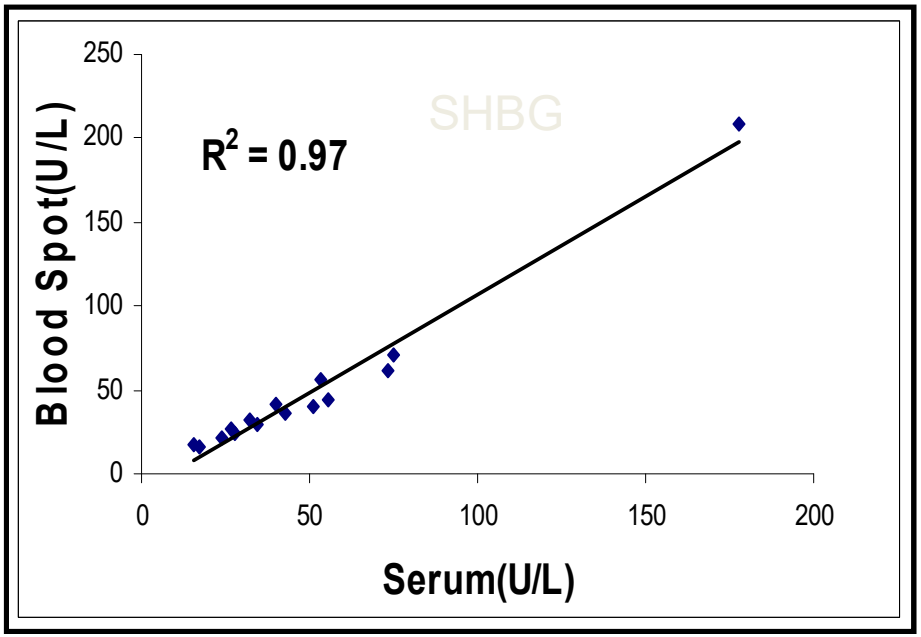


Blood Spot vs. Serum Correlations









Venous vs. Capillary Blood Progesterone Levels Following Topical Progesterone Use

Case Example

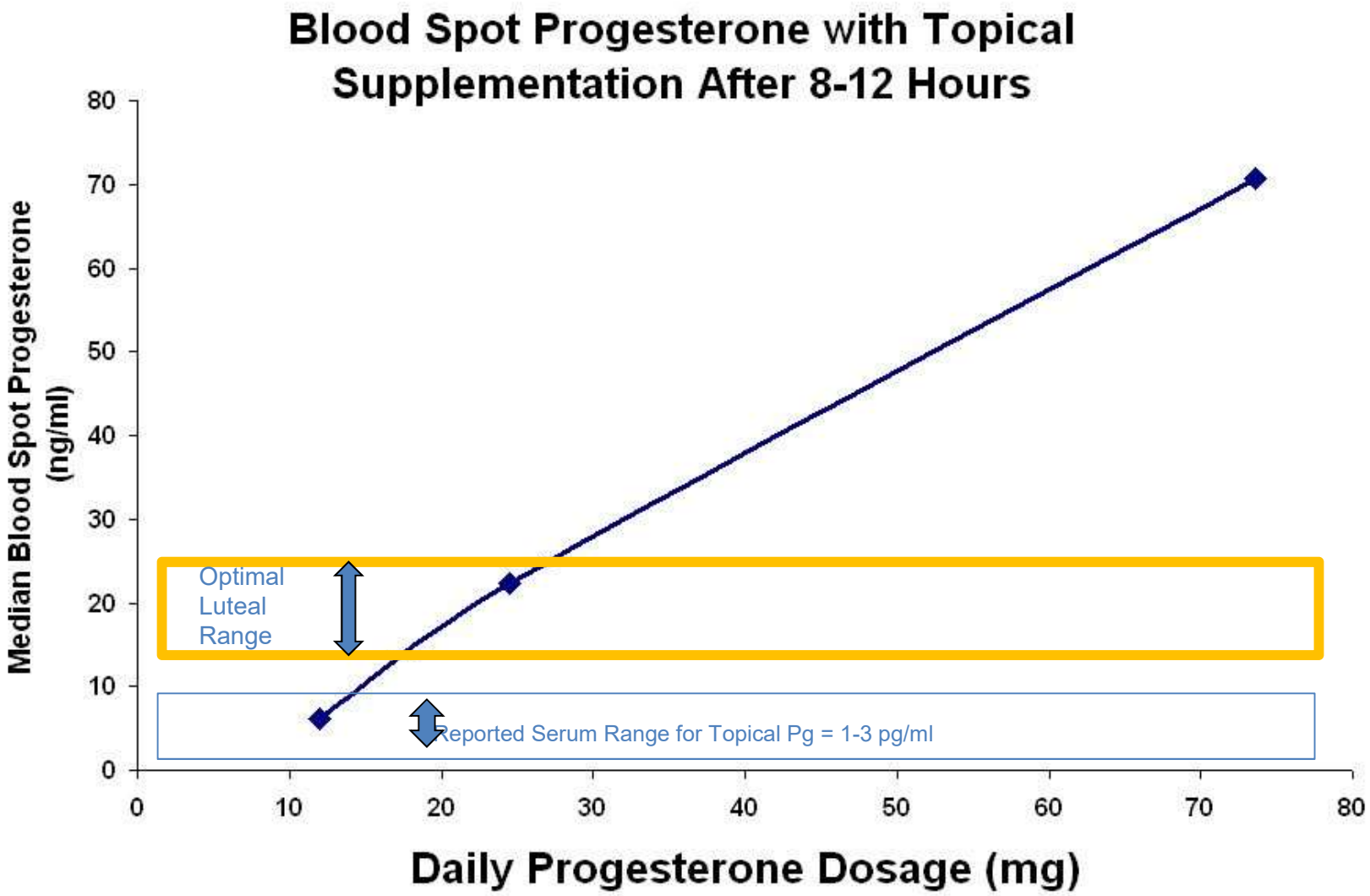
Patient using 20 mg topical progesterone BID (12 hr last used)

Body Fluid Type	Progesterone Level	Luteal Range
Serum	1.9 ng/ml	3-25 ng/ml
Blood Spot	24.6 ng/ml	3.3-22.5 ng/ml
Saliva	1291 pg/ml	75-350 pg/ml

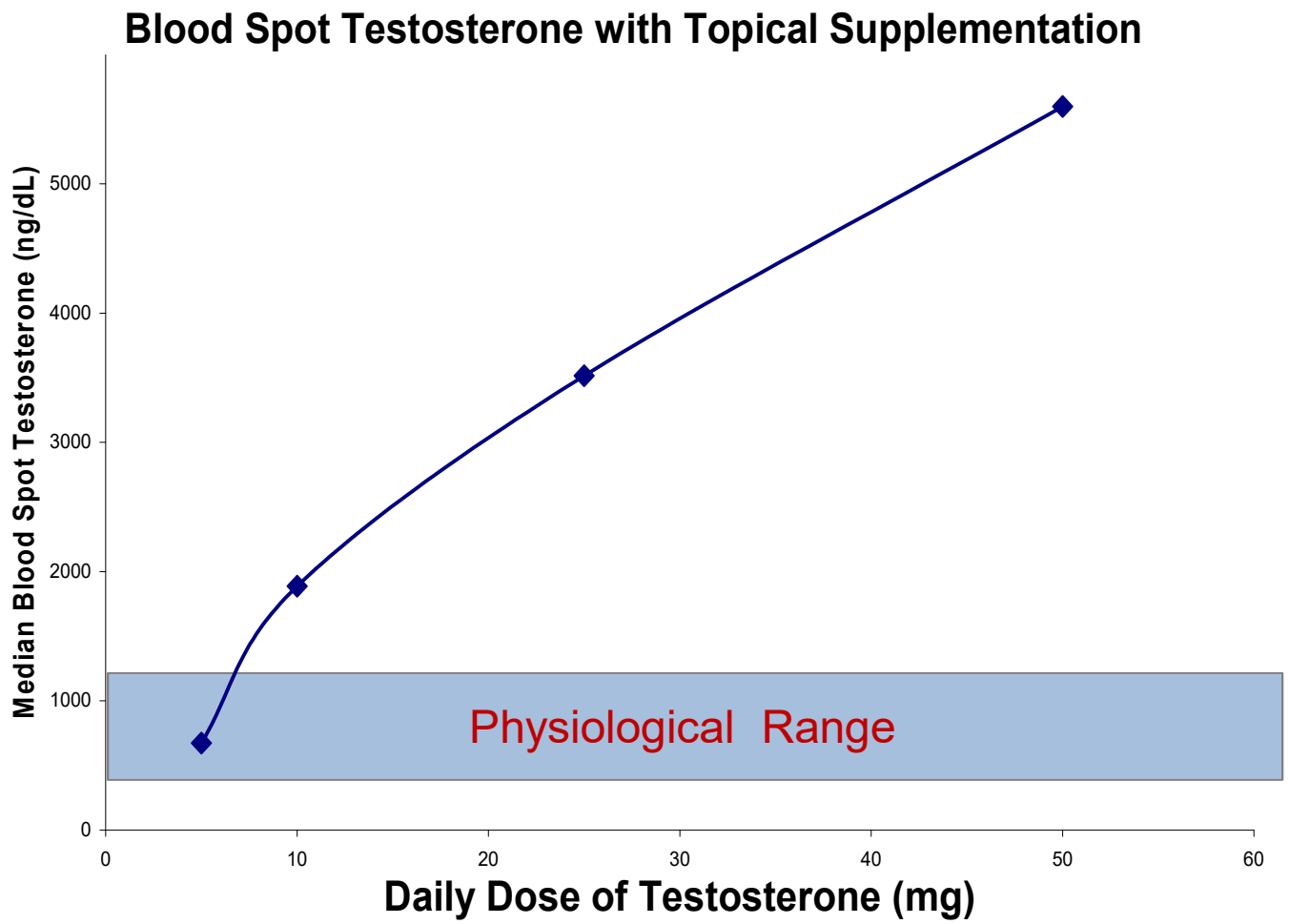
Summary: Capillary blood level of progesterone is 10x serum!

Serum grossly underestimates tissue uptake of progesterone delivered topically

Physiological Dosing (20-30 mg) with Topical Progesterone Results in Physiological (Luteal) Levels (15-25 ng/ml) of Progesterone in Capillary Blood



Linear Increase in Capillary Blood Testosterone with Increasing Topical Testosterone Dosage



Why is this so important to understand?

Need to have a hormone test that accurately reflects how much hormone is being taken up by tissue, not how much is circulating in venous blood.

With topically delivered hormones *venipuncture* serum and urine grossly underestimate (10x) tissue uptake of hormone. Underestimating tissue uptake, based on venous serum results, often leads to overdosing.

Prevents overdosing of patients with topically delivered hormones and allows for a more physiological delivery of hormones to target tissues

A Guide to Steroid Hormone Testing in Different Body Fluids Following Different Routes of Hormone Administration

Type of Body Fluid	None Endogenous Steroids	Oral Steroids	Topical Steroids	Vaginal Steroids	Troche Steroids	Pellet/IM Steroids
Serum	Yes	Yes (1)	No (2)	No (2)	Yes	Yes
Saliva	Yes	Yes (3)	Yes (3)	Yes	No (4)	Yes
Urine	Yes	Yes (1)	No (2)	No (5)	No (2)	Yes (1)
DBS	Yes	Yes	Yes (6)	Yes	Yes	Yes

1) Overestimation: Metabolites interfere with immunoassays- LC-MS or GC-MS ok

2) Underestimation: Hormone levels not reflective of tissue uptake

3) Overestimation: Requires range adjustment

4) Overestimation: Direct contamination of oral mucosa/saliva

5) Overestimation: Direct contamination of urine

6) Overestimation: IF fingertips contaminated with topical hormones

Thank You

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Accurate Testing of Hormones Opens New Doors to Better Hormone Replacement Therapy